Development of captive rearing techniques for decorator crab, *Camposcia retusa*, for marine aquarium trade

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Abstract

The marine ornamental trade is a rapidly growing industry but currently heavily relies on wild harvesting. At present, the majority of traded specimens for marine aquariums are sourced from coral reefs, which are already subjected to severe threats from increasing anthropogenic activities and climate change. Captive breeding of popular marine ornamental species, supported by robust scientific research, is believed crucial to the future sustainability of the global marine ornamental trade. It is also believed that relevant research outcomes also help fill the knowledge gaps on the basic biology of the species concerned, which aids coral reef management and conversation.

Marine ornamental crustaceans are an important group of marine ornamentals, they are popular as a part of reef aquariums due to their unique appearance and behaviour. Among them, species belonging to the superfamily Majoidea are among the most traded marine ornamental brachyurans. However, literature related to the biology and larval culture techniques of these species is scarce. Within the superfamily Majoidea, the decorator crab *Camposcia retusa*, is a species that displays unique appearance and behaviour (i.e. decorating behaviour) with good market values, hence making it a good candidate for investigating its captive breeding techniques. Therefore, this thesis addresses multiple aspects of captive breeding of *C. retusa*, including description of larval and juvenile morphology, establishment of reliable feeding regimes and identifying optimal cultural environments for each larval stage, and juvenile survival and growth pattern. The thesis consists of a total of 7 chapters.

Chapter 1 reviews the current status of larval culture techniques for marine ornamental crustaceans, with a particular focus on brachyuran species. As marine ornamental aquaculture is still in its infancy, this chapter identified the bottlenecks that hinder the development of the captive culture of the marine ornamental crabs and highlights the areas where research efforts should be focused on for future study. The key areas identified include the establishment of

the reliable larval feeding regime and optimisation of larval culture conditions, such as physical environmental conditions and potential settlement cues for megalopae, through scientific studies.

In Chapter 2, the morphology of larvae and the first stage juvenile (C_1) of *C. retusa* is described for the first time based on captive-bred specimens. Newly hatched larvae obtained from the wild-captured broodstocks were successfully reared to settle as C_1 crabs. The morphology of each larval stage was compared with a previous description that relied on wild-collected planktonic specimens. Due to substantial differences between the two studies, it was suggested that some specimens used by the earlier study may not be those of *C. retusa*. In addition, the morphology of each larval stage was also compared with the corresponding stages of other species from family Inachidae reported previously.

Chapter 3 reports a series of three experiments conducted to establish a larval feeding regime for *C. retusa*. In the first experiment, *C. retusa* larvae were offered rotifers at 30–90 ind./ml. For newly hatched Zoea 1 (Z_1) larvae, no significant difference was detected in larval survival (ranging from 58.3 to 65.0%) and moulting interval between all treatments, including the unfed control. However, no Zoea 2 (Z_2) larvae were able to survive to the megalopal stage. Therefore, it was concluded that rotifer is not a suitable live prey for *C. retusa* larval rearing. In the second experiment, live prey offered to *C. retusa* larvae were newly hatched *Artemia* nauplii at the density of 5, 10 and 15 ind./ml, as well as the combination of *Artemia* nauplii and pelagic copepods at 5 ind./ml each. Survival to the megalopae was significantly higher in treatment provided 10 *Artemia*/ml as prey (91.3 ± 3.1%), while the supplement of copepods to co-fed with *Artemia* did not show any positive effects on larval performance. Despite general high survival to megalopae, high mortality (> 80%) occurred during the megalopal stage in all treatments. The third experiment investigated if enrichment of *Artemia* might improve the survival of *C. retusa* megalopae. Four feeding treatments with unenriched newly hatched nauplii and enriched *Artemia* metanauplii were provided at 5 and 10 ind./ml, respectively, to feed the larvae. Again, the highest survival (65.1 ± 6.3%) obtained from the treatment used unenriched newly hatched

Artemia nauplii at 10 ind./ml. Interestingly, larvae fed enriched *Artemia* had significantly lower survival to the megalopal stage when compared to those fed unenriched *Artemia* nauplii at the same density ($p = 0.001$). Similar to the previous experiments, more than 80% of megalopae did not survive to the C_1 stage, suggesting that environmental factors rather than food may be the main contributors to high mortality observed during the megalopal stage.

In all the above experiments, at least 60% unfed larvae from the controlled moulted to Z_2, it was also observed Z_1 larvae showed organ colour in their guts, hence the Z_1 larvae of *C. retusa* are considered as facultative lecithotrophic.

In Chapter 4, the first two experiments were conducted to identify the optimal salinity condition for *C. retusa* larval culture. The zoeal larvae were reared under 7 salinities of 26, 29, 32, 35, 38, 41 and 44. The results showed a complete mortality of zoeae within 3 days when salinity was between 26–32, however, when salinity was between 35–38, zoeae showed high survival to megalopae (76.7–81.7%), which was significantly higher than that of salinity 44 treatment (61.7 ± 3.3%; $p < 0.05$). However, there was no significant difference in zoeal duration across all salinity treatments. In the subsequent experiment, newly moulted megalopae were subjected to 6 salinities of 26, 29, 32, 35, 38 and 41. The results showed that at salinity 32, the megalopal survival was the highest (84.0 ± 11.7%), which was significantly higher than those from salinities 26, 38 and 41 ($p < 0.05$). Meanwhile, megalopal durations were significantly shorter under lower salinities (26–32) than those reared under higher salinities (35–41; $p < 0.05$). The polynomial regression suggested that the optimal salinity for *C. retusa* larvae shifted with the ontogenetic development of larvae (36.3 for zoeae vs. 32.7 for megalopae). To better understand the underlying mechanism of salinity effects on megalopae, 24 h ingestion rate of individual megalopa was investigated under 3 salinity conditions of low (salinity 27), optimal (salinity 32) and high (salinity 37) within their tolerable range based on the previous experiment. The results suggested that salinity strongly influence the feed intake pattern of *C. retusa* megalopae. In particular, the food consumption of megalopae reared in their unfavourable salinities was suppressed, especially during the

first 1/3 of their stage interval ($p < 0.01$). However, the total number of *Artemia* consumed by megalopae reared in different salinities did not show a significant difference ($p > 0.05$). Considering the higher energy cost of larvae for osmoregulation when reared in unfavourable salinities, suggesting that a substantially lower amount of energy was channelled for development and growth for those megalopae reared under unfavourable salinities, hence higher mortality rates.

Chapter 5 describes four experiments conducted to evaluate potential other environmental factors that may influence the survival of *C. retusa* megalopae. The first experiment examined the effects of stocking density and basal area of culture vessels on megalopal survival. Megalopal survival was found significantly improved by reducing stocking density or increasing the basal area of cultural vessels while megalopal duration was not affected. In the following two experiments, whether the presence of conspecific adult and juvenile exudates influenced larval settlement and metamorphosis was assessed. The results demonstrated that the presence of adult exudates improved larval survival and accelerated development; on the other hand, the presence of juvenile exudates delayed the metamorphosis of the megalopae. The last experiment investigated if the presence of artificial substrates (50 to 800 μm nylon mesh) affected settlement and metamorphosis of *C. retusa* megalopae. Megalopal survival increased with the increase of mesh size, and a significant difference was found between the control (absence of substrate) and the 800 μm nylon mesh treatment. On the other hand, the presence of nylon mesh did not influence megalopal duration.

In chapter 6, the survival, growth pattern, and sexual dimorphism of *C. retusa* juvenile was investigated, modelled, and illustrated based on captive-bred specimens. *C. retusa* juveniles were reared individually from the first to the tenth (C_1–C_{10}) stage. The moulting interval of each juvenile stage increased in an exponential manner from 5.2 ± 0.1 to 47.5 ± 4.4 days. Meanwhile, the pattern of carapace length and width increases and also followed the exponential model, which showed that newly settled C_1 crabs can be reared to reach market size in about a 6-month time. Based on the morphology of pleopods, i.e. the

appearance of four pairs of pleopods in females while only two pairs of pleopods in males, sexual differentiation of *C. retusa* juveniles was observed at the C_5 stage.

Chapter 7 summarises the thesis results and discusses them in a broader context.

In summary, the research presented in this thesis achieved the aim of developing a reliable larval culture protocol for the popular marine ornamental species, the decorator crab *C. retusa*. With the techniques developed through this study, *C. retusa* larvae can now be reliably reared with good survival and development using newly hatched *Artemia* nauplii provided at 10 ind./ml. *Camposcia retusa* larvae were also revealed to be rather stenohaline and culture salinity should be kept between 35–37 for zoeal larvae, then reduced to 30–33 at the megalopal stage. During the megalopal stage, reducing stocking density, increasing the basal area of cultural vessels, and providing conspecific exudates can improve the production of C_1 crabs. After the larvae settled as juveniles, they can reach market size in about 6 months. This thesis also expands the knowledge on larval morphology and other general larval biology of *C. retusa.*

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Chapter 1. General introduction: Review on development of larval rearing techniques for marine ornamental crabs

The global trade of aquatic organisms and associated products for home and public aquarium has become a multi-billion-dollar industry, known as aquatic ornamental industry (AOI). Although the accurate number is difficult to obtain, the annual trade value of AOI is estimated to be between 15 to 30 billion US dollars (Bartley 2000; Palmtag 2017). Within the AOI, the marine species only make up less than 10% of the total volume of the aquatic ornamental trade, however, owing to their high unit price, their percentage in terms of value is much higher (King 2019; Livengood & Chapman 2007; Whittington & Chong 2007). A number of important technological improvements over the past two decades, such as commonly available of commercial instant ocean products (salts that can be used to make seawater suitable for keeping marine ornamentals) and highly efficient recirculating aquarium systems, have facilitated the popularity of marine aquarium keeping (Calado 2006; Calado *et al.* 2003a; Pinnegar & Murray 2019; Wood 2001). Based on recent estimations, over 46 million marine ornamental organisms representing 2,500 species are traded annually with a value exceeding 300 million US dollars (King 2019; Palmtag 2017).

The continual expansion of marine aquarium trade reveals in the voracious appetite for a wide range of marine ornamental species (Cohen *et al.* 2013). However, unlike their freshwater counterparts, where more than 90% of species are captive bred, it is estimated that about 90–95% of marine ornamentals are currently sourced from the wild, mainly from tropical coral reefs (King 2019; Olivotto *et al.* 2016; Wabnitz *et al.* 2003). Such heavy reliance on wild-caught individuals and the selective collection of high value species are likely to have negative impacts on the biodiversity of the fragile coral reef ecosystem (Olivotto *et al.* 2016).

The current marine ornamental trade is characterised by sourcing the bulk of reef

dwelling organisms from the tropical developing countries to export to developed countries, such as US, EU countries and Japan (Pinnegar & Murray 2019; Thornhill 2012). For example, the Coral Triangle Region, including the waters off the Pacific countries (e.g. Indonesia, Malaysia, the Philippines, Papua New Guinea, Solomon Islands and Timor-Leste), supplies about 2/3 of the marine ornamentals for the global trade (Koldewey & Martin-Smith 2010; Murray *et al.* 2012). In some countries of this region, destructive capture method, such as sedation of fish using chemicals (e.g. cyanide etc.), using explosions, and smashing the coral rocks to drive animals into the nets, are widely practiced (Cohen *et al.* 2013; Moorhead & Zeng 2010). At the point of collection, these destructive collecting practices inflict severe, and sometimes, permanent damages to the coral reef ecosystems (Cohen *et al.* 2013; Palmtag 2017). The extension of toxic chemicals and the physical damage to reefs also contribute to the habitat destruction and likely will kill non-targeted species (Palmtag 2017; Thornhill 2012). The over-exploitation of reefs in destructive, unsustainable manners has been reported to affect the recovery of exploited reefs, and thus, reduce the yields of both ornamental and food species from reefs (Ziemann 2001). In fact, in some cases, the marine ornamental market itself has been directly impacted by the localised reduction of several popular species (Ziemann 2001). In addition, the compound effect of chronic chemical toxicity, poor handling and stress during transport also results in high post-collection mortality (King 2019; Militz *et al.* 2018; Wood 2001), which may also affect the reputation of collectors and the whole marine ornamental industry (Dawes 2003).

The ill-managed marine ornamental fishery is likely to add to the woe of global climate change and increased human activities that are threatening the very existence of the world coral reefs (Militz *et al.* 2018; Olivotto *et al.* 2016; Olivotto *et al.* 2011; Wood 2001). The necessity for multi-faceted management to control the loss of reefs and stimulate recovery is obvious (Morcom *et al.* 2018; Olivotto *et al.* 2016). The fact that the majority of coral reefs are classified as threaten may lead some to believe that eliminating the marine ornamental trade would help improve the well-being of the coral reefs; however, scientists who study coral reefs pointed out that the public aquarium play the pivotal role in public awareness on the dire situation of reefs and the urgent need for reef conservation (Tlusty *et al.* 2013). In

fact, marine ornamental aquaculture has great potential and can alleviate the collection pressure on the reefs while offering sustainability to the marine ornamental trade industry (King 2019; Murray & Watson 2014). Captive breeding of marine ornamentals not only could reduce the wild collection, but also may help reef restoration via controlled releasing captive-bred fingerlings/juveniles of heavy-harvested species. Additionally, captive breeding of marine ornamentals requires good knowledge on reproductive and larval biology of the species to be bred, which should help fill the knowledge gaps that are existing for the majority of reef-dwelled species, hence improving our understanding of reef ecosystems (Olivotto *et al.* 2016; Olivotto *et al.* 2011; Palmtag & Holt 2007).

Over the last couple of decades, significant progress has been made in the breeding of marine ornamental species (King 2019). CORAL Magazine summarised the species of ornamental fish that has been bred successfully in captive conditions, with 39 new species added in 2019, bringing the total number to 398 species (Sweet & Pedersen 2019). However, for nearly 90% of these species (i.e. 352 out of 398), the efforts of captive breeding are still only achieved in small-scale or in laboratory trials with low survival rates, hence are not commercially viable. It would be quite some time before they can be supplied to the market at reasonable prices through commercial aquaculture production (Sweet & Pedersen 2019). One of the reasons behind this phenomenon is the general poor understanding of the biology of marine ornamentals and the lack of systemic and scientifically rigorous research (e.g., successful breeding often accomplished by hobbyists with low repeatability), which severely limits the establishment of reliable breeding techniques that is essential for commercial marine ornamental aquaculture (Murray & Watson 2014; Tlusty 2002).

While greater effort has been made more recently to develop breeding techniques for marine ornamental decapods, this sector lags far behind ornamental fish, which even itself is poorly studied (Calado *et al.* 2003a). In addition, current research on marine ornamental decapods culture is largely bias toward ornamental shrimps. For the ornamental crabs, the paucity of information on both reproductive biology and larval culture is clear since, thus far, only two species (i.e.: *Mithraculus forceps* and *Mithraculus sulptus*) have been studied in

certain degree of detail for their captive culture techniques (Penha-Lopes *et al.* 2005; Rhyne *et al.* 2005; summarised in Table 1.1). To reach commercial success, marine ornamental crab aquaculture needs to develop reliable techniques that can yield large quantity and high quality of eggs and rear larvae to marketable sizes with decent survival rates (Tlusty 2002). To achieve this, robust scientific experiments are required. The aim of this chapter is to highlight the major bottlenecks of the marine ornamental crab larval aquaculture. Due to the lack of literature on captive breeding of ornamental crabs, techniques and methods developed for food crabs, as well as other decapods, that have the potential to be adapted for marine ornamental crabs are also included. Overall, this chapter attempts to provide an overview on the current status, major challenges and prospects of captive breeding of marine ornamental crabs.

1.1 Marine ornamental crabs: taxonomy and current status on their captive breeding research

Decapods are one of the most popular groups of invertebrates currently traded in the marine aquarium market (Calado *et al.* 2003a). Their popularity is not due exclusively to their dazzling colouration and unique body forms, marine ornamental decapods usually are hardy and "reef safe" (i.e., not harming corals and other organisms in the same aquarium set-up; Calado 2006). Moreover, some ornamental decapods can be used as "cleaning crews" to control nuisance organisms (e.g. glass anemones, hair algae, etc.) growing in the aquarium (Calado 2006; Calado *et al.* 2003a; Olivotto *et al.* 2011; Penha-Lopes *et al.* 2005).

Among all ornamental crabs supplied to the marine ornamental market, small majoid crabs and hermit crabs are likely experiencing the most intensive capture effort. Table 1.1 shows currently traded marine ornamental crabs based on available information: in total, 59 species are traded and among them, 27 are from the infraorder Brachyura, also known as "true crabs". Among the ornamental brachyuran crabs, the highest number of species (7) are

spider/arrow crabs from family Inachidae. As shown in the Table 1.1, many of the ornamental crabs display special behaviours (i.e. associative or decorating behaviours) or can be employed as cleaner crews in aquaria. For example, herbivorous brachyuran crabs from genus *Mithraculus* and Anomuran crabs from family Diogenidae, are particularly popular due to their efficiency in controlling the proliferation of nuisance algae within aquaria (Calado 2006; Calado *et al.* 2003a; Rhyne *et al.* 2005).

However, larval development for the majority of ornamental crab species is either unknown or only partially known (Table 1.1). Even in the relatively well-understood group, for example the majoid crabs, information on their larval development is only available for a few numbers of species (Table 1.1). The typical larval development for ornamental brachyuran crabs includes several zoeal and one megalopal stage (Anger 2001). The swim of brachyuran zoeal larvae relies exclusively on natatory exopods of the maxillipeds, as their pereiopods and pleopods are not functional (Clark *et al.* 1998; Martin *et al.* 2014; Møller *et al.* 2020). On the other hand, megalopal larvae have well-developed chelae and walking legs (pereiopods), which are more similar to the benthic juveniles in appearance; and they and also swim with natatory pleopods (Anger 2001; Martin *et al.* 2014; Møller *et al.* 2020).

Table 1.1. Marine ornamental crab species for aquarium trade and current status of knowledge on their larval development and captive breeding techniques

	Superfamily	Family	Scientific name	Common name	Distribution	LD	CBT	PB
Brachyura	Dromioidea	Dromiidae	*Cryptodromiopsis antillensis*	Hairy sponge crab	Western & Central Atlantic	K[1]	BD[2]	AI
			Dromia marmoreal	Marble sponge crab	Eastern Atlantic	U	NA	AI
			Dromia personata	Linnaeus's sponge crab	Eastern Atlantic	K[3]	NA	AI
	Majoidea	Inachidae	*Camposcia retusa*	Decorator spider crab	Indo-Pacific	K[4]	E[4]	DB
			Inachus phalangium	Anemone spider crab	Eastern Atlantic	K[5]	NA	AI
			Inachus dorsettensis	Decorator spider crab	Eastern Atlantic	K[6]	NA	DB
			Macropodia rostrata	Spider crab	Eastern Atlantic	K[6]	NA	DB
			Stenorhynchus debilis	Panamic arrow crab	Eastern Atlantic	U	NA	
			Stenorhynchus lanceolatus	Arrow crab	Eastern Atlantic	K[7]	NA	AI
			Stenorhynchus seticornis	Yellowline arrow crab	Western Atlantic	K[8]	NA	AI
		Mithracidae	*Leptopisa setirostris*		Southwest Atlantic	U	NA	AI
			Mithraculus forceps	Ruby red crab	Western Atlantic	K[10]	E[9]	CC
			Mithraculus sculptus	Emerald crab	Western Atlantic	U	E[9]	CC
		Epialtidae	*Lissa chiragra*	Decorator crab	Eastern Atlantic	K[11]	NA	DB
			Pisa armata	Decorator crab	Eastern Atlantic	K[12]	NA	DB
			Pelia mutica	Cryptic teardrop crab	Western Atlantic	U	NA	AI
	Grapsoidea	Plagusiidae	*Percnon gibbesi*	Sally lightfoot	Atlantic	K[13]	BD	AI
	Portunoidea	Portuniidae	*Lissocarcinus laevis*	Harlequin crab	Indo-Pacific	U	NA	AI

			Lissocarcinus orbicularis	Harlequin crab	Indo-Pacific	U	NA	AI
	Pseudozioidea	Pseudoziidae	*Euryozius bouvieri*	Strawberry crab	Atlantic	U	NA	
	Trapezioidea	Trapeziidae	*Trapezia ferruginea*	Rusty guard crab	Indo-Pacific	U	NA	AI
			Trapezia rufopunctata	Rust-spotted guard crab	Indo-Pacific	U	NA	AI
			Trapezia wardi	Red-spotted guard crab	Indo-Pacific	U	NA	AI
	Xanthoidea	Xanthidae	*Liomera cinctimana*	Colourful reef crab	Indo-Pacific	U	NA	
			Lybia edmonsoni	Hawaiian pom-pom crab	Pacific	U	NA	AI
			Lybia tessalata	Boxer crab	Indo-West Pacific	U	NA	AI
			Platypodiella picta	Gaudy clown crab	Eastern Atlantic	U	NA	AI
Anomura	Galatheoidea	Porcellanidae	*Neopetrolisthes alobatus*	Porcelain crab	Indo-West Pacific	U	NA	AI
			Neopetrolisthes maculatus	Porcelain crab	Indo-West Pacific	PK[14]	BD	AI
			Neopetrolisthes ohshimai	Anemone crab	Indo-West Pacific	PK[14]	BD	AI
			Porcellana sayana	Spotted porcelain crab	Western Atlantic	U	NA	AI
	Paguroidea	Coenobitidae	*Coenobita clypeatus*	Caribbean hermit crab	Western Atlantic	K[15]	NA	CC
			Coenobita compressus	Ecuadorian hermit crab	Eastern Pacific	K[16]	NA	
			Coenobita perlata	Red hermit crab	Central Indo-Pacific	U	NA	
			Coenobita variabilis	Australian land hermit crab	Indo-Pacific	U	NA	
	Paguroidea	Diogenidae	*Aniculus aniculus*	Scaly-legged hermit-crab	Western Pacific	U	NA	CC
			Aniculus maximus	Hairy yellow hermit crab	Indo-Pacific	U	NA	

	Calcinus californiensis	Red-leg hermit	Eastern-Pacific	U	NA	
	Calcinus elegans	Electric blue knuckle hermit crab	Indo-Pacific	U	NA	
	Calcinus laevimanus	Dwarf zebra hermit crab	Western Pacific	U	NA	
	Calcinus tibicen	Pacific red legged hermit crab	Western Atlantic	K[17]	NA	
	Calcinus tubularis	Sedentary hermit crab	Eastern Atlantic	K[18]	NA	
	Ciliopagurus striatus	Halloween hermit crab	Indo-Pacific	U	NA	
	Clibanarius aequabilis	Dwarf red tip hermit crab	Eastern Atlantic	K[19]	NA	
	Clibanarius erythropus	Dwarf red/yellow tip hermit crab	Eastern Atlantic	K[19]	NA	
	Clibanarius tricolor	Blue legged hermit crab	Western Atlantic	U	NA	
	Clibanarius virescens	Thin stripe hermit crab	Indo-West Pacific	U	NA	AI
	Dardanus deformis	Anemone carrying hermit crab	Indo-Pacific	U	NA	AI
	Dardanus guttatus	Anemone carrying hermit crab	Indo-Pacific	U	NA	AI
	Dardanus lagopodes	Anemone carrying hermit crab	Indo-West Pacific	U	NA	AI
	Dardanus megistos	Anemone carrying hermit crab	Indo-West Pacific	U	NA	AI
	Dardanus pedunculatus	Anemone carrying hermit crab	Indo-West Pacific	U	NA	AI

		Paguristes cadenati	Red legged hermit crab	Western Atlantic	U	NA	
		Paguristes eremita	Hermit crab	Eastern Atlantic	K[18]	NA	
		Petrochirus diogenes	Giant hermit crab	Western Atlantic	K[20]	NA	
Paguroidea	Paguridae	*Manucomplanus varians*	Staghorn hermit crab	Pacific	U	NA	AI
		Paguritta gracilipes	Coral hermit crab	Pacific	U	NA	AI
		Pagurus prideaux	Prideaux's hermit crab	Eastern Atlantic	K[21]	NA	AI
		Phimochirus operculatus	Polka dot hermit crab	Western Atlantic	U	NA	CC

Abbreviations: LD: larval development： K: known; PK: partly known; CBT: captive breeding techniques： E: established; BD: being developed; NA: not addressed; U: unknown; PB: particular behaviour： AI: associated with invertebrate; DB: decorating behaviour; CC: cleaning crew.

References: [1] Rice and Provenzano 1966; [2] Calado *et al.* 2003b; [3] Rice *et al.* 1970; [4] current thesis; [5] Lebour 1928; [6] Ingle (1992); [7] Paula and Cartaxana 1991; [8] Yang 1976; [9] Rhyne *et al.* 2005; [10] Wilson *et al*. 1979; [11] Guerao *et al*. 2003; [12] Ingle and Clark 1980; [13] Paula and Hartnoll 1989; [14] Fujita and Osawa, 2003; [15] Provenzano 1962b; [16] Brodie and Harvey 2001; [17] Provenzano 1962a; [18] Pike and Williamson 1960; [19] Bartilotti *et al*. 2008; [20] Provenzano 1968; [21] Goldstein and Bookhout 1972.

1.2 Larval culture of marine crabs

Larval culture often presents itself as the most significant challenge and bottleneck for aquaculture development of many species (Dhert *et al.* 2001; Moorhead & Zeng 2010). The reliable technique of rearing larvae to the juvenile stage is essential for a sustainable commercial production of any aquaculture species (Moorhead & Zeng 2010), and marine ornamental crabs are no exception. Research and techniques of larval rearing of marine ornamental crabs lag far behind that of edible crabs and other marine ornamental groups (i.e. fish and shrimps; Calado *et al.* 2003a; Calado *et al.* 2003b). The obstacles of the larval culture for marine ornamental crabs lie on the lack of knowledge on the larval biology and development, including requirements of feeds, nutrition, and cultural environment, of most traded ornamental species (Calado *et al.* 2003a; Calado *et al.* 2003b; Calado *et al.* 2017; Olivotto *et al.* 2011).

1.2.1 Larval feeds

Most decapod larvae require external feeding to acquire dietary nutrition for normal growth and development (Anger 2001). Food is arguably the most important factor that affect the success of larval culture of marine crabs, while many physical and chemical aspects determine whether a diet is suitable for the larvae of a target species (Oliver *et al.* 2017; Ruscoe *et al.* 2004). The physical characteristics of the larval feeds include size, shape, mobility and density, while the chemical features include the nutrition contents, digestibility and also attractants (Anderson & De Silva 2011; Holme *et al.* 2009; Ruscoe *et al.* 2004; Waiho *et al.* 2018). It is well recognised that high mortality due to suboptimal larval feed represents an impediment for the development of both marine ornamental and edible crabs (Dan *et al.* 2016b; Hamasaki *et al.* 2002b; Rhyne *et al.* 2005; Ruscoe *et al.* 2004). However, as studies of the nutritional requirements of aquatic crustacean larvae face several technical difficulties (Anger 2006), information on this area is limited. For literatures addressing the

issue of larval feeds, feed size, type, density of feeds used for feeding and nutrition value has typically been the focus of these studies (Anderson & De Silva 2011; Anger 2001; Støttrup & McEvoy 2003), which will be discussed in the following sections.

1.2.1.1 Feed type, size, and feeding density

One of the key questions facing decapod larval cultures is to identify both optimal type and quantity of feeds for feeding different stages of larvae (Tsuji *et al.* 2015; Zhang *et al.* 1998b). As for edible crabs, establishing the optimal larval feeding regime is arguably the most critical step in developing the captive breeding techniques for a target ornamental crab species (Calado *et al.* 2017; Daly *et al.* 2009; Ruscoe *et al.* 2004). Since inert feeds (formulated feeds) are generally not successful for the larval culture of crabs, particularly early larvae (Waiho *et al.* 2018), live planktonic prey are still the primary food source for larvae of most marine decapod species (Fileman *et al.* 2014). Currently, crab hatcheries rely largely on traditional live prey, i.e. rotifers and *Artemia*, for larval rearing (Jeffs & O'Rorke 2020; Oliver *et al.* 2017; Støttrup & McEvoy 2003; Waiho *et al.* 2018). While copepods are the natural prey for many marine organisms during the larval stage, and there has been substantially increased research interest in their massive culture and application in finfish larval culture, including ornamental fishes; the necessity of their use and benefits in crab larval rearing have not been established (Arts *et al.* 2001; Støttrup & McEvoy 2003).

To establish a larval feeding regime, a wide range of factors, including live prey species selection, their optimal feeding densities, and the best timing for transiting from one prey to another to meet larval ontogenetic requirements must be addressed. As larvae develop rapidly, the optimal live prey often needs to be changed within a very short timeframe (often in days) (Tsuji *et al.* 2015; Zhang *et al.* 1998a). In general, a suitable feeding regime for a particular larval stage should meet at least following three criteria: 1) appropriate size and locomotor pattern of the prey that can be easily captured and ingested by larvae; 2) appropriate concentration of the prey is provided and supplies sufficient amount of feed

without the detrimental effects of reducing water quality; and 3) prey containing sufficient dietary nutrition that can be effectively digested and assimilated by the larvae.

In general, larvae of marine crabs are described as primarily raptorial feeders (Anger 2001). However, studies investigated the ingestion of crab larvae on natural plankton reported that their natural diets included a wide range of prey, including phytoplankton, protists, copepods, bivalve veligers, as well as mangrove detritus (Epifanio *et al.* 1994; Fileman *et al.* 2014; Hinz *et al.* 2001; Schwamborn *et al.* 2006). For the early larvae (zoeae) in particular, significant amounts of phytoplankton ingestion have been reported (Epifanio *et al.* 1994; Fileman *et al.* 2014). Similarly, rearing trials also indicated that including microalgae in early zoeal diets produced a better yield of megalopae in some crab species, such as *Mithraculus forceps* (Penha-Lopes *et al.* 2006) and edible mud crab *Scylla paramamosain* (Nghia *et al.* 2007). However, it is unclear whether the ingestion of microalgae is by accident and if they provide any signficant nutritional benefits to larvae. For the vast majority of species, hatchery feeds for crab larvae are reliant heavily on the two most commonly used live prey in commercial hatchery, namely rotifers (*Brachionus* spp.) and *Artemia* (Dan *et al.* 2016a; Holme *et al.* 2009; Ruscoe *et al.* 2004; Støttrup & McEvoy 2003). There are multiple advantages on using rotifers and *Artemia* as live prey for marine crab larvae, including their planktonic nature and small size, relatively easy for mass production, and last but not least, their non-selective filter-feeding nature that enables the improvement of their nutritional profile via enrichment (Anderson & De Silva 2011; Ferreira *et al.* 2008; Figueiredo *et al.* 2009; Navarro *et al.* 1999; Yoshimura *et al.* 2003).

Table 1.2 summarises the feeding regimes reported in the literature for both ornamental and edible crabs. It appears that rotifers are generally used as the first feed for brachyuran larvae, particularly in the case of portunid crabs. The success of rotifers as feed in marine crab hatchery is manifold. The swimming ability of early zoea is relatively weak (Ruscoe *et al.* 2004), thus, larvae may not be able to catch a highly mobile zooplankton prey so easily. As slower swimmers, the low mobility and swimming behaviour of rotifers make them more likely to be captured than other more mobile organisms, including copepods (Lumasag *et al.*

2007). Additionally, the relatively small (45–200 μm) size of rotifers makes them ideal feeds for early zoea larvae whose feeding appendages are still rudimentary and probably too small to capture newly hatched *Artemia* nauplii, which is bigger and display a stronger swimming ability (Jeffs & O'Rorke 2020; Lumasag *et al.* 2007; Støttrup & McEvoy 2003). Moreover, some authors suggested that rotifers may be more digestible to early crab larvae than *Artemia* (Davis *et al.* 2005). During early zoeal stages, brachyuran crab larvae usually exhibit low level of enzyme activity and digestive ability (Andrés *et al.* 2010b; Jones *et al.* 1997; Sui *et al.* 2008). The larval digestive system is not well-developed in many crab species during their early life stage (Jeffs & O'Rorke 2020). For example, the forming of features, such as the gastric mill and highly vacuolated epithelial cells of the midgut, take place only during 3rd and 4th zoeal stages in *Scylla serrata* (Lumasag *et al.* 2007) and *Carcinus maenas* (Spitzner *et al.* 2018), respectively; and for *Dyspanopeus sayi*, this procedure occurs even later, not until after the metamorphosis to megalopal stage (Castejón *et al.* 2015a). Thus, for species whose digestive system is undeveloped during early life stages, a highly digestible first prey is essential (Lumasag *et al.* 2007). In fact, previous research has clearly suggested the necessity of rotifers as live feed for early larvae of most of crabs (e.g. Baylon 2009; Dan *et al.* 2016a; Davis *et al.* 2005; Suprayudi *et al.* 2002).

Table 1.2. Summarization of larval feeding regimes reported for ornamental and edible crabs

Species	**Larval stage**	**Feed (prey density)**	**References**
Callinectes sapidus	Z_1–Z_2	R (50 ind./ml)	Zmora *et al.* (2005)
	Z_3–Z_4	R (50 ind./ml) & AN (300 ind./L) & FC (5g/m^3)	
	Z_5–Z_6	R (50 ind./ml) & AN (300 ind./L) & EA[1] (500 ind./L) & FC (5g/m^3)	
	Z_7–Z_8	R (50 ind./ml) & AN (300 ind./L) & EA (800 ind./L) & FC (5g/m^3)	
	M	EA (1000 ind./L) + FC (10 g/m^3)	
Chionoecetes opilio	Z–M	R (5 ind./ml) + EA[2] (0.5–1 ind./ml)	Kogane *et al.* (2007)
Eriocheir sinensis	Z_1	R (15 ind./ml)	Sui *et al.* (2008)
	Z_2	R (20 ind./ml)	
	Z_3	AN (3 ind./ml)	
	Z_4	AN (5 ind./ml)	
	Z_5	AN (8 ind./ml)	
Maja brachydactyla	Z_1–M	EA[3] (60 ind./larva)	Andrés *et al.* (2007)
M. brachydactyla	Z_1–Z_2	AN (60 ind./larva)	Guerao and Rotllant (2010)
	M	*Artemia* adult & frozen mysis	
Maja squinado	Z & M	EA[4] (60 ind./larva)	Durán *et al.* (2012)
Menippe nodifrons	Z_1–Z_3	R (40 ind./ml)	Guarizo *et al.* (2020)
	Z_4–Z_5–M	AN (0.6 ind/ml)	
Mithraculus forceps	Z & M	AN (7 ind./ml)	Penha-Lopes *et al.* (2005)
Ranina ranina	Z_1	AN (0.5 ind./ml)	Minagawa and Murano (1993a)
	Z_3	AN (0.6 ind./ml)	
	Z_5	AN (1.8 ind./ml)	
	Z_7	AN (2.3 ind./ml)	
Scylla paramamosain	Z_1–Z_2	ER[5] (30–45 ind./ml, increasing gradually)	Nghia *et al.* (2007)
	Z_3	EA[6] (10 ind./ml)	
	Z_4–Z_5	EA (15 ind./ml)	

Scylla serrata	Z_1	ER[7] (7 ind./ml)	Hamasaki *et al.* (2002b)
	Z_2	ER (9 ind./ml)	
	Z_3	ER (10 ind./ml) & AN (0.5 ind./ml)	
	Z_4	ER (12 ind./ml) & AN (0.5–1 ind./ml)	
	Z_5	ER (15 ind./ml) & AN (1 ind./ml)	
	M	minced mysis (10–100 g/kL/day) + AN (1 ind./ml)	
S. serrata	Z_1–Z_2	ER[8] (40 ind./ml)	Suprayudi *et al.* (2002)
	Z_3	AN (1.5 ind./ml)	
	Z_4–Z_5	AN (2 ind./ml)	
Scylla tanquebarica	Z_1–Z_2	R (20 ind./ml) & AN (4 ind./ml)	Baylon (2009)
	Z_3	R (20 ind./ml) & AN (2 ind./ml)	
	Z_4	AN (2 ind./ml)	
	Z_5	AN (1 ind./ml)	

Abbreviations: Z: zoea; M: megalopa; ind.: individuals; R: rotifers; ER: enriched rotifers; AN: newly hatched *Artemia* nauplii; EA: enriched *Artemia* metanauplii; FC: frozen copepods.

Live feed enriching ingredients: [1] Algamac2000 (Biomarine, USA) or AquaGrow (Advanced BioNutrition, USA); [2] commerial oil (Marine omega, Japan); [3] commercial oil (EasySelco, INVE, Belgium); [4] commercial oil (Easy DHA Selco, INVE, España); [5] *Chlorella* & Dry Immune Selcos (DIS®, INVE Aquaculture); [6] Dry Immune Selcos (DIS®, INVE Aquaculture); [7] beer yeast (Kirin Brewery, Japan); [8] *Nannochloropsis* sp.

On the other hand, larger *Artemia* (400–500 μm) are a more suitable prey for later larval stages (Genodepa *et al.* 2004b; Støttrup & McEvoy 2003). As larvae grow bigger, they prefer larger prey because it would become increasingly difficult to maintain a positive energy budget due to higher foraging energy expenditure on small prey (Tsuji *et al.* 2015). The disproportional size of food particles and larval feeding appendages may even incapacitate the larvae to feed on small prey and may finally die due to depletion of their energy reserves (Guarizo *et al.* 2020). Ideally, the size of prey should allow for it to be easily grasped and consumed by the mouthparts of the larvae; choosing the prey based on the mandibular width of larvae could be a useful approach since the mandibles are the most important structures for processing food physically before it enters the digestive track (Jeffs & O'Rorke 2020; Lumasag *et al.* 2007). In addition, increasing foraging and swimming ability of larvae also facilitates a shift in prey preference to larger ones (Zhang *et al.* 1998b). During late larval stages, larger prey can also result in higher feeding efficiency and thus help to reduce cannibalism (Baylon 2009; Romano & Zeng 2017; Sui *et al.* 2008; Waiho *et al.* 2018). In addition to their size, the lipid content of *Artemia* nauplii is typically higher than rotifers, which is essential for late larvae for both metabolic maintenance and the production of hormones that regulate upcoming critical metamorphosis (Anderson & De Silva 2011; Sui *et al.* 2008).

Although rotifers and *Artemia* are widely used in marine crab hatcheries nowadays, their nutritional profile have long been recognised to be insufficient for crab larvae (Oliver *et al.* 2017; Støttrup & McEvoy 2003). To improve their nutrition values (e.g. highly unsaturated fatty acids or HUFAs content), the enrichment of rotifers and *Artemia* is commonly adopted in aquaculture hatcheries (Ferreira *et al.* 2008; Figueiredo *et al.* 2009; Støttrup & McEvoy 2003). Different methods have been developed for rotifer enrichment, such as using HUFA-rich microalgae (e.g. *Chlorella, Nannochropsis, Pavlova, Tetraselmis*), HUFA-enriched yeasts, fish oil emulsions, or enrichment microcapsules (Ferreira *et al.* 2008; Olivotto *et al.* 2017). For instance, the use of microalgae as enrichment media was reported to have positive effects on larval survival and the development of edible brachyuran crabs (Baylon 2009;

Nghia *et al.* 2007). Similar results have also been reported in ornamental crustaceans, for instance, rotifer enrichment was shown to enhance larval survival of the cleaner shrimp *Lysmata amboinensis* when compared to those fed unenriched rotifers (82 ± 8% vs. 62 ± 3.7%) (Cunha *et al.* 2008). However, a recent study on the mud crab *Portunus armatus* larvae suggested that enriching rotifers and *Artemia* with lipid emulsion increased DHA (docosahexaenoic, 22:6n-3) had limited effect on larval survival, growth or development (Basford *et al.* 2021). Moreover, Hamasaki *et al.* (2002a) observed advanced morphogenesis in the last larval stage zoeal larvae of mud crab *S. serrata* (i.e. the larvae possessing megalopal features) when the larvae were fed *Nannochropsis* enriched rotifers during early zoeal stages. The authors suggested that the occurrence of such abnormal morphology was due to the excess HUFA intake originated from *Nannochropsis,* which accelerated larval development (Hamasaki *et al.* 2002a). The zoeae with advanced morphogenesis had a significantly higher tendency to fail during crucial metamorphosis moulting and die (Hamasaki 2002; Hamasaki *et al.* 2002a).

Same as rotifers, *Artemia* nauplii have long been recognized as lacking essential fatty acids. For example, unenriched *Artemia* only contain a very low level (0.33%) of EPA (eicosapentaenoic, 20:5n-3), while their DHA is at an undetectable level (Suprayudi *et al.* 2004a). Zoeae of *S. serrata* fed unenriched *Artemia* showed a prolonged intermoult period and many of them died at the last zoeal stage without exhibiting any sign of metamorphosis (Suprayudi *et al.* 2004a). Similarly, feeding zoeal larvae of *Portunus trituberculatus* with unenriched *Artemia* was reported to result in the occurrence of morphologically immature megalopae (i.e. retaining some features of zoeal larvae, such as dorsal spin and telson folk) (Dan *et al.* 2016b; Dan *et al.* 2016c).

Besides fatty acids, the protein content of *Artemia* has also been reported to significantly be improved via enrichment with a mixture of multiple species of microalgae (*Nannochloropsis oculata*, *Tetraselmis suecica*, *Dunaliella salina*, *Isochrysis galbana* and *Chaetoceros gracilis*); and such enriched *Artemia* fed to larvae can result in a remarkably increase in both survival and growth of first stage zoeae of the southern surf crab *Ovalipes*

trimaculatus (Martelli *et al.* 2020).

Although rotifer and *Artemia* enrichment is commonly practiced and believed to benefit the survival and growth of decapod larvae (McEvoy *et al.* 1996; McEvoy *et al.* 1995; Navarro *et al.* 1999; Olivotto *et al.* 2017), mass mortality around the time of metamorphosis, which is known as moult-death syndrome (MDS, i.e. larvae fail to shed the exuvia completely and eventually die during moulting), are still often reported during larval culture among various decapod species (Bermudes *et al.* 2008; Dan *et al.* 2013; Hamasaki 2002; Hamasaki *et al.* 2007; Hamasaki *et al.* 2011). Within brachyuran crab larval culture, MDS appears to be a common issue as it has been reported in various species, including *Portunus pelagicus* (Maheswarudu *et al.* 2008), *P. trituberculatus* (Dan *et al.* 2013), *Scylla tranquebarica* (Baylon 2009), and *Ucides cordatus* (Silva *et al.* 2012). However, the precise mechanisms of this moulting failure are not fully understood despite excess HUFA intake has been revealed as one of them (Dan *et al.* 2016c; Hamasaki 2002; Hamasaki *et al.* 2002a). Clearly, further research on how to maintain a balance of improving live prey quality by enrichment while avoiding excess overdose that may lead to detrimental effects, such as resulting in advanced morphological development, is warranted.

As one of the likely natural diets for brachyuran larvae (Fileman *et al.* 2014), copepods usually show a wide range of size at different developmental stages, making them ideal to cover different stages of larval development (Ajiboye *et al.* 2011; Støttrup & McEvoy 2003). More importantly, copepods are believed to possess nutrient profiles that match larval development requirements (Corner & O'Hara 1986; Dhont *et al.* 2013; Støttrup 2003). In particular, copepods are known to generally contain high levels and a balanced ratio of HUFAs and carotenoids, as well as generally higher digestive enzyme activities as compared to rotifers and *Artemia* (Corner & O'Hara 1986; Støttrup 2003). Clearly, despite a general lack of research, there is a good potential for copepods to be used as live prey in marine decapod larval culture, as the few studies that have been conducted so far generally show positive outcomes. For example, Sudharma and Edirsinghe (2016) introduced calanoid copepods to be co-fed with *Artemia* in fire shrimp *Lysmata debelius* larval culture and

reported that they have improved larval survival to the first juvenile stage (up to 18.35 ± 0.15%) with shorter larval duration (50–90 days) when compared to a previous report where copepods were not used (ca. 10% larval survival with larvae settled between 75–158 days; Palmtag & Holt 2001). In edible crustaceans, faster growth, greater length and weight gain, and higher astaxanthin content were observed when tiger prawn (*Penaeus monodon*) larvae were fed a mixture of three copepods (*Macrosetella gracilis*, *Pseudodiaptomus* sp. and *Oithona rigida*) as compared to feeding exclusively on *Artemia* nauplii (reviewed by Waiho *et al.* 2018). However, these studies often lack scientific vigour as they either attempted to make comparations between different studies/trials or the species of copepods used were not provided.

A major hurdle on the potential use of copepods as live prey in marine crab hatchery is the high cost and difficulty for copepod intensive culture (van der Meeren *et al.* 2014). It is still very difficult to culture copepods at high densities (Drillet *et al.* 2011; Støttrup 2003). Although the cultivation of copepods has attracted great interest in recent years and noticeable progress in culture techniques has be achieved (e.g. Alajmi *et al.* 2015; Camus & Zeng 2012; Pinto *et al.* 2001; Ribeiro & Souza-Santos 2011; Souza-Santos *et al.* 2006), most copepod species, especially calanoids, are still produced at substantially low quantities as compared to rotifers and *Artemia*, thus often being unable to satisfy the numbers required by commercial hatcheries (Drillet *et al.* 2011; van der Meeren *et al.* 2014). Therefore, future studies on selecting suitable high productive copepods as live prey and further improving their mass culture technology is important to their potential use in marine crab hatcheries.

Unlike fish, decapod larvae are generally not dependent on visual cues for hunting and catching prey; rather, the chance of random encountering with prey is believed to play a key role (Calado *et al.* 2008; Ruscoe *et al.* 2004). This highlights the importance of providing larvae with an optimal prey density, which is particularly relevant to early larvae who have weak swimming ability. Based on the results of larval prey ingestion rate studies in several brachyuran zoeal larvae, including *Hyas araneus* (Anger & Dietrich 1984), *Ranina ranina* (Minagawa & Murano 1993a), and *S. serrata* (Suprayudi *et al.* 2002), larval ingestion rates

generally showed a linear relationship with *Artemia* nauplii density provided until a saturation level is reached. Similar results were also reported among other decapod species, including the mysis larvae of prawn *Metapenaeus ensis* (Chu & Shing 1986), the zoeal larvae of freshwater prawn *Macrobrachium rosenbergii* (Barros & Valenti 2003) and the cleaner shrimp *Lysmata wurdemanni* (Zhang *et al.* 1998a). These studies showed that, in general, prior to reaching the saturation level, a higher density of live prey not only had a positive effect on larval survival and growth, but also larval development with reduced intermoult duration (Anger 2001; Minagawa & Murano 1993a; Suprayudi *et al.* 2002). However, excess food can have adverse effects on larvae, probably due to water quality degradation and/or bacterial proliferation (Sui *et al.* 2009; Zhang *et al.* 1998a). Dan *et al.* (2016a) further reported that an excessive high *Artemia* density induced morphological abnormality in the last zoeal stage larvae of *P. trituberculatus*, which led to MDS. Therefore, it is important to identify the optimal density of live prey that satisfies larvae rearing requirements while avoid overfeeding. It is also important to bear in mind that such optimal density is likely to change with the rapid ontogenetic development of larvae.

1.2.1.2 Larval nutrition

Providing adequate nutrition via ingested diets is essential to successful rearing of crustacean larvae. However, the technical difficulties (e.g. the small size of larvae, leaching of nutrients from tested diet, etc) in studying nutritional requirements of aquatic crustacean larvae have led to relatively limited understanding on the ontogenetic nutritional requirements of decapod larvae (Anger 2006). It is well known that lipid, protein, and carbohydrate are three macro-nutrients for various organisms (Guillaume 1997). In general, the marine environment is rich in lipids and proteins but scarce in carbohydrates; and marine animals have adapted to this nutritional background through evolution (Anderson & De Silva 2011). Thus, lipids and protein are arguably the two most important nutrients for decapod crustacean larvae (Anger 2001; Anger 2006).

1.2.1.2.1 Lipids

Lipids is an important energy source for marine organisms, and crustaceans are no exception (Anger 2001; Arts *et al.* 2001). As the main energy source during embryonic and larval development, lipids provide a densest form of energy sources, yielding up to 2/3 more energy than carbohydrates or proteins per unit mass (Parrish 2013). Previous studies have shown that dietary lipid level could significantly impact survival, growth, as well as biochemical and fatty acid (FA) compositions of decapod larvae and juveniles (Beder *et al.* 2018; Wen *et al.* 2006; Xu *et al.* 1994). Therefore, dietary lipid is an important factor to consider for decapod larval culture.

In crustaceans, lipids are present in three major classes, namely triacylglycerols (TAGs), phospholipids (PLs), and sterols (STs). TAGs make up the major form of energy storage and is the major group of lipids found in the adipose tissues (Budge *et al.* 2006; Reppond *et al.* 2008). PLs is the main structural component of cell membrane, and they also play an important role in antioxidant function and buoyancy control (Budge *et al.* 2006; Coutteau *et al.* 1997). In particular, the dietary contribution of PLs, especially phosphatidylcholine, has been considered essential for maintaining normal larval development and growth (Coutteau *et al.* 1997), even though crustacean larvae appear to be able to synthesize PLs from HUFAs (Fegan 2004). The third class of lipid, STs play a key role in larval growth and metabolic maintenance; they are also the precursors of important hormones, such as moulting hormones (Andrés *et al.* 2010a).

Essential fatty acids (EFAs) are fatty acids that cannot be synthesized by crustaceans, and hence must be acquired via their diets to maintain survive and normal development (Parrish 2013; Suprayudi *et al.* 2004b). Dietary EFAs have been reported to influence survival, growth, immunity, stress resistance, ion balance and buoyancy of decapod larvae (Anger 2001). Two essential highly unsaturated fatty acids (HUFAs), DHA and EPA, have attracted special research interest since they have been proven to significantly influence performance

of crab larvae. Interestingly, both deficient or excessive dietary level of DHA have been reported to result in low survival rate and prolonged intermoult period in *S. serrata* larvae (Suprayudi *et al.* 2004a; Suprayudi *et al.* 2004b), while the lack of dietary EPA led to significantly longer developmental period in zoeal larvae of *Erimacrus isenbeckii* (Jinbo *et al.* 2013). In addition, the ratio of DHA and EPA is another important factor for consideration. For example, inappropriate DHA/EPA ratios reportedly decreased survival and growth of *S. serrata* larvae (Davis *et al.* 2005; Suprayudi *et al.* 2002). Similarly, in the Chinese mitten crab *Eriocheir sinensis*, feeding larvae on a diet with a higher DHA/EPA ratio of 4.0 enhanced larval survival, body weight and salinity tolerance as compared with those fed a diet with DHA/EPA ratio of 0.6 (Sui *et al.* 2007). Moreover, the elevated dietary DHA and EPA levels have been revealed to increase the occurrence of advanced morphologies (i.e. the enlarged chelae) in the last zoeal stage of *S. serrata*, leading to higher rate of moult failures during metamorphosis to the megalopal stage (Dan & Hamasaki 2011; Hamasaki *et al.* 2002b; Suprayudi *et al.* 2004a; Suprayudi *et al.* 2004b). The similar phenomenon was also found in *P. trituberculatus* (Dan *et al.* 2016c). These results suggest that the requirement of HUFAs by brachyuran larvae are complicated and that multiple factors need to be considered.

1.2.1.2.2 Protein

Protein is an essential nutrient for all animals, during the early life-history stages of crustaceans when rapid tissue synthesis occurs, dietary requirement of proteins could be higher (Anger 2001). Protein typically is the highest and also the most expensive component of aquatic feed (Anderson & De Silva 2011), therefore, a good understanding of protein requirement by crab larvae is important to establish a well-balanced and cost-efficient larval feeding regime, as well as knowledge base for the development of formulated diets (Holme *et al.* 2009).

Table 1.3. Dietary protein levels used in past nutritional studies on decapod larvae and juveniles

Species	**Stage**	**Crude protein (%)**	**References**
Penaeus monodon	zoea	37.8	Sheen and Huang (1998)
	mysis	45.9	
Penaeus japonicus	larvae	55.8	Moe *et al.* (2004)
P. japonicus	larvae	50	Kanazawa *et al.* (1985)
Macrobrachium rosenbergii	larvae	56.9–57.6	Kamarudin and Roustaian (2002)
Litopenaeus setiferus	larvae	52.7	Gallardo *et al.* (2002)
Homarus americanus	larvae	50–57	Fiore and Tlusty (2005)
Eriocheir sinensis	juvenile	39.0–42.5	Mu *et al.* (1998)
Portunus pelagicus	megalopa	49.85–57.50	Castine *et al.* (2008)
P. pelagicus	juvenile	50	Noordin *et al.* (2018)
Portunus trituberculatus	juvenile	49.5	Jin *et al.* (2015)
P. trituberculatus	juvenile	51.5	Jin *et al.* (2013)
P. trituberculatus	juvenile	51.27	Huo *et al.* (2014)
Scylla paramamosain	juvenile	47.06–52.09	Zheng *et al.* (2020)
Scylla serrata	megalopa	79.4	Genodepa *et al.* (2004a)
S. serrata	megalopa	55	Holme *et al.* (2006)
S. serrata	juvenile	34.2–51.8	Catacutan (2002)
S. serrata	juvenile	46.9–47.03	Unnikrishnan and Paulraj (2010)

However, information available on protein requirement by brachyuran crab larvae is relatively limited (Table 1.3). As the results, Table 1.3 includes dietary protein requirements of larvae and juvenile from some decapod species other than crabs. In general, the crude protein in most diets used for the larval and juvenile decapod rearing ranges between 30–60% (Table 1.3), and an excessive protein level may result in reduction of growth rate (Catacutan 2002; Jin *et al.* 2013). As can be seen from Table 1.3, the suitable protein level in larval diets varies among species and developmental stages. Such variations could be related to differences in feeding habitats through evolution and adaption, for example, carnivorous species are often expected to have higher protein requirements than those of herbivorous species (Guillaume 1997). Unfortunately, in crab species, most studies on pertain requirement were conducted during juvenile stage, with studies during larval phase having been conducted only for the megalopal stage of *S. serrata* (Table 1.3; Castine *et al.* 2008; Genodepa *et al.* 2004b; Holme *et al.* 2006). In the two studies on *S. serrata* megalopae, micro-bound diets (MBD) containing a crude protein level of 55% and 79.4%, respectively, obtained high survival rates (up to 90%) (Genodepa *et al.* 2004b; Holme *et al.* 2006). For *Portunus pelagicus*, protein source based on fish meal showed significantly better effect on megalopal growth and development than protein source based on squid or soybean meal (Castine *et al.* 2008). However, the limited information on both the optimal level and source of dietary protein for the various larval stages of crabs suggests that significant research efforts are still needed to fill the knowledge gaps in these areas.

Amino acid composition and identification of essential amino acids (i.e. amino acids that cannot be synthesised by animals and hence have to be obtained from the diet) is another aspect of the protein nutrition (Guillaume 1997). The source and amino acid composition can strongly influence larval performance (Anderson & De Silva 2011; Guillaume 1997). Studies on larval amino acids requirements have been conducted with juveniles or adults of several decapod species, including the crab *Metacarcinus magister* (previously known as *Cancer magister*, Lasser & Allen 1976), and the shrimps *Palaemon serratus* (Cowey & Forster 1971) and *Penaeus japonicus* (Kanazawa *et al.* 1981), but again not for larvae. Based on previous

research on juveniles or adults, ten amino acids were considered as essential: arginine, histidine, isoleucine, leucine, lysine, methionine, threonine, tryptophan, phenylalanine, and valine. The presence of other amino acids in the diet is considered less crucial as non-essential amino acids (Anger 2001; Guillaume 1997). However, the requirements of amino acids may vary at different life stages (Andrés *et al.* 2010a). Similarly, research on optimal dietary inclusion levels of amino acids for crabs are also based on juveniles, two recent studies showed that the optimal dietary level of lysine and arginine being 2.41% and 2.77%, respectively, for the culture of *P. trituberculatus* juveniles (Jin *et al.* 2015; Jin *et al.* 2016). The lack of research on quantitative requirements of amino acids by crab larvae is probably related to the fact that the traditional methods for determining amino acid requirements are unsuitable for marine larvae due to their small size (Bengtson 1993).

1.2.1.2.3 Carbohydrates

Compared with lipids and protein, dietary carbohydrate requirement is often considered less important due to the low utilization of carbohydrate by marine animals (Anderson & De Silva 2011). Consequently, few studies have been conducted on carbohydrates requirement of crustaceans, and information is largely lacking for crab larvae. Previous studies on adults and juveniles suggested that for most crustaceans, there is no specific requirement on dietary carbohydrates (Fegan 2004; Jeffs & O'Rorke 2020). In practice, carbohydrates are often used as binders to formulate feeds or added to reduce feed costs through lipid or protein sparing effect (Holme *et al.* 2009; Jeffs & O'Rorke 2020).

1.2.2 Cultural environment

The culture environment is another important aspect that should be considered for crab larval culture. However, the information in this area for marine ornamental crabs is scarce,

which means that the following information will be largely drew broad from literature based on larval culture of edible crabs.

1.2.2.1 ‘Greenwater’ vs. ‘clearwater’ technique

‘Greenwater’ technique (i.e. adding microalgae into larval rearing water) is often practiced in larval culture of marine animals (Brown & Blackburn 2013). The speculated benefits of ‘greenwater’ technique include water quality improvement via enhanced oxygen production and pH stabilisation; light attenuation (considered especially beneficial to phototactic larvae); prebiotic effects; and the maintenance of larval live prey nutrition (Brown *et al.* 1997). Table 1.4 lists the rearing conditions adopted by previous studies on various crabs, it appears that the application of the ‘greenwater’ method in the larval culture of crabs is not as common as for finfish (Moorhead & Zeng 2010). Unlike fish, the development and growth of crustacean larvae are discontinuous processes, accompanying a series of moults (Anger 2001). Previous studies have shown that microbes, such as filamentous bacteria, can foul the exoskeleton of crustacean larvae and affect their swimming, foraging ability as well as respiration; in severe cases, they can cause unsuccessful moulting and ultimately lead to death of the larvae (Swingle *et al.* 2013). Adding microalgae into culture media potentially can lead to such consequences, which may explain why ‘greenwater’ is not so commonly used in marine crab larval culture.

One of the potential benefits of using ‘greenwater’ is to maintain the nutritional value of live prey (Brown *et al.* 1997). As the primary producer in the aquatic food chain, microalgae are the source of nutrients, including HUFAs, which are believed to be essential for marine crab larvae (please refer to Section 1.2.1.2.1). Although crab larvae are often carnivorous and do not actively feed on microalgae, the fatty acids within microalgae can be transferred to them via zooplankton (Anderson & De Silva 2011; Arts *et al.* 2001). Additionally, microalgae in the cultural environment could be beneficial to larval culture of some species. For example, (Penha-Lopes *et al.* 2006) reported that when microalgae *Amphora* sp. was present

during the larval development of *M. forceps*, larval intermoult duration decreased and growth at each moult improved. Nghia *et al.* (2007) also concluded that the use of microalgae in the culture of *S. paramamosain* can improve the performance of larvae by enhancing live prey quality and helping to control pathogen level.

Table 1.4. Summary of larval culture conditions reported for brachyuran species

Species	Larval stage	Culture method	Salinity	Temperature (°C)	Photoperiod (hours)	Reference
Callinectes sapidus	Z, M	GWT	30	22	12L: 12D	Zmora *et al.* (2005)
Cancer irroratus	Z	USW	29–32	15 ± 1	NP	Charmantier-Daures and Charmantier (1991)
	M	CWT	29–32	19 ± 1	NP	
Charybdis feriatus	Z, M	CWT	25-35	26–32	NA	Baylon and Suzuki (2007)
Chionoecetes opilio	Z, M	CWT	30-33	12–14	NA	Kogane *et al.* (2007)
C. opilio	Z	CWT	33	5–16	NA	Yamamoto *et al.* (2014)
	M	CWT	33	5–14	NA	
C. opilio	Z	CWT	20–38	11	NA	Yamamoto *et al.* (2015a)
	M	CWT	28–36	8	NA	
Maja brachydactyla	Z, M	CWT	35	21	12L: 12D	Castejón *et al.* (2015b)
Maja squinado	Z	GWT	34 ± 1	18 ± 1	NP	Guerao and Rotllant (2010)
M. squinado	Z, M	GWT	37	19.6 ± 0.6	24L: 0D	Durán *et al.* (2012)
Menippe nodifrons	Z, M	CWT	30	25 ± 1	12L: 12D	Guarizo *et al.* (2020)
Mithraculus forceps	Z, M	CWT	35	28 ± 0.5	14L: 10D	Penha-Lopes *et al.* (2005)
M. forceps	Z, M	CWT	35	28 ± 0.5	14L: 10D	Rhyne *et al.* (2005)
Mithraculus sculptus	Z	CWT	35	28 ± 0.5	14L: 10D	Rhyne *et al.* (2005)
Portunus pelagicus	Z	CWT	22	28 ± 1	14L: 10D	Wu *et al.* (2014)
	M	CWT	33–35	28 ± 1	14L: 10D	

P. pelagicus	Z	CWT	27 ± 1	29 ± 1	18L: 6D	Andrés *et al.* (2010c)
Ranina ranina	Z	CWT	32–34	28.8–29.2	12L: 12D	Minagawa (1994)
Scylla serrata	Z	CWT	25–35	26–32	NA	Baylon (2010)
	M	CWT	25–35	32	NA	
S. serrata	Z, M	CWT	20–30	28–30	16L :8D	Nurdiani and Zeng (2007)
S. serrata	Z	GWT	30	20.8–27.8	NA	Ruscoe *et al.* (2004)
	M	GWT	25	20.8–27.8	NA	

Abbreviations: Z: zoea; M: megalopa; GWT: green water; CWT: clear water; USW: unfiltered seawater; NP: natural photoperiod; NA: not addressed.

However, there are opposite results reported on using microalgae for marine crab larval rearing. Hamasaki *et al.* (2002b) revealed that high concentration of the microalga *Nannochloropsis* sp. could result in higher mortality of *S. serrata* larvae due to an accelerated development caused by excessive HUFA intake via prey on rotifers who filter feed on *Nannochloropsis* sp. In *P. trituberculatus*, when algal supplementation was applied, it was reported that megalopae tended to present an immature morphology (i.e., retains zoeal morphological features, such as the dorsal spine or telson furcae) and resulted in mass mortality (Dan *et al.* 2013; Dan *et al.* 2016c). Such a phenomenon is believed to be associated with the presence of microalgae *Chlorella* sp., especially during the last zoeal stage, during larval rearing (Dan *et al.* 2016c). It was suggested that the possible mechanism of high rate of immature forms of megalopae could be a result of disrupted endocrine pathways of the larvae due to the teratogenic factors produced by microalgae (Dan *et al.* 2016c). Indeed, the conflicting results suggest that the effect of microalgae supplementation probably is both species-specific and larval stage specific for crabs. Clearly, further research is needed to clarify the role of microalgae in crab larval rearing, as well as to screen and select the appropriate species and concentration to be used in larval culture water.

1.2.2.2 Temperature and salinity

Temperature and salinity are among the most important abiotic factors that directly affect marine crab larvae (Zeng *et al.* 2020). Temperature, in particular, exerts strong effects on the frequency of moulting in crustacean larvae, which is directly related to their development and growth (Anger 2001). As other ectotherms, life-history characteristics of crabs (size at maturation, reproductive lifespan, growth rate, ageing, etc.) are remarkably sensitive to temperature variation (Atkinson 1994). Within a thermal tolerance range, higher temperatures result in faster growth but ultimately lead to a smaller body size (Atkinson 1994; Zeng *et al.* 2020). This trend, also known as ‘temperature-size rule’, has been observed in various taxa, including bacteria, protozoans, plants, and animals (Angilletta & Dunham 2003).

Elevated temperature has been shown to influence both the intermoult period (IMP) and moult increment (or growth per moult, GPM) in brachyuran crabs. Within the tolerance range, higher temperature has consistently been reported to lead to shorter IMP during larval development of various crab species, including the ruby crab *M. forceps* (Penha-Lopes *et al.* 2005), the common spider crab *Maja brachydactyla* (Castejón *et al.* 2015b), the mud crab *S. serrata* (Baylon 2010; Nurdiani & Zeng 2007), the swimmer crab *Charybdis feriatus* (Baylon & Suzuki 2007), the knobbed crab *Mithrax caribbaeus* (Lárez *et al.* 2000), the Chinese mitten crab *E. sinensis* (Anger 1991), and the snow crab *Chionoecetes opilio* (Yamamoto *et al.* 2014). The effects of temperature on GPM of larvae, however, is not as consistent. For example, in the swimming crab *P. trituberculatus,* larvae had a lower carapace length increment when they were reared under a higher temperature (Dan *et al.* 2013), whereas for *M. forceps,* larvae showed no significant difference in the GPM of carapace size when reared under different temperatures (Penha-Lopes *et al.* 2005). Yet for the shore crab *C. maenas* larvae, the relationship between GPM of larval biomass and ambient temperature was reportedly largely dependent on food availability (Torres & Giménez 2020). The different effects of temperature on larval growth of different crab species may reflect the complex influences of temperature on larval activity. For example, the locomotor activity of larvae of *Charybdis feriatus* decreased with decreasing temperature, which eventually impacted their growth due to a decline in feeding (Baylon & Suzuki 2007). On the other hand, the metabolic level of larval *Carcinus maenas* was reported higher at elevated temperature, which resulted in less energy reserves and growth (Dawirs & Dietrich 1986). The inconsistency of larval growth responses to temperature suggests further investigation on the effects of temperature on larval growth, especially over different larval stages and temperature ranges, is needed.

Another important environmental factor, salinity, is also known to exert a strong effect on the survival of decapod larvae (Anger 2001). In general, salinity tolerance of crab larvae is often associated with their natural distribution, i.e., estuarine, coastal or offshore. For example, the larvae of the tropical crab *Armases miersii*, which reproduce in supratidal rock pools where salinity fluctuate greatly, are able to survive under a wide range of salinity (15–

45) with no significant effect on larval development (Anger *et al.* 2000). On the contrary, the larvae of fully marine species normally have a narrow salinity tolerance range. For instance, larvae of the marine spanner crab *R. ranina* can only survive within a much narrower salinity range of 27–34 (Minagawa 1992). Compared with temperature, salinity typically exerts a weaker influence on the duration of larval development but a stronger effect on larval survival (Anger 2001). For example, for larvae of extremely euryhaline crab species, their developmental delay is usually only detectable under extremely stressful salinity conditions (Anger 1996; Anger *et al.* 2000). While under unfavourable salinity conditions, the intermoult period of crab larvae is usually prolonged (Anger 2003; Anger *et al.* 1998), reduced salinity can sometime accelerate the larval moulting cycle of particular larval stages in some species. For example, reduced salinity displayed a stimulating effect on metamorphosis moult and settlement in the megalopae of the blue crab *Callinectes supidus* (Forward *et al.* 1994). Such effect is often associated with the recruitment of megalopae from offshore to coastal or estuarine environments, which act as nursery grounds for juveniles. Moreover, salinity also interacts with other factors, such as temperature and food availability, to exert its effects on larval survival and development (Baylon & Suzuki 2007; Baylon 2010; Castejón *et al.* 2015b; Dan & Hamasaki 2011; Huchin-Mian *et al.* 2018; Lárez *et al.* 2000; Nurdiani & Zeng 2007). Among these factors, temperature interactions with salinity are better studied, and it has been shown that under unfavourable temperature conditions, larval tolerance to salinity stress typically decrease, as was reported for *E. sinensis* (Anger 1991), *M. brachydactyla* (Castejón *et al.* 2015b), and *S. serrata* (Nurdiani & Zeng 2007).

Unfavourable salinity conditions have also been reported to cause intraspecific morphological variation (Giménez 2006). For example, the larval development of *E. sinensis* and *R. ranina* reared under unfavourable low salinity tended to undergo extra zoeal and/or megalopal stages, and as the result, less morphological changes between successive larval stages (Anger 1991; Minagawa 1992). Meanwhile, stressful salinity conditions may cause malformations of larval morphology. For instance, in *S. serrata*, the relative length of chela (i.e. the ratios of chela length to carapace length) of the last zoeal stage (Z_5) larvae reportedly increased with increasing salinity, which was attributed to larvae being unable to accumulate

sufficient nutrients when subjected to low salinity stress, therefore leading to a delayed morphogenesis (Dan & Hamasaki 2011).

Variation in growth rates under different salinities may indicate that the uptake and conversion of food is affected by salinity (Anger 2001). In some cases, the depression of growth under unfavourable salinity conditions is a consequence of reduced feeding. For example, in the zoeal larvae of *R. ranina*, food ingestion was depressed in both hypo and hypersaline conditions (Minagawa 1992). Meanwhile, the growth of larvae might also be affected by unfavourable salinity conditions via increased metabolism and energy expenditure for osmoregulation (Dan & Hamasaki 2011; Rey *et al.* 2015), and such effect could be chronic. For example, *C. maenas* juveniles moulted from megalopae exposed to a salinity of 10 displayed a significantly inferior carapace width (CW: 4.87 ± 0.28 mm) and wet weight (WW: 28.95 ± 4.62 mg) at the fifth juvenile stage as compared with those from megalopae developed under higher and normal salinity of 25 (CW: 5.90 ± 0.33 mm and WW: 50.89 ± 8.14 mg) (Rey *et al.* 2015). Extreme salinity stress can cause negative growth in larvae, which is presumably due to disturbed synthetic processes and increased energy costs for osmoregulation (Torres *et al.* 2002). Such effect was observed, for instance, in the stenohaline crab *Cancer pagurus* first stage zoeae exposed to reduced salinities (Torres *et al.* 2002): In treatments where salinity was 15 or 25, larvae lost significant amounts of lipid, protein, as well as biomass, especially when larvae were exposed to low salinity conditions immediately after hatching (Figure 1.1 a–f; derived from Torres *et al.* 2002). On the contrary, the larvae of the highly euryhaline crab *Chasmagnathus granulate* subjected to similar conditions showed significantly lower loss in biomass and biochemical composition than *C. pagurus* (Figure 1.1 g–l; derived from Torres *et al.* 2002). These results suggest that the extent of salinity effects on larval growth under hypoosmotic stress largely reflects the status of eury- or steno-halinity of the crab species concerned.

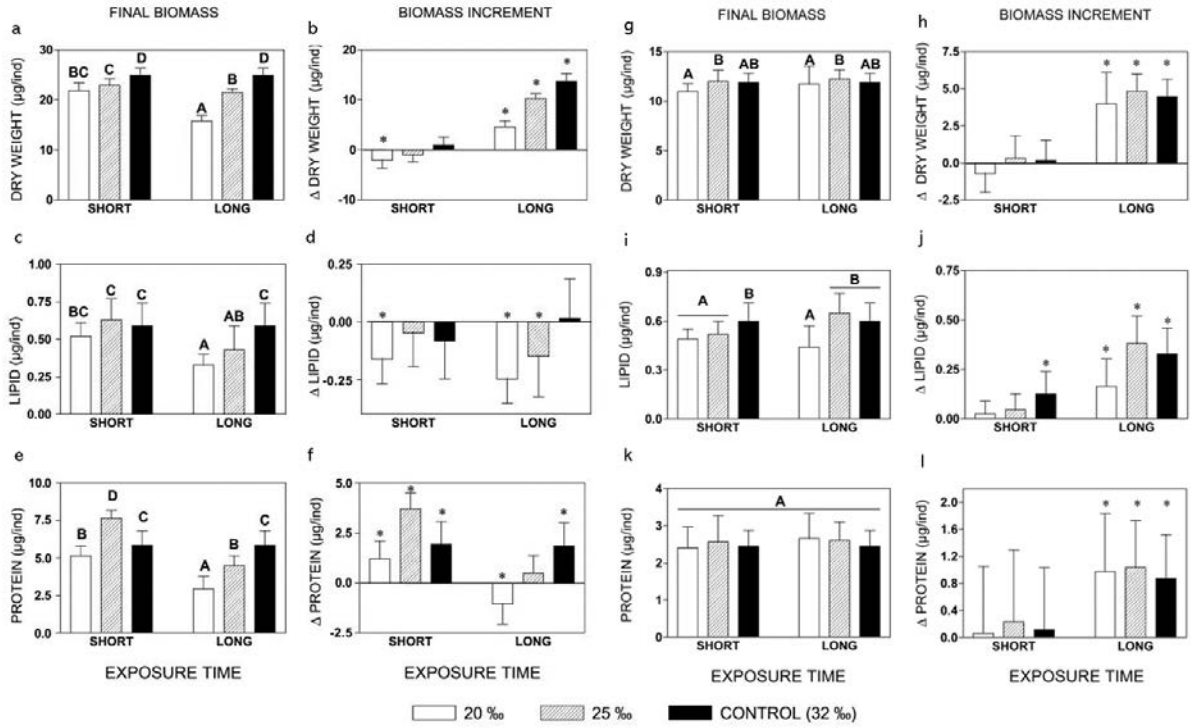

Figure 1.1. Larval biomass (dry weight) and proximate compositions (lipid, protein content per individual) of the first zoeae of *Cancer pagurus* and *Chasmagnathus granulate* exposed to different salinities. a–f: *C. pagurus*; g–l: *C. granulate*. Short exposure: 40–50% of the moulting cycle; long exposure: 50–100% of the moulting cycle. Different letters or asterisks show significant differences. Modified from Torres *et al*. (2002).

1.2.2.3 Light

Light is another important environmental factor that can affect crab larval rearing success since it shapes biological rhythms, feeding, and behaviour of larvae (Anger 2001). Compared to temperature and salinity, far less attention has been paid to the effects of light on decapod larval culture. Photoperiod and light intensity have been reported to affect larval survival and development of several brachyuran species, including *R. ranina* (Minagawa 1994), *P. pelagicus* (Andrés *et al.* 2010c), and *Pseudocarcinus gigas* (Gardner & Maguire 1998), as well as other decapods, such as the cleaner shrimps *Lysmata* spp. (Calado *et al.* 2008), the coconut crab *Birgus latro* (Hamasaki *et al.* 2016), and the spiny lobsters *Sagmariasus verreauxi* (Fitzgibbon & Battaglene 2012) and *Jasus edwardsii* (Bermudes *et al.* 2008).

In general, decapod larvae have been reported to be able to survive under constant darkness, which is probably related to the fact that they are non-obligate visual feeders (Andrés *et al.* 2010c; Calado *et al.* 2008; Gardner & Maguire 1998; Minagawa 1994), that is, they do not rely on visual cues but either chemosensory detection or random encounter to capture prey (Jeffs & O'Rorke 2020). However, the presence of light does appear to stimulate larval feeding behaviour (Jeffs & O'Rorke 2020; Minagawa 1994). For example, zoeal larvae of *R. ranina* ingested 2.6–2.8 times higher numbers of *Artemia* under the presence of light than those reared in darkness (Minagawa & Murano 1993b). Gardner and Maguire (1998) also reported that the feeding behaviour of *P. gigas* was enhanced by the presence of light. A possible explanation for the increase in ingestion rate during the light phase is that light stimulated larval swimming behaviours, hence increasing the chance of encounter between larvae and prey (Andrés *et al.* 2010c; Gardner & Maguire 1998; Minagawa 1994; Minagawa & Murano 1993b). As consequence, larvae reared under constant darkness usually show lower survival, longer development time, and lower dry weight than those cultured under photoperiods with the presence of a light phase (Andrés *et al.* 2010c; Castejón *et al.* 2018; Hamasaki *et al.* 2016). In some cases, constant darkness might even have a carry-over effect. For example, in *M. brachydactyla*, completely darkness experienced during zoeal stages led

to prolonged megalopal development under a light: dark cycle (L: D = 12: 12) (Castejón *et al.* 2018).

On the other hand, the other extreme photoperiod condition, constant light, is also reported to reduce the survival and growth in some crab larvae (Castejón *et al.* 2018; Fitzgibbon & Battaglene 2012; Hamasaki *et al.* 2016). Moreover, under constant light, zoeal larvae of *R. ranina* showed retarded development (less segmentation on some appendages) from the fifth stage onwards (Minagawa 1994). Such results may be explained by increased swimming activities and metabolism during the prolonged light phase, which were not sufficiently compensated by increased feeding rates (Andrés *et al.* 2010c; Hamasaki *et al.* 2016). These examples suggested that extreme photoperiods of either constant darkness or constant light typically do not benefit brachyuran larval culture, hence an alternate light-dark regime is recommended. Although the optimal photoperiod varies between species and larval stages, increased light phase has been reported to produce multiple beneficial effects in various crab species, including higher larval survival (Ichikawa *et al.* 2018), faster development (Andrés *et al.* 2010c; Gardner & Maguire 1998; Ichikawa *et al.* 2018), larger body size (Fitzgibbon & Battaglene 2012; Ichikawa *et al.* 2018), and a more synchronous metamorphosis (Andrés *et al.* 2010c; Matsuda *et al.* 2012).

Light intensity is another parameter that may influence larval survival and development of decapods. For example, the metabolic feeding efficiency (i.e. ratio of feed intake: oxygen consumption) of early phyllosomae of the spiny rock lobster *J. edwardsii* was higher under dim light (7.7×10^{16} quanta m^{-2} s^{-1}) than those assessed under higher irradiance (3.9×10^{18} quanta m^{-2} s^{-1}), suggesting low light intensity is preferred by *J. edwardsii* early larvae (Bermudes *et al.* 2008). On the contrary, higher light intensities (3000 lx and 500 lx, respectively, i.e. 2.5×10^{19} quanta m^{-2} s^{-1} and 6.0×10^{18} quanta m^{-2} $s^{-1,}$ according to the light sources used in experiments) were shown to benefit larval development and weight gain of crabs *M. brachydactyla* and *P. gigas* (Castejón *et al.* 2018; Gardner & Maguire 1998).

In addition to photoperiod and intensity, another factor related to light, the colour of

cultural vessels, might also influence crab larval culture. For instance, Rabbani and Zeng (2005) found darker culture vessels produced higher larval survival than light-colour ones for *S. serrata*. Although decapod larvae do not rely on vision to feed, the possibility of them utilizing visual cues to capture their preys cannot be precluded (Jeffs & O'Rorke 2020). In the case of *S. serrata* larvae, it was speculated that dark backgrounds may have improved prey visibility; and hence larval feeding efficiency (Rabbani & Zeng 2005). Another possible explanation was that the reflection of light in light-colour tanks may create an unnatural light distribution, which caused disorientation and chronic stress to the larvae (Rabbani & Zeng 2005; Shi *et al.* 2019). In summary, the responses to light condition by decapod larvae appear to be diverse, which may reflect their diverse feeding mechanisms (Rabbani & Zeng 2005). This is clearly an interesting field that warrants further research.

1.2.2.4 Cues stimulating metamorphosis and settlement

The life cycle of crabs typically includes a planktonic larval phase and a benthic juvenile and adult phase (Anger 2001). One of the most critical moments during their life cycle is the transition from planktonic to the benthic habitat. Two processes, metamorphosis and settlement, are normally associated with this transition (Gebauer *et al.* 2020). In various crab species, larval metamorphosis and settlement are triggered by specific cues, including both chemical and physical ones (Forward *et al.* 2001). The absence of settlement cues may prolong the time to metamorphosis (TTM) for several days, in larvae of some crab species and in extreme cases may eventually lead to mortality (Gebauer *et al.* 2003). Such a flexibility of the timing of metamorphosis has been considered as a selective advantage since it could improve the probability of larvae to settle in a favourable location (Anger 2001; Forward *et al.* 2001; Gebauer *et al.* 2020). However, in aquaculture settings, such a delay in metamorphosis increases costs and the risk of larval rearing, and it may further affect subsequent early juvenile survival and growth due to extra energy expenditure during prolonged larval duration (Diele & Simith 2007; Rhyne & Lin 2004; Simith *et al.* 2013b).

Therefore, despite past research on this field being largely from an ecological and biological perspective (Andrews *et al.* 2001; Fitzgerald *et al.* 1998; Forward *et al.* 2001; Simith *et al.* 2013b; Simith *et al.* 2010; Zeng *et al.* 1997), it is equally important to identify such cues under aquaculture settings.

1.2.2.4.1 Chemical cues

For most crab species, larvae and juveniles/adults live in different habitats; therefore, chemical cues derived from juvenile/adult habitats or produced by juvenile/adult themselves might serve as cues to trigger metamorphosis (Anger 2001; Forward *et al.* 2001). Previous studies have shown that water-soluble exudates from conspecific adults or juveniles could accelerate the metamorphosis in various crab species, including *Uca pugnas* (O'Connor & Gregg 1998; O'Connor & Van 2006; Welch *et al.* 2016), *Uca cordatus* (Diele & Simith 2007; Simith *et al.* 2017), *Uca pugilator* (Welch *et al.* 2016), *Uca minax* (Welch *et al.* 2016), *Uca vocator* (Simith *et al.* 2010), *Hamigrapsus takanoi* (Geburzi *et al.* 2018), *Hamigrapsus sanguineus* (Geburzi *et al.* 2018), *Armass roberti* (Anger *et al.* 2006), *Panopeus herbstii* (Andrews *et al.* 2001; Rodríguez & Epifanio 2000), *Chasmagnathus granulata* (Gebauer *et al.* 1998), *Rhithropanopeus harrisii* (Fitzgerald *et al.* 1998) (Table 1.5). However, different results have also been reported in some other species, such as *Callinectes sapidus*, *C. maenas*, *Menippe mercenaria* and *M. brachydactyla* megalopae, as the presence of conspecific adult odours was found not to affect their metamorphosis (Castejón *et al.* 2019; Forward *et al.* 1994; Krimsky & Epifanio 2008; Zeng *et al.* 1997). The fact of early juveniles and adults of these crab species not sharing the same habitat in the wild was suggested as one of the possible explanations for the latter cases (Forward *et al.* 1994).

In addition to the odours secreted by conspecifics, megalopae may also respond to odours from congeners or closely related species. Geburzi *et al.* (2018) demonstrated that the megalopae of *H. takanoi* responded to the presence of larger individuals of both conspecific and congeneric crab *H. sanguineus*, while the existence of *C. maenas*, which is

phylogenetically more distant, had no such an effect. A similar example is that for *P. herbstii* megalopae, their TTM reduced when the adult odour of either conspecifics or closely related species *Dyspanopeus sayi* (both from family Panopeidae) were present, but not affected by the adult odour from distantly related the fiddler crab *Uca pugnax* (from family Ocypodidae) (Rodríguez & Epifanio 2000). These results indicate that the acceleration of metamorphosis seems to become weaker with increased phylogenetic distance. However, cross-species responses of exudates from closely related species are not universal and may depend on whether these species are sympatric. For example, the megalopae of *Uca pugnax* maintained in congeneric adult water of *Uca minax* did not reduce their TTM (O'Connor & Gregg 1998). In fact, the cross-effect of exudates from co-exist species could be disadvantageous since it potentially increases interspecific competition.

Aside from the odours produced directly by conspecific or congeneric individuals, other water-soluble chemicals have also been reported to have various effects on metamorphosis and settlement of megalopae. For instance, biofilm related to conspecific adult habitat has been found to stimulate metamorphosis and settlement of megalopae in several crab species, including *Uca cordatus*, *H. sanguineus*, *P. herbstii*, and *R. harrisii* (Anderson & Epifanio 2009; Fitzgerald *et al.* 1998; Rodríguez & Epifanio 2000; Simith *et al.* 2017; Steinberg *et al.* 2008). Similarly, there was a slight reduction in the TTM when *P. herbstii* megalopae were exposed to odour from their potential prey, the oyster *Crassostrea virginica* (Rodríguez & Epifanio 2000). On the contrary, in the presence of adverse chemical cues, such as high concentration of ammonium and the odour of predators, megalopae might postpone their settlement (Forward *et al.* 2003; Geburzi *et al.* 2018; Welch *et al.* 1997). It shows that competent megalopae (i.e. being able to respond to settlement cues) are likely able to distinguish microhabitats to avoid adverse locations via sensing of chemical cues. In general, cues from juvenile or adult habitat may induce metamorphosis, while cues that delay metamorphosis are usually linked to adverse environments.

Table 1.5. Chemical cues that have been reported to influence time to metamorphosis (TTM) of brachyuran megalopae

Species	**Settlement habitat**	**Cues**	**Effects on TTM**	**Reference**
Armass roberti	freshwater	conspecific adult exudates	+	Anger *et al.* (2006)
Callinectes sapidus	estuarine	predator exudates	-	Welch *et al.* (1997)
C. sapidus		estuary humid acid	+	Forward *et al.* (1997)
		ammonium chloride	-	
C. sapidus	estuarine	estuary water	=	Forward *et al.* (1996)
		vegetation	+	
C. sapidus	estuarine	vegetation	+	Forward *et al.* (1994)
		conspecific adult exudates	=	
Carcinus maenas	coastal	predator exudates	-	Geburzi *et al.* (2018)
		conspecific juvenile exudates	-	
Chasmagnathus granulata	estuarine	conspecific adult exudates	+	Gebauer *et al.* (1998)
Hemigrapsus sanguineus	coastal	conspecific adult exudates	+	Geburzi *et al*. (2018)
		congeneric exudates	+	
		C. maenas exudates	=	
H. sanguineus	coastal	conspecific adult exudates	+	Geburzi *et al*. (2018)
		congeneric exudates	+	
		C. maenas exudates	=	
H. sanguineus	coastal	biofilm	+	Anderson and Epifanio (2009)
H. sanguineus	coastal	biofilm	+	Steinberg et al. (2008)
H. sanguineus	coastal	conspecific juvenile exudates	+	(Anderson *et al.* 2010)
H. sanguineus	coastal	conspecific adult exudates	+	O'Connor (2007)
		C. maenas adult odour	+	
		biofilm	+	
Maja brachydactyla	Seafloor (0–30m)	conspecific adult exudates	=	Castejón *et al.* (2019)
		vegetation	=	

		annelids	=	
		biofilm	=	
Menippe mercenaria	subtidal	conspecific adult exudates	=	Krimsky and Epifanio (2008)
		vegetation	+	
		habitat exudates	+	
		food odour	+	
		biofilm	+	
Panopeus herbstii	estuarine	conspecific adult exudates	+	Andrews *et al.* (2001)
P. herbstii	estuarine	biofilm	+	Rodríguez and Epifanio (2000)
		conspecific adult exudates	+	
		Dispanopeus sayi exudates	+	
		food odour	+	
P. herbstii	estuarine	biofilm	+	Weber and Epifanio (1996)
		exudates from other species	=	
		predator exudates	=	
		habitat exudates	+	
Rhithropanopeus harrisii	estuarine	biofilm	+	Fitzgerald *et al.* (1998)
		conspecific adult exudates	+	
Uca cordatus	coastal	biofilm	+	(Simith *et al.* 2017)
		conspecific adult exudates	+	
U. cordatus	coastal	conspecific adult exudates	+	Diele and Simith (2007)
U. cordatus	coastal	conspecific juvenile exudates	+	Simith *et al.* (2013a)
Uca minax		habitat exudates	+	O'Connor and Judge (2004)
U. minax		conspecific adult exudates	+	Welch *et al.* (2016)
		congeneric exudates	+	
Uca pugilator	coastal	conspecific adult exudates	+	Welch *et al*. (2016)
		congeneric exudates	-	
U. pagnax	coastal	conspecific adult exudates	+	O'Connor and Gregg (1998)
		congeneric exudates	=	

		vegetation exudates	=	
U. pugnax	coastal	conspecific adult exudates	+	Welch *et al*. (2016)
		congeneric exudates	+	
U. pugnax	coastal	habitat exudates	+	O'Connor and Van (2006)
Uca vocator	coastal	habitat exudates	+	Simith *et al.* (2010)
		polluted habitat exudates	-	
		conspecific adult exudates	+	

Abbreviations: +: positive; -: negative; =: no effect.

There were also attempts to characterise the chemical cues that induce megalopae metamorphosis. Andrews *et al.* (2001) found that the odour from adult *P. herbstii* has a molecular weight of <1 kDa and remained active after being boiled at 100 °C or frozen at -20 °C. Another study suggested that the odour from *H. sanguineus* was proteinaceous and started to degrade within two days in room temperature at around 25 °C (Anderson *et al.* 2010). Moreover, the effect of chemical cues has also been shown to be dose-dependent. For example, the TTM of both *R. harrisii* and *P. herbstii* decreased with increasing concentration of adult odour (Andrews *et al.* 2001; Fitzgerald *et al.* 1998). However, in the natural environment, the concentrations of such water-soluble cues are expected to be considerably lower than even the lowest level tested in those studies (Andrews *et al.* 2001) because exudates from conspecific adults would be diluted rapidly by water movement. Hence, characterisation of chemical cues, as well as the identification of their effective concentration, is an area that still needs further study.

1.2.2.4.2 Physical cues

As summarised in Table 1.6, several abiotic factors, including tactile and acoustic cues, have been reported to influence the TTM of brachyuran megalopae. Firstly, the lack of a suitable substrate has been shown to lead to prolonged TTM or even mortality in some species. Anomuran crabs are one of the typical examples of this – suitable shells must be available to allow metamorphosis to occur successfully (Hamasaki *et al.* 2014; Oba & Goshima 2004). For brachyuran crabs, substrates that provide appropriate texture can induce metamorphosis. For instance, the TTM of *H. sanguineus* megalopae was reportedly reduced when abiotic rock or nylon mesh was provided as substrate and their effects were further enhanced when the biofilm was formed on the surface (Anderson & Epifanio 2009; O'Connor 2007; Steinberg *et al.* 2008). Rhyne *et al.* (2005) suggested that at the late megalopa stage of *M. sculptus*, the absence of substrate may force the larvae to spend more energy searching for suitable habitats to settle, resulting in smaller juveniles, or even the eventual death of the

megalopae.

Table 1.6. Physical cues that have been reported to influence time to metamorphosis (TTM) of brachyuran megalopae

	Settlement habitat	**Cues**	**Effects on TTM**	**Reference**
Callinectes sapidus	estuarine	artificial vegetation	=	Forward *et al.* (1996)
Chasmagnathus granulata	estuarine	mud	+	Gebauer *et al.* (1998)
		nylon mesh	=	
Cyclograpsus lavauxi	coastal	habitat sounds	+	Stanley *et al.* (2012)
C. lavauxi	coastal	habitat sounds	+	Stanley *et al.* (2009)
Cymo andreossyi	coastal	habitat sounds	+	Stanley *et al*. (2012)
Grapsus tenuicrustatus	coastal	habitat sounds	+	Stanley *et al*. (2012)
Hemigrapsus sanguineus	coastal	rocky texture	+	Anderson and Epifanio (2009)
H. sanguineus	coastal	nylon mesh	+	Steinberg *et al.* (2008)
		rocky texture	+	
H. sanguineus	coastal	rocky texture	+	O'Connor (2007)
		nylon mesh	+	
H. sexdentatus	coastal	habitat sounds	+	Stanley *et al*. (2012)
H. sexdentatus	coastal	habitat sounds	+	Stanley *et al*. (2009)
Maja brachydactyla	seafloor 0–30m	nylon mesh	=	Castejón *et al.* (2019)
		sand	=	
		mud	=	
		shells	=	
		stone	=	
Macrophthalmus hirtipes	coastal	habitat sounds	+	Stanley *et al*. (2009)
Panopeus herbstii	estuarine	artificial vegetation	=	Weber and Epifanio (1996)
		rock/shell	+	
		sand	-	

Schizophrys aspera	coastal	habitat sounds	+	Stanley *et al*. (2012)
Uca cordatus	coastal	mud	+	Diele and Simith (2007)
Uca pagnax	coastal	glass beads	=	O'Connor and Gregg (1998)

Abbreviations: +: positive; -: negative; =: no effect.

However, for many crabs, structure cues do not accelerate metamorphosis and settlement of megalopae. Artificial structural mimics of natural substrates and seaweeds, such as nylon mesh (for *C. granulata*, Gebauer *et al.* 1998), glass beads (for *Uca pagnax*, O'Connor & Gregg 1998), ribbons (for *Callinectes sapidus*, Forward *et al.* 1996), and plastic strips (for *P. herbstii*, Weber & Epifanio 1996), free from chemical cues have shown no effect on TTM. The ineffectiveness of structure cues on metamorphosis was also observed in *C. sapidus*, where TTM remained unchanged when megalopae were given a structural mimic with or without plant odour (Forward *et al.* 1996).

Underwater sound is another abiotic cue that may induce metamorphosis and settlement of decapods but with only very limited studies available to date. As one of the most robust cues that guide onshore orientation for pelagic larvae, underwater sound is directional, independent of water current, and able to carry significant biological information about the habitat (Radford *et al.* 2010). The ability to interpret and respond to habitat-related sounds has been found in megalopae of both tropical and temperate brachyuran species from Families Grapsidae, Xanthidae, and Macrophthalmidae (Stanley *et al.* 2009; Stanley *et al.* 2011; Stanley *et al.* 2012). When exposed to sound from their optimal settlement habitat, the TTM of megalopae reduced up to 47% compared with those exposed to the sounds from other habitat types (Stanley *et al.* 2012). A similar pattern was also observed in the spiny lobster *J. edwardsii*: the lack of acoustic cues from their settlement habitat not only caused a delayed metamorphosis, but also led to lower survival and poorer nutritional conditions during early juvenile stages (Stanley *et al.* 2015). These results suggest that the sounds emanating from specific habitats may play an important role in inducing metamorphosis and settlement of pre-settlement larvae of decapods. Further study on this area is clearly warranted.

1.2.2.4.3 Temporal window of being ‘competent’

The moment when larvae become capable to respond to settlement cues is referred to as being 'competent' (Forward *et al.* 2001). Although the megalopae of crabs are usually considered as being competent, the exact temporal window during which a particular cue become effective on TTM is an area that still requires further research. However, experiments designed to determine the earliest moment and the length of time required to contact with a stimulus that result in a positive larval response is scarce. The temporal window of megalopae being competent to cues has been investigated for *Chasmagnathus granulate* and *Sesarma curacaoense* (Gebauer *et al.* 2004; Gebauer *et al.* 2005). The results showed that for *C. granulata*, metamorphosis of megalopae could only be induced when they were in contact with settlement cues during the first 1/3–1/2 of the megalopal stage duration, and the contact period required was about 5 days (Gebauer *et al.* 2004). Similarly, for megalopae of *S. curacaoense*, if their contact with cues occurred after about 65% of the moulting cycle had passed (i.e., 6–7 days), or the contact time with cues was less than one day, metamorphosis could not be induced (Gebauer *et al.* 2005). In both species, megalopae were receptive to settlement cues during the intermoult (stage C, about 30–50% of a moulting cycle) and premoult period (Stage D_0, about 45–75% of a moulting cycle), coincident with the period that the secretion of moulting hormone occurs (Anger 2001). Thus, the control of metamorphosis may involve an interaction of extrinsic stimulation (cues) with intrinsic factors (hormones).

In conclusion, multiple types of cues can affect TTM of crab megalopae. Cues that have accelerate effects on metamorphosis and settlement are generally linked to the juvenile or adult habitats or their odours (Diele & Simith 2007; Geburzi *et al.* 2018; O'Connor 2007; Simith *et al.* 2017; Simith *et al.* 2010; Welch *et al.* 2016). While structural mimics of natural sediments or vegetation could be effective (Anderson & Epifanio 2009; Stanley *et al.* 2009; Stanley *et al.* 2011; Stanley *et al.* 2012; Steinberg *et al.* 2008), such physical textural cues in general appear to be less effective than chemical ones (Anderson & Epifanio 2009; Steinberg *et al.* 2008; Weber & Epifanio 1996). Previous studies on this area mainly focused on species with juvenile or adult habitats in estuarine or coastal areas (summarised in Table 1.5). Since most ornamental species inhabit coral reefs and many show a prolonged larval duration under

culture condition (reviewed by Calado *et al.* 2017), settlement cues could be highly important for the successful culture of these species. Unfortunately, no information is currently available on this area for ornamental crustaceans, including brachyuran crabs.

1.3 Conclusion

The marine ornamental aquaculture is currently still in its infancy. The development of larval rearing techniques for marine ornamental crabs is constrained by the presence of multiple bottlenecks and an overall lack of research. Tapping into existing knowledge on edible crab species has allowed a rudimentary development of the industry. This chapter reviews the available literature and areas in which further research efforts should be invested to improve larval culture success of marine ornamental crabs; of which areas of particular importance are:

- Optimisation of larval feeding regimes, including both quality and quantity of live prey to be used, as well as live prey nutritional enhancement methods, in order to maximise larval performance.

- Exploitation of alternative, nutritive new live prey other than traditional ones (rotifers and *Artemia*) and developing their mass culture techniques.

- Optimisation of physical environment for larval rearing that maximises larval feeding, growth, health, and survival.

- Investigation of settlement cues of megalopae, in order to avoid the delay of metamorphosis and settlement and associated negative effects.

As the interest in the marine ornamental industry grows, research efforts on the above areas should stand to contribute significantly not only towards the development of a viable marine ornamental aquaculture industry, but also the overall knowledge of crustacean

aquaculture and general biology, as well as reef conservation.

1.4 About this thesis

1.4.1 Candidate species of this thesis

The decorator crab *Camposcia retusa* (Figure 1.2), also known as Velcro crab, is one of the most popular marine ornamental crabs due to its fascinating decorating behaviour. This species is widely distributed in the tropical and sub-tropical waters of the Indo-Pacific, from the eastern coast of Africa to south and east Asia and the northern and eastern coasts of Australia (Fishelson 1971; Gohar & Al-Kholy 1957; Griffin 1974; Haswell 1880; Kumar & Wesley 2012; Vidhya *et al.* 2017). The taxonomy of *C. retusa* (Latreille, 1829) is as follows:

Phylum: Arthropoda

Subphylum: Crustacea

Superclass: Multicrustacea

Class: Malacostraca

Order: Decapoda

Infraorder: Brachyura

Superfamily: Majoidea

Family: Inachidae

Genus: *Camposcia*

Species: *Camposcia retusa* (Latreille, 1829)

The decorating behaviour of *C. retusa* is widely known, as they collect materials from their habitats and attach these items on their hook-like setae as a way of camouflage (Hultgren & Stachowicz 2011; Wicksten 1993). Hence, the exterior appearance of *C. retusa* may vary substantially in shapes and colours depending on what they use for decoration. Indeed, such fascinating behaviour make the species a popular target for the marine ornamental trade (Calado 2006; Calado *et al.* 2003a). For this reason, *C. retusa* was chosen as

the candidate species for the present study, which aims to develop reliable techniques for its larval culture. Despite its popularity, there have been no reports so far on the successful larval culture of *C. retusa*. An early work by Gohar and Al-Kholy (1957) reported their attempts to rear *C. retusa* larvae for morphological description, but unfortunately no larvae survived to the second zoeal stage.

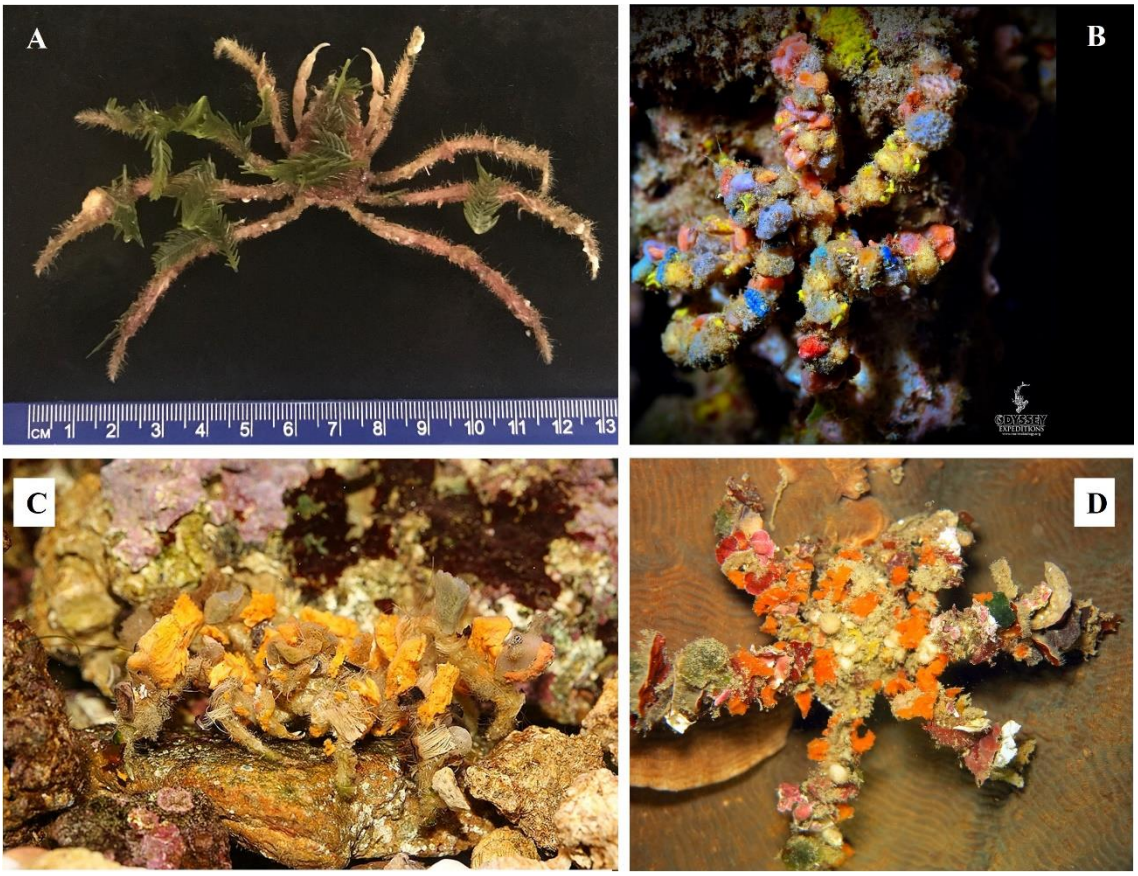

Figure 1.2. Adult decorator crab (*Camposcia retusa*) with different decorations. A: Captive-bred and photo by Tian Xu; B: from Buchheim (2004); C: photo by S. J. Huang (2014); D: photo by G. Y. Chen (2014).

1.4.2 Outlines of thesis chapters

The overarching objective of this thesis is to develop reliable captive culture techniques for *C. retusa*. The following section provides a snapshot of each chapter. It is worth noting that chapter 2 has already been published in a peer reviewed journal (Zootaxa, doi: 10.11646/zootaxa.4577.2.4), but minor editing was carried out to fit the format of the thesis and include updated information. In addition, to ensure the adequate and logic flow of each chapter, some information of the Introduction and Materials and Methods sections may be repeated in subsequent chapters.

Chapter 1 outlines the current status of the marine ornamental trade and aquaculture, and the relevance of this thesis research. Past literature on marine crab larval rearing is reviewed and major knowledge gaps are identified.

Chapter 2 for the first time describes and illustrates the morphology of larval and first-stage juvenile of *C. retusa* reared under captive condition. Moreover, the morphological characters of the larval stages of *C. retusa* are compared with the corresponding stages of other species from family Inachidae previously reported in the literature.

Chapter 3 establishes the feeding regime for *C. retusa* larval rearing. Experiments were conducted to firstly evaluate the suitability of two traditional hatchery live prey, rotifers and *Artemia*, on larval feeding of *C. retusa*; the optimal *Artemia* feeding density for *C. retusa* larvae were subsequently determined. Finally, the effect of copepod co-feeding with *Artemia* nauplii and *Artemia* enrichment on *C. retusa* larval culture were also investigated.

Chapter 4 assesses the effects of different salinity conditions on *C. retusa* larval culture. Two experiments were conducted to identify the optimal salinity for zoeal and megalopal stages of *C. retusa*. A subsequent experiment was conducted to investigate the underlying mechanism of significant salinity effects on megalopal survival and development via determining the patterns of ingestion rate of megalopae under different salinities.

Chapter 5 reports a series of experiments investigating the effects of stocking density and bottom areas of culture containers, various artificial substrates, size of mesh as artificial substrates, and presence of settlement cues on survival and development of megalopae of *C. retusa*.

Chapter 6 describes the development and growth of juvenile *C. retusa* under captive conditions. It also describes the sexual dimorphism of *C. retusa* and determines the age of its appearance. The information presented is useful for evaluating potential commercial breeding of this species for the marine ornamental trade.

Chapter 7 summarises the main results of this thesis and discusses their significances in a broader context. It also points out future research directions.

Chapter 2. Morphological descriptions of the larval and first juvenile stages of the decorator crab *Camposcia retusa* (Latreille, 1829) from laboratory-reared material

2.1 Abstract

The complete larval and first crab stages of the decorator crab *Camposcia retusa* (Latreille, 1829) are described and illustrated based on laboratory-reared material for the first time. Specimens were obtained from larvae hatched from adult crabs collected from coral reefs of Queensland, Australia. Newly hatched larvae were successfully reared to settlement as first-stage crabs. Larval development consisted of two zoeal stages and one megalopal stage. The morphology of each larval stage was compared with those available from a previous study using material from the Red Sea. Due to substantial differences in morphology of the second zoeal and megalopal stages between the two studies, we argue that the larval stages described by the earlier report may not be that of *C. retusa*. Finally, the morphological characters of both larval and first crab stages of *C. retusa* are also compared with the corresponding stages of previously reported Inachidae.

2.2 Introduction

The decorator crab, *Camposcia retusa* (Latreille, 1829), is one of the popular marine ornamental crustaceans but is poorly studied (Calado *et al.* 2003a). This species is widely distributed in the tropical and subtropical shallow waters of the Indo-West Pacific, from eastern Africa to south and east Asia and the northern and eastern coast of Australia (Fishelson 1971; Gohar & Al-Kholy 1957; Griffin 1974; Haswell 1880; Kumar & Wesley 2012; Vidhya *et al.* 2017). *Camposcia retusa* decorates itself with materials collected from their habitat as a method of camouflage (Hultgren & Stachowicz 2011; Wicksten 1993).

Hence the appearance of *C. retusa* may differ in colour and shape depending on what it uses for decoration; such character of the crab makes it a popular target of the marine ornamental trade (Calado 2006; Calado *et al.* 2003a).

Despite its popularity in the marine aquarium trade, there have been no reports so far on successful larval culture of *C. retusa*. Gohar and Al-Kholy (1957) described the larval stages of *C. retusa*, only the prezoeal and the first zoeal (Z_1) stages were described based on specimens hatched in the laboratory since they failed to rear the larvae past the Z_1 stage. The description of remaining larval stages was based on plankton specimens collected at the Red Sea. In addition, the first crab (C_1) stage was not described because these authors were unable to obtain the specimens. Moreover, due to the low number of specimens (e.g. only 3 megalopae), and the knowledge and technical limitations at the time, the larval descriptions by Gohar & Al-Kholy (1957) were insufficient in details and accuracy, with only one-page of small and poorly detailed illustrations that do not conform to updated standards (viz. Clark *et al.* 1998). Additionally, while more than 30 types of setae of crustacean larvae are recognised today (Ingle 1992; Martin *et al.* 2014; Vieira *et al.* 2013), in Gohar & Al-Kholy's (1957) description, they were merely referred to vaguely as 'setae, bristles, or spines'.

In our efforts to breed *C. retusa* in captivity to reduce collection pressure on wild populations, newly hatched larvae of the species were successfully reared to settle as first stage crabs. Since this appears to be the first known successful larval rearing of *C. retusa* to settlement, we describe and illustrate the morphology of the larval and the first juvenile stages of *C. retusa* based on laboratory-reared material.

2.3 Material and methods

2.3.1 Broodstock maintenance

A total of 6 adult *C. retusa* were purchased from a commercial collector (Cairns Marine,

Cairns, Australia) as broodstock. The crabs had been collected from reefs of the Coral Sea, Australia and air-freighted to James Cook University, Townsville. On their arrival at the laboratory, the crabs were acclimatised for 1 h before being kept in several 50 L rectangular acrylic tanks connected to a recirculating seawater system in the Marine and Aquaculture Research Faculty Unit (MARFU) at James Cook University. The crabs were kept in pairs in separate tanks and shelters in the form of seaweed, rocks and dead coral were provided in each tank. Water temperature was maintained at 26.7–28.9 °C and salinity at 35–37. Chopped prawn and mussels, as well as frozen blood worms were fed to the broodstock twice daily, and uneaten food was removed the following morning. The females were checked for spawning every second day. Once a female was found carrying eggs, it was left in the aquarium with the male crab but closely monitored for larval hatching. It was found that egg incubation generally took 21–25 days under the described conditions.

2.3.2 Larval rearing

Larvae normally hatched overnight, and occasionally in the early morning. When the berried female was judged to be near hatching, a banjo filter with fine mesh was plugged to the outlet of the aquarium in the late afternoon to prevent newly hatched larvae from flowing out. In the morning of the day of hatching, aeration was first stopped, then the room light was turned off, and a torch was directed to an upper corner of the tank to attract the positively phototactic larvae. The larvae gathered were then gently scooped with a small beaker with as little water as possible.

Twenty newly hatched larvae were subsequently transferred to each of several 500 mL beakers for rearing. Enriched brine shrimp (*Artemia*) metanauplii (INVE Thailand, Amphoe Wachirabarami, Thailand) were provided at 10 individuals/mL daily to feed the crab larvae throughout larval development. A 100% water exchange was carried out daily by moving live larvae with a pipette to a new beaker filled with fresh seawater and *Artemia* when larval moulting and mortality was monitored and recorded. Throughout larval culture, temperature

was maintained at 27.7 ± 0.6 °C and salinity 35–37.

2.3.3 Live feed culture

Larvae were fed with *Artemia* nauplii (INVE Thailand, Amphoe Wachiabarami, Thailand) in this experiment. *Artemia* nauplii were hatched daily from cysts. The hatching period lasted at least 23h with the temperature and salinity was maintained between 26–28 °C and 34–35, respectively. Newly hatched *Artemia* nauplii were harvested next morning for either feeding larvae immediately or subsequent enrichment. The enrichment of *Artemia* nauplii was carried out using S.presso (Selco S.presso®, INVE Aquaculture, UT, USA) enrich emulsion following the manufacture's instruction. Following enrichment, enriched *Artemia* metanauplii were collected the next morning and thoroughly rinsed before being counted for density. The density of *Artemia* was estimated via averaging three 1 ml sub-samples taken from the beaker. The number of each sub-sample was counted using a Sedgewick-Rafter counting chamber under microscope (Leica LME).

2.3.4 Description of morphology and measurements

Larval and juvenile specimens used for morphological description were fixed in 10% formalin. Description of each stage was based on 5–12 specimens. Line illustrations were made under a compound microscope equipped with differential interference contrast optics (Leica DM LS2). Prior to dissection, specimens were prepared with lactic acid to dissolve internal tissue. The sequence of the larval description is from anterior to posterior, and the setation on appendages is described from proximal to distal segments, following Clark *et al.* (1998).

Measurements were taken with a calibrated ocular micrometre and based on lateral view of zoeal larvae but dorsal view for megalopae. The following parameters were measured and

recorded: carapace length (CL = the distance from the frontal to the posterior margin of the carapace); rostrodorsal length (RDL = from the tip of the rostral spine to the tip of the dorsal spine) of zoeal larvae; and carapace width (CW = the widest distance of the carapace) of the megalopal and first stage crab. All sizes were presented as mean ± standard deviation (SD).

Specimens of adult crabs, moults and undissected larvae of each larval stage were preserved as voucher specimens at the College of Science and Engineering, laboratory BD032-006, James Cook University, Australia (labelled 24122017), while all dissected larvae were discarded. The morphology of all larval stages was compared with what was described by Gohar & Al-Kholy (1957) for the species, and the corresponding stages of other previously reported inachid larvae, i.e. *Platymaia wyvillethomsoni* Miers, 1886 (viz. Oh & Ko 2012), *Macrocheira kaempferi* Temminck, 1836 (viz. Clark & Webber 1991), *Macropodia czernjawskii* Brandt, 1880 (viz. Marco-Herrero *et al.* 2012), *Dorhynchus thomsoni* C.W. Thomson, 1873 (viz. Ingle 1992), *Inachus aguiarii* Brito Capello, 1876 (viz. Guerao & Abelló 2007), and *Inachus communissimus* Rizza, 1839 (viz. Guerao & Abelló 2007).

2.4 Results

Based on our first successful larval rearing of the species in the laboratory, the larval development of *C. retusa* was confirmed to include two zoeal and one megalopal stage. Herein the first zoeal stage is fully described. For the subsequent stages, including the newly settled first-stage crab, only new features and differences from the previous stage are described in detail.

Description

2.4.1 Zoea I

Size: RDL = 1.94 ± 0.10 mm; CL = 1.18 ± 0.09 mm. (Number of examined specimens: 12).

Carapace (Figure 2.1A). With 1 short curved dorsal spine, 1 straight rostral spine, and 1 lateral spine on each side of the carapace; each lateroventral margin with 1 densely plumose 'anterior seta', which is defined as 'majid seta' (Clark *et al.* 1998), and followed by 5 sparsely plumose setae; eyes sessile.

Antennule (Figure 2.1B). Unsegmented, smooth, conical; 3 aesthetascs present at the terminal end; endopod absent.

Antenna (Figure 2.1C). Biramous; protopod long and pointed, with 2 rows of tiny spinules; endopod bud presents; exopod unsegmented, with spiny distal processes; 2 plumose setae present about half-way from the tip.

Mandible (Figure 2.1D). Asymmetric; with median toothed molar and lateral incisor processes, areas between incisor and molars have serrulate processes; palp absent.

Maxillule (Figure 2.1E). Coxal endite bearing 6 plumodenticulate setae; basial endite with 7 plumodenticulate setae; endopod bi-segmented, with 1 and 6 plumodenticulate setae on proximal and distal segment, respectively.

Maxilla (Figure 2.1F). Coxal endite bilobed, with 4 and 5 plumodenticulate setae on the proximal and distal lobe, respectively; basial endite bilobed, with 5 and 4 plumodenticulate setae on the proximal and distal lobe, respectively; endopod unsegmented, bearing 8 plumodenticulate setae; exopod (scaphognathite) with 17 – 18 plumose setae distribute around the margin, plus 1 stout distal process.

Maxilliped I (Figure 2.1G). Coxa naked; basis bearing 9 plumodenticulate setae arranged at 2 + 2 + 2 + 3; endopod five-segmented and the segments from proximal to distal with 3, 2, 1, 2, 5 plumodenticulate setae, respectively; exopod incompletely bi-segmented, proximal segment naked and distal segment with 4 long plumose terminal natatory setae.

Maxilliped II (Figure 2.1H). Coxa naked; basis bearing 3 plumodenticulate setae arranged at 1 + 1 + 1; endopod three-segmented and the segments from proximal to distal with 0, 1, 4 plumodenticulate setae, respectively; exopod incompletely bi-segmented, bearing 4

long plumose terminal natatory setae on the distal end.

Maxilliped III (Figure 2.1I). Undeveloped, present as a small biramous bud.

Pereiopods (Figure 2.1J). Undeveloped, present as small buds. The first pereiopod bilobed, appears in a shape of chela.

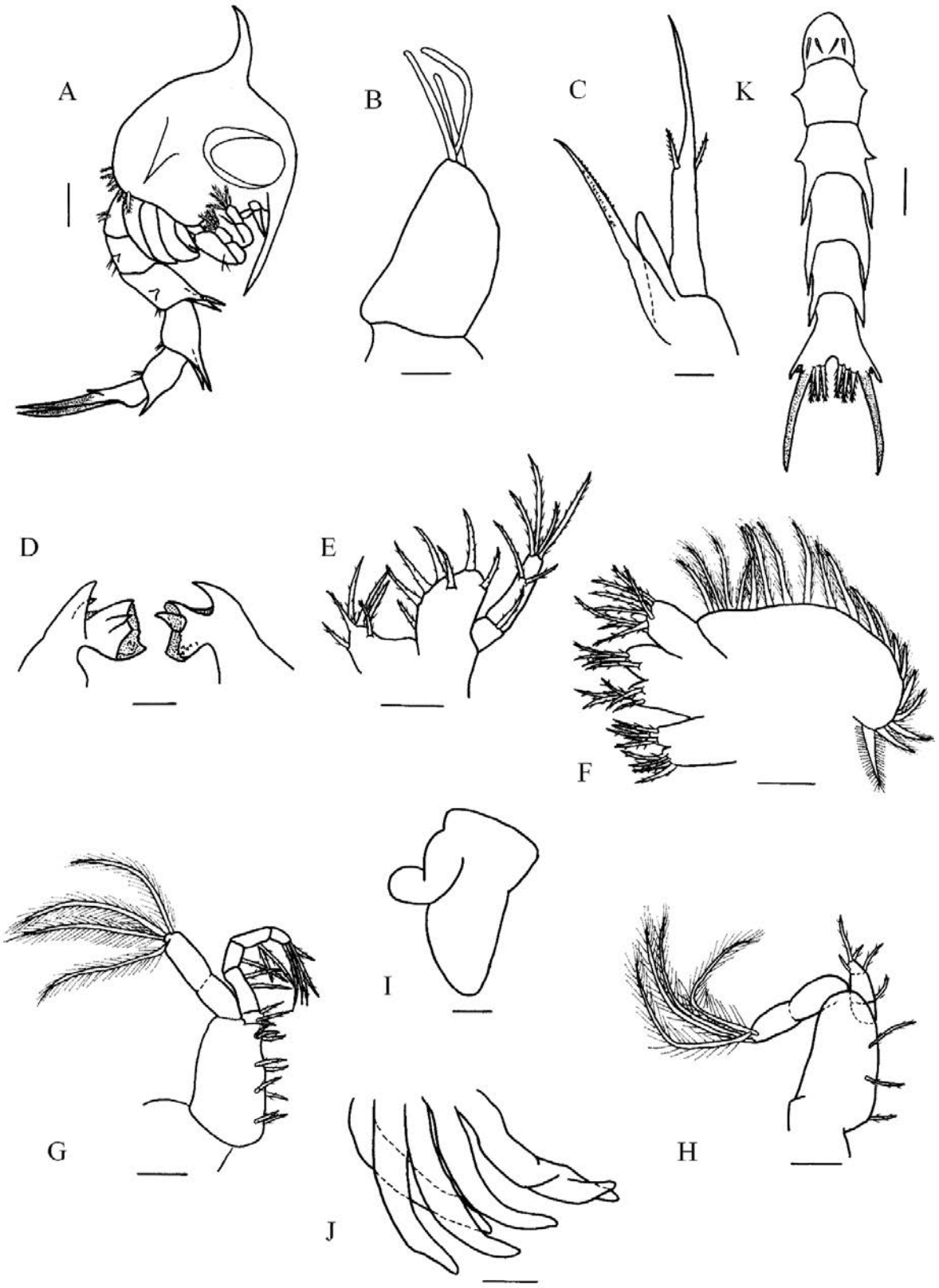

Figure 2.1. *Camposcia retusa* zoea I: A) lateral view; B) antennule; C) antenna; D) mandible; E) maxillule; F) maxilla; G) maxilliped I; H) maxilliped II; I) maxilliped III; J) pereiopods 1–5; and K) pleon and telson (dorsal view). Scale bars: A, and K: 300 μm; G, H, and J: 150 μm; B, C, D, E, and I: 75 μm; F: 30 μm.

Pleon (Figure 2.1K). From somites 1 to 5, there are 4, 2, 2, 2, 2 simple setae on dorsal surface of each somite, respectively; somites 2 – 5 with pleopod buds.

Pleopods. Absent.

Telson (Figure 2.1K). Bifurcated with a U-shape cleft; 3 pairs of serrulate setae on the middle of posterior margin; 1 pair of lateral acicular spines and 2 pairs of dorsolateral spinules present on the base of furcal shaft; furcal shafts covered with rows of spines from proximal to terminal.

2.4.2 Zoea II

Size: RDL = 2.24 ± 0.06 mm; CL = 1.42 ± 0.05 mm. (Number of examined specimens: 12).

Carapace (Figure 2.2A). Lateroventral margin with 1 densely plumose 'anterior seta' plus 6 sparsely plumose setae; eyes stalked.

Antennule (Figure 2.2B). Presents 2 terminal buds, coxa and basis are not differentiated, exopod with 8 aesthetascs present on the distal end.

Antenna (Figure 2.2C). Endopod bud enlarged.

Mandible (Figure 2.2D). Palp presents, naked, unsegmented.

Maxillule (Figure 2.2E). Coxal endite bearing 7 plumodenticulate setae; basial endite bearing 9 plumodenticulate setae, with 1 plumose seta present on external margin; endopod bi-segmented, with 1 and 6 plumodenticulate setae on proximal and distal segment, respectively.

Maxilla (Figure 2.2F). Coxal endite bilobed, with 4 and 3 plumodenticulate setae on the proximal and distal lobe, respectively; basial endite bilobed, with 4 and 5 plumodenticulate setae on the proximal and distal lobe, respectively; endopod unchanged; scaphognathite marginally with 32–34 plumose setae, including one distal stout process.

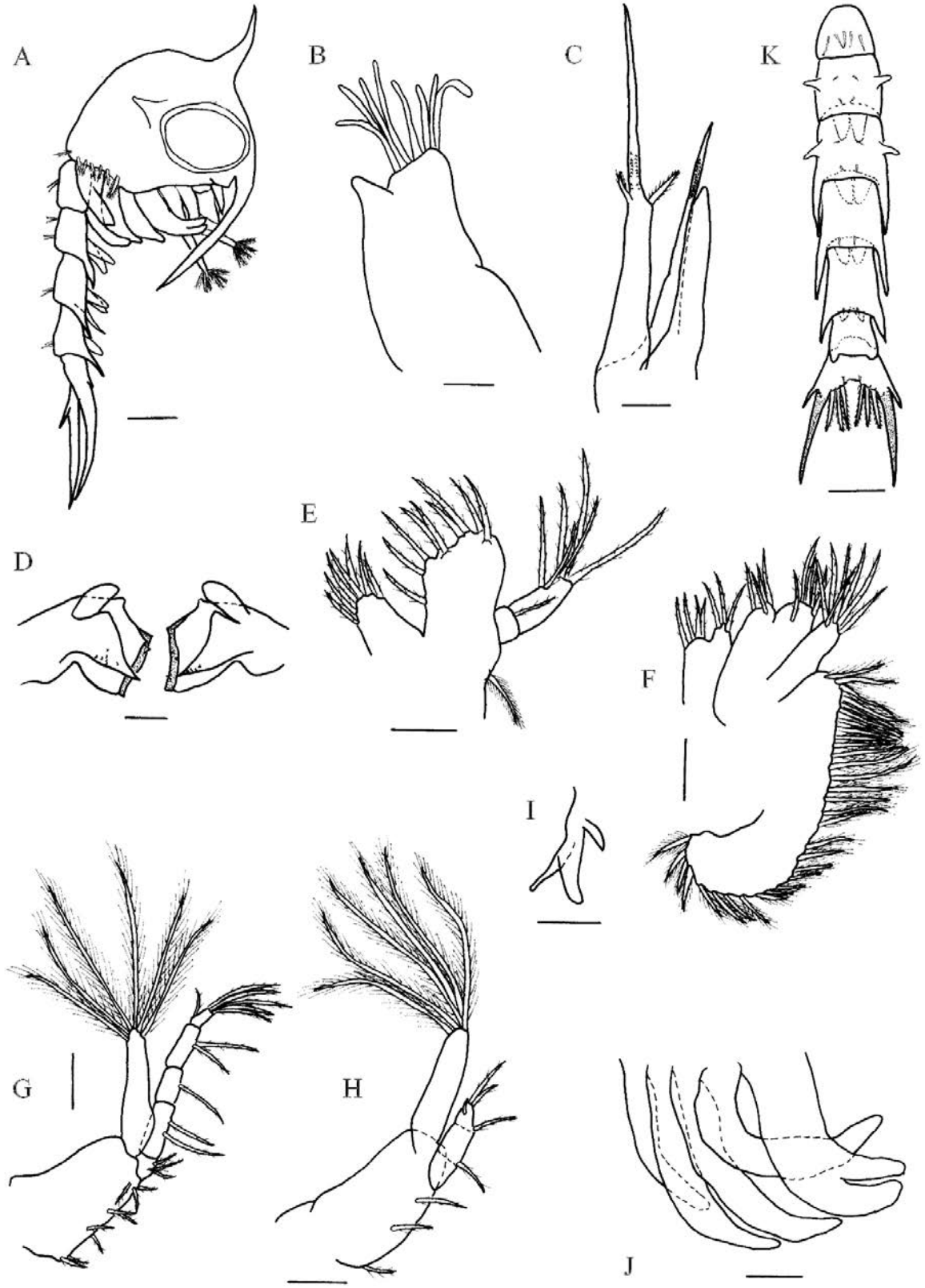

Figure 2.2. *Camposcia retusa* zoea II: A) lateral view; B) antennule; C) antenna; D) mandible; E) maxillule; F) maxilla; G) maxilliped I; H) maxilliped II; I) maxilliped III; J) pereiopods 1–5; and K) pleon and telson (dorsal view). Scale bars: A and K: 300 μm; G, H, I and J: 150 μm; B, C, D, E and F: 75 μm.

Maxilliped I (Figure 2.2G). Coxa, basis and endopod unchanged, except endopod distal segment now with 6 plumodenticulate setae; exopod distal segment with 6 terminal plumose natatory setae.

Maxilliped II (Figure 2.2H). Exopod distal segment with 6 terminal plumose natatory setae; other parts largely unchanged.

Maxilliped III (Figure 2.2I). Developing, a triple-lobed bud present.

Pereiopods (Figure 2.2J). Developing, longer. A distinct but non-functional cheliped present.

Pleon (Figure 2.2K). 6 somites; from somites 1 to 6, there are 5, 4, 4, 2, 2, 0 simple setae on dorsal surface of each somite, respectively; somites 2–5 with pleopod buds.

Pleopods (Figure 2.2A). Present as small biramous buds.

Telson (Figure 2.2K). Unchanged except 1 pair of simple setae present on the U-shape cleft.

2.4.3 Megalopa

Size: CL = 1.45 ± 0.15 mm; CW = 0.95 ± 0.09 mm. (Number of examined specimens: 8).

Carapace (Figure 2.3A). More crab-like; length longer than width, dorsoventrally compressed; dorsal surface covered sparsely with simple setae; one posterior median process present, and two anterolateral processes present on the dorsal surface; anterolateral margin has 2 simple setae; one process mid-posterior margin; posterior margin with 3 pairs of simple setae.

Antennule (Figure 2.3B). Peduncle with three segments and the segments from proximal to distal with 0, 0, 2 simple setae, respectively; endopod developed, with 3 simple setae; exopod two-segmented, the proximal segment bearing 3 – 4 long aesthetascs, and the distal segment with 4 aesthetases plus 1 simple setae.

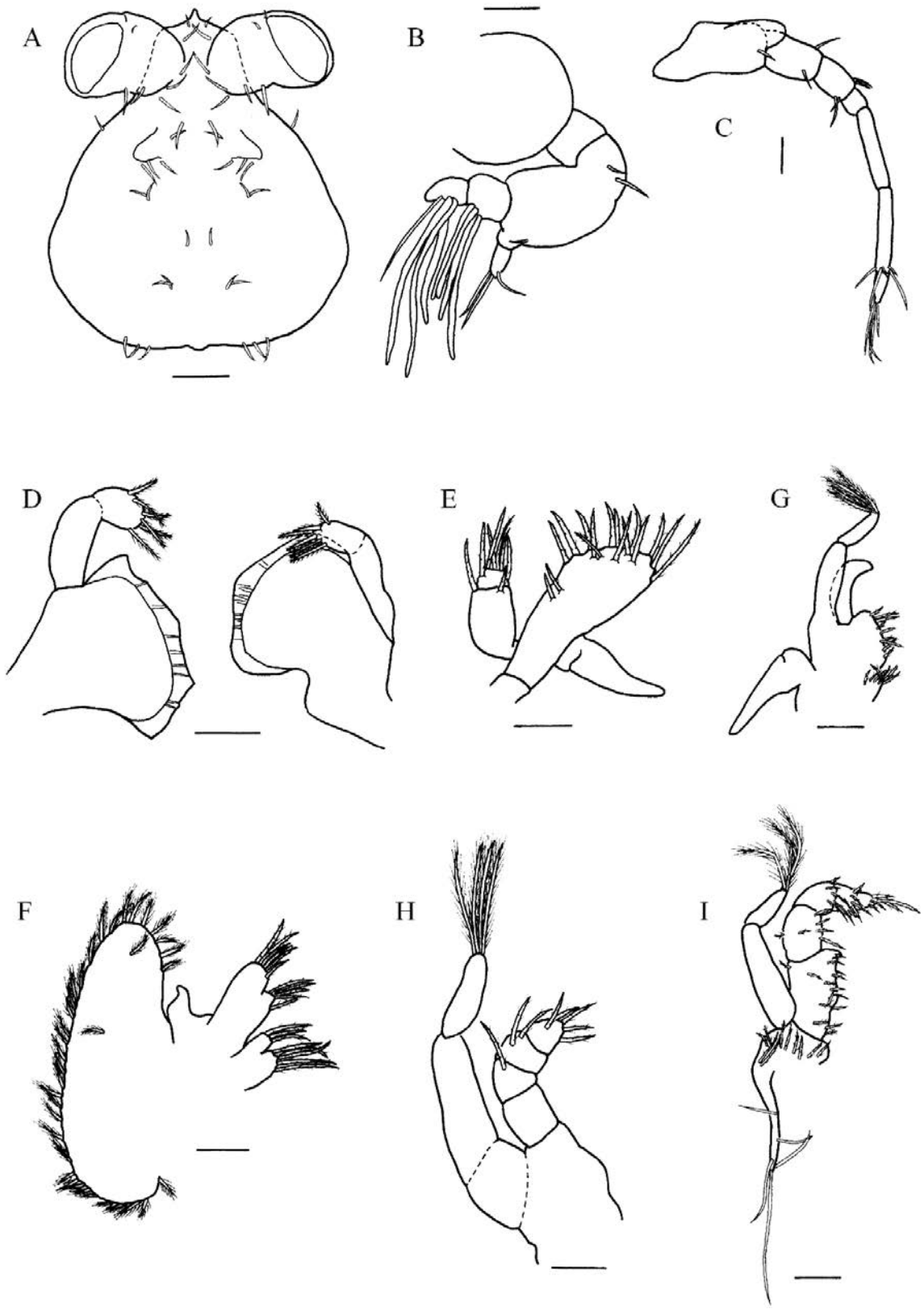

Figure 2.3. *Camposcia retusa* megalopa: A) carapace dorsal view; B) antennule; C) antenna; D) mandible; E) maxillule; F) maxilla; G) maxilliped I; H) maxilliped II; I) maxilliped III. Scale bars: A: 300 μm; B–I: 75 μm.

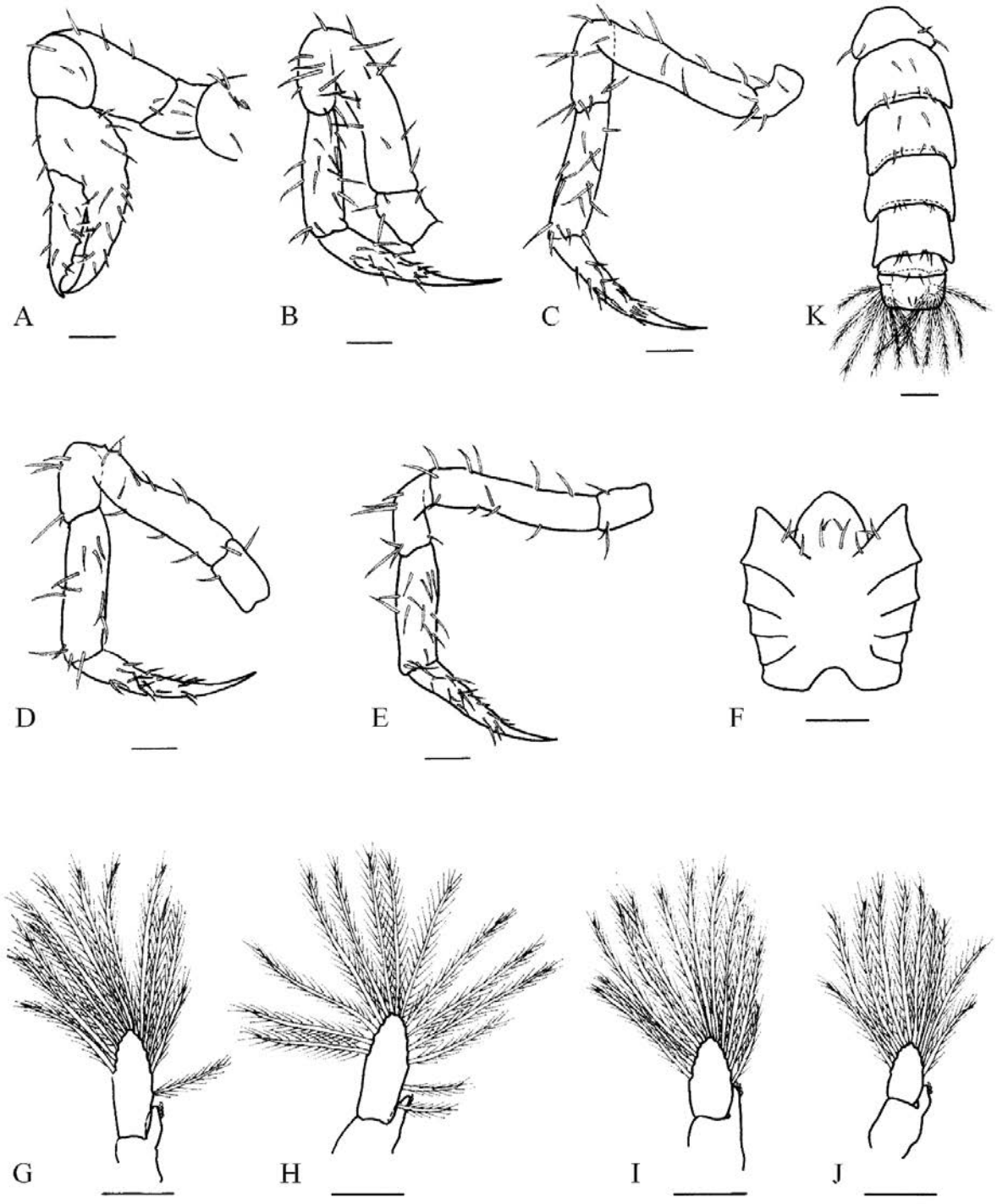

Figure 2.4. *Camposcia retusa* megalopa: A)–E) pereiopods 1–5; F) sternum; G)–J) pleopods 1–5; and K) pleon, telson and uropods (dorsal view). Scale bars: A–E and G–K: 150 μm; F: 300 μm.

Antenna (Figure 2.3C). Segments 1 – 7, progressing proximally to distally, each with 1, 2, 4 (2+2), 0, 0, 5 (2 short and 3 long, terminally), 4 (1 short and 3 long, terminally) simple setae, respectively; exopod peduncle present on basis.

Mandible (Figure 2.3D). Asymmetrical; the left has wedge-shape blade with cutting edge; the right blade rounded with cutting edge; both mandibles with incompletely two-segmented palp, distal segment with 6 terminal plumodenticulate setae.

Maxillule (Figure 2.3E). Coxal endite with 8 apical plumodenticulate setae; basial endite with 16 mostly plumodenticulate setae distally; endopod partly bi-segmented and reduced, lacking setae.

Maxilla (Figure 2.3F). Coxal endite with 5 + 3 plumodenticulate setae; basial endite with 6 + 6 plumodenticulate setae; endopod naked and reduced; scaphognathite bearing 33 – 35 marginal plumose setae and 2 plumodenticulate setae on the blade.

Maxilliped I (Figure 2.3G). Coxa and basis with 7 and 9 plumodenticulate setae, respectively; endopod unsegmented and naked; exopod bi-segmented, proximal segment with 0 – 1 plumodenticulate seta, and distal segment with 4 long plumose setae; epipod triangular and naked.

Maxilliped II (Figure 2.3H). Coxa and basis without seta and are not clearly differentiated; endopod with an indistinct basis and subsequent four segments, from proximal to distal, each segment bearing 0, 1, 2, and 5 plumodenticulate setae, respectively; exopod with a naked proximal segment, and distal segment with 4 long plumose setae; epipod absent.

Maxilliped III (Figure 2.3I). Coxa and basis are not clearly differentiated, with 10 plumodenticulate setae; endopod with 5 segments and the segments from proximal to distal with 15, 7, 5, 6, and 5 plumodenticulate setae, respectively; ischium margin shows crista dentate, and distal margin with one acicular process; exopod with two segments, proximal segment naked, distal segment bearing 4 long plumose setae; epipod prolonged into branchial chamber, with 5 long grooming setae.

Thoracic sternum (Figure 2.4F). Somites 1–3 fused and form a single plate, with a spade-

shape anterior margin and 2 pairs of simple setae on the plate; segment 4 with 3 pairs of simple setae on the anterior margin; subsequent segments naked; posterior margin convex forward.

Pereiopods (Figure 2.4A–E). All pereiopods covered with dense simple setae. Cheliped (pereiopod 1) well-developed, dactylus bearing three acicular processes; pereiopod 2 with one acicular process on anterior margin of propodus; pereiopod 3 with one small, rounded process on anterior margin of carpus; pereiopod 4 has one lateral acicular process on merus; pereiopod 5 has one small process on anterior margin of carpus and one acicular process on anterior margin of merus.

Pleon (Figure 2.4K). With six somites plus telson, dorsal surface of somite 1–6 with 4, 6, 6, 6, 6, and 2 simple setae, respectively; somites 2–5 with functional natatory setose pleopods, and the last somite bearing one pair of uropods.

Pleopods (Figure 2.4G–K). Five pairs, present on somites 2–5 of the pleon; endopod of pleopod 1–4 bearing 3–4 cincinnuli each; exopod of pleopod 1–4 with 13–15, 13, 12–13, and 10–11 long plumose natatory setae, respectively; pleopod 5 (i.e. uropod) reduced, segments fused, with 6 plumose natatory setae in terminal end.

Telson (Figure 2.4K). Rectangular, round posteriorly, 1 pair of simple setae dorsally, forming tail fan with uropods.

2.4.4 Crab I

Size: CL = 2.05 ± 0.20 mm; CW = 1.32 ± 0.12 mm. (Number of examined specimens: 5).

Carapace (Figure 2.5A). Length longer than width, narrowing anteriorly; two triangular processes posterior to eyes; one rounded tubercle on the middle of dorsal surface; two knob-like protuberances near the border of gastric area; the border of cardiac region and posterolateral margin slightly elevated; surface covered with numerous long simple setae and hooked setae.

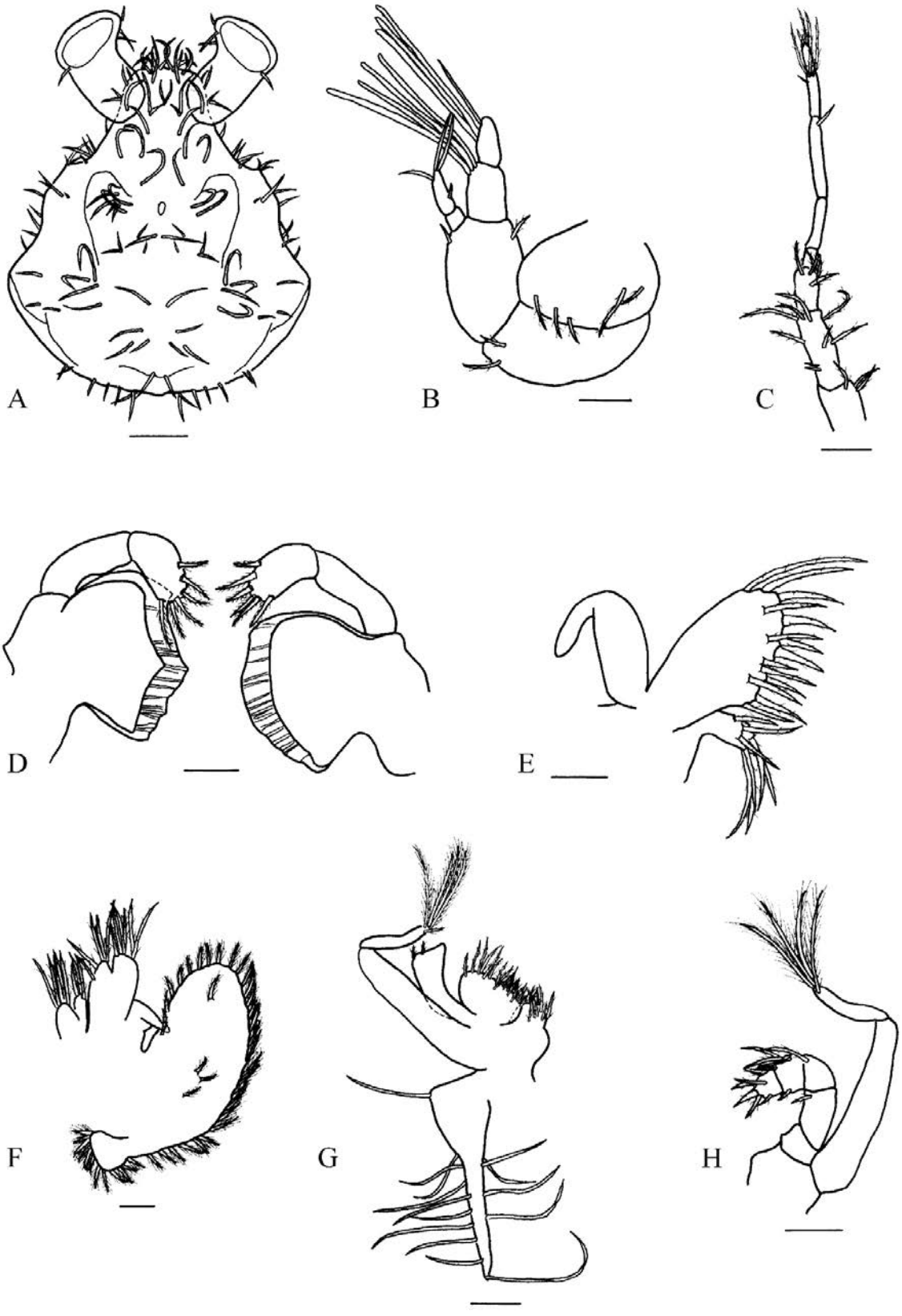

Figure 2.5. *Camposcia retusa* first crab: A) carapace dorsal view; b) antennule; C) antenna; D) mandible; E) maxillule; F) maxilla; G) maxilliped I; and H) maxilliped II. Scale bars: A: 300 μm; B, D and F: 75 μm; C, G and H: 150 μm.

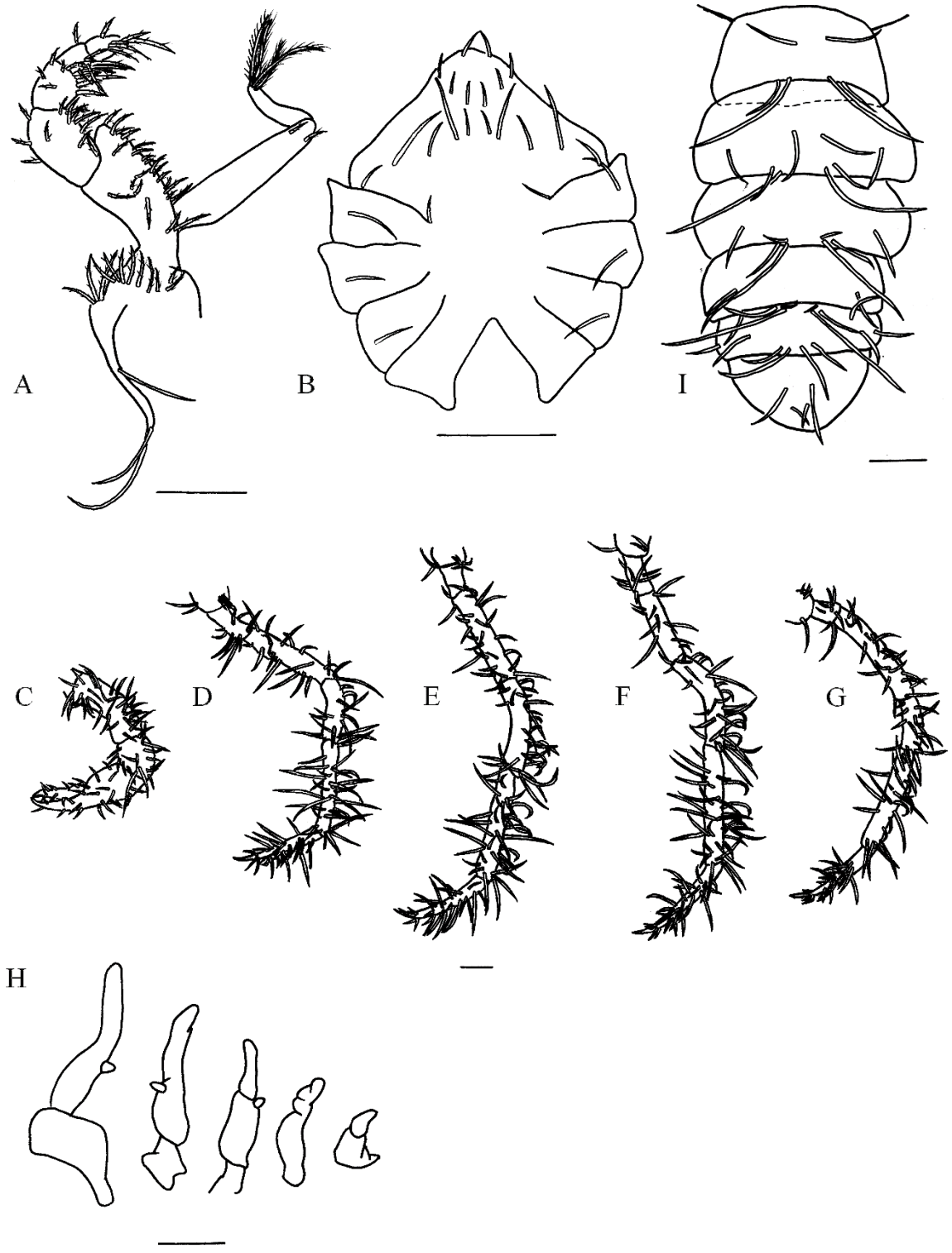

Figure 2.6. *Camposcia retusa* first crab: A) maxilliped III; B) sternum; C)–G) pereiopods 1–5; H) pleopods 1–5; and I) pleon and telson (dorsal view). Scale bars: A, H and I: 150 μm; B–G: 300 μm.

Antennule (Figure 2.5B). Peduncle three-segmented with 5, 2, 2 plumose setae, respectively; endopod bi-segmented, proximal segment naked and distal segment with 3 + 1 setae; exopod bi-segmented, proximal segment bearing 4 aesthetascs and distal segment with 3 aesthetascs plus 1 simple seta.

Antenna (Figure 2.5C). Segments 1–8 from proximal to distal has 4, 8, 5, 0, 0, 1, 4, and 3 setae, respectively, all setae are plumose except that 2, 1, 1, and 3 setae on the second, sixth, seventh and last segments are simple.

Mandible (Figure 2.5D). Asymmetric; blades are the same as for megalopa except that size has increased; palp bi-segmented with 0 and 6 plumodenticulate setae.

Maxillule (Figure 2.5E). Basial endite with 14 plumodenticulate setae; endopod unsegmented and naked.

Maxilla (Figure 2.5F). Both proximal and distal lobe of basial endite have 5 plumodenticulate setae; Endopod naked and reduced; scaphognathite bearing 41 plumose marginal setae, and 5 plumose setae on the blade.

Maxilliped I (Figure 2.5G). Coxa bearing 14 plumodenticulate setae; basis with 18 plumodenticulate setae; endopod reduced, with 2–3 short plumodenticulate setae; exopod bi-segmented, proximal segment naked; distal segment with 5 setae, 1 short, plumodenticulate and 4 long, plumose; epipod triangular and prolonged, with 14 long grooming setae.

Maxilliped II (Figure 2.5H). Coxa and basis naked and not differentiated; endopod five-segmented, from proximal to distal, each segment with 0, 1, 1, 5, 6 plumodenticulate setae, respectively, one process presents on the margin of the first segment; exopod with two segments, the proximal segment without setae, and the distal segment bearing 4 long terminal plumose setae; epipod absent.

Maxilliped III (Figure 2.6A). Coxa and basis not differentiated, bearing 12 plumodenticulate setae; endopod five-segmented and from proximal to distal, each segment with 23, 12, 10, 8, 7 plumodenticulate setae, respectively, with crista dentate on the margin of

ischium; exopod bi-segmented, the proximal segment with 2 plumodenticulate setae and the distal segment with 4 plumose setae; epipod bearing 3 long simple setae.

Pereiopods (Figure 2.5C–G). Covered with curved simple setae and hooked setae, both density and length of setae increased compared to megalopa; no process present on segments.

Thoracic sternum (Figure 2.5B). Somites 1–3 fused and forming a plate, with 10 pairs of simple setae; subsequent segments from proximal to distal bearing 1, 1, 1, and 0 pairs of simple setae, respectively.

Pleon (Figure 2.5I). With six somites; somites 2–5 each with 6, 8, 8, 2, and 16 simple setae, respectively.

Pleopods (Figure 2.5H). Five pairs; reduced and no seta presents.

Telson (Figure 2.5I). Triangular, with a rounded posterior margin, bearing 4 simple setae.

2.5 Discussion

2.5.1 Larval stages

Superfamily Majoidea Samouelle, 1819 is one of the biggest taxonomic groups within infraorder Brachyura (Order Decapoda) with more than 900 species identified so far (Ng *et al.* 2008). Though the species of Majoidea are distributed across various marine habitats with a wide variety of larval forms, all of them appear to share a number of common features that distinguish them from other brachyuran superfamilies. These common features include a larval development characterised by two zoeal stages and one megalopal stage. The first zoeal stage has one pair of anterior setae on the ventral margin of carapace, and nine or more plumose marginal setae on the scaphognatite of the maxilla. In the second zoeal stage, pleopods are present as well-developed buds (Clark *et al.* 1998; Rice 1980). Megalopa lack sensory setae on the dactylus of the fifth pereiopod, and if uropods are present, the number of

setae is no more than eight (Rice 1988). Based on this study, *C. retusa* shares all these features.

The decorator crab *C. retusa* belongs to the majoid family Inachidae MacLeay, 1838, which includes 37 genera and 206 species (Ng *et al.* 2008). Larval morphology has been used to study the phylogeny and familial relationships of crabs from majoid families Inachidae, Inachoididae Dana, 1851, Majidae Samouelle, 1819, and Pisidae Dana, 1851 (Clark & Webber 1991; Marques & Pohle 2003; Pohle & Marques 2000; Rice 1980), however, the complete descriptions of larval development and morphology are limited for family Inachidae, and mostly are for species from genus *Inachus* Weber, 1795 (Clark 1983; Guerao *et al.* 2002; Ingle 1992). Therefore, it is probably still too early to define larval features that characterise this family.

While the larval development and morphology of *C. retusa* share the general features of Majoidea, in comparison to other species in family Inachidae, *C. retusa* shows the following major differences: the Z_1 stage: 1) five setae present on the ventral margin of the carapace in addition to the anterior setae; 2) the exopod of the antennule presents 3 aesthetascs and no seta; 3) the coxal endite of maxillule; 4) the setation of the endopod and scaphognathite of the maxilla; 5) the coxa of maxilliped I; and 6) the setation on the first somite of the pleon (Table 2.1). On the other hand, the similarities of *C. retusa* Z_1 larvae to the other Inachidae species include: 1) the setation on exopod of antenna; 2) the basis and endopod of maxilliped I; 3) the basis of maxilliped II; and 4) the cleft of telson (Table 2.1).

For the second zoeal (Z_2) stage, *C. retusa* differs from the other described species of family Inachidae in the following ways: 1) the terminal armature of the antennule exopod presents as 8 aesthetascs and no seta; and 2) the number of plumose marginal setae on the scaphognathite of maxilla (Table 2.2). Thus far, it appears that only the setal formula of the basis and the endopod of maxilliped I are consistent for the Z_2 stage in all Inachidae species that larval morphology have been described (Table 2.2).

Table 2.1. Comparison of major larval morphology of the first zoeal larva of species from the family Inachidae.

Species	***Platymaia wyvillethomsoni***	***Macrocheira kaempferi***	***Macropodia czernjawskii***	***Dorhynchus thomsoni***	***Inachus aguiarii***	***Inachus communissimus***	***C. retusa***
References	Oh and Ko (2012)	Clark and Webber (1991)	Marco-Herrero *et al.* (2012)	Ingle (1992)	Guerao and Abelló (2007)	Guerao and Abelló (2007)	Present study
Carapace							
Rostral sp	+	+	+	+	-	-	+
Lateral sp	-	+	-	+	-	-	+
Dorsal sp	+	+	+	+	+	+	+
Ventral margin (ps)	5	8	3	12	2	1	6
Antennule							
Exo (ae/s, terminal)	3 / 2	5 / 0	4 / 1	4 / 0	4 / 0	4 / 0 – 1	3 / 0
Antenna							
Exo (s)	2 subterminal	2 terminal	2 medial	n/d	2 medial	2 medial	2 medial
Maxillule							
Cox (pds)	7	8	7	7	7	7	6
Endo (pds)	1, 6	1, 6	0, 3	0, 5	0, 4	0, 4	1, 6
Maxilla							
Endo (pds)	5	6	4	6	4	4	8
Sca (ps, marginal)	9	14	9	11	11	11	18–19
Maxilliped I							
Cox (pds)	1	n/d	0	n/d	1	1	0
Bas (pds)	2+2+2+3	2+2+2+3	2+2+2+3	2+2+2+3	2+2+2+3	2+2+2+3	2+2+2+3
Endo (pds)	3, 2, 1, 2, 5	3, 2, 1, 2, 5	3, 2, 1, 2, 5	3, 2, 1, 2, 5	3, 2, 1, 2, 5	3, 2, 1, 2, 5	3, 2, 1, 2, 5
Maxilliped II							

Bas (pds)	1+1+1	1+1+1	1	1	0	0	1+1+1
Endo (pds)	0, 1, 4	1, 1, 6	0, 0, 4	0, 1, 4	0, 1, 4	0, 1, 4	0, 1, 4
Pleon							
Somite I (s)	2	2	0	2	0	0	4
Telson							
Furcae surface	spinulated	smooth	spinulated	smooth*	spinulated	spinulated	spinulated
Cleft (pairs)	3ss	3ss	3ss	3ss	3ss	3ss	3ss

Abbreviations: Sca: scaphognathite; cox: coxa or coxal endite; bas: basis or basial endite; exo: exopod; endo: endopod; seg: segments; sp: spine(s); s: simple setae; ps: plumose setae; pds: plumodenticulate setae; ss: serrated setae; ae: aesthetascs; n/d: not described; (*): based on the figure in Ingle (1992).

Table 2.2. Comparison of major larval morphology of the second zoeal larva of species from the family Inachidae.

Species	***Platymaia wyvillethomsoni***	***Macropodia czernjawskii***	***Macropodia rostrata***	***Dorhynchus thomsoni***	***Inachus dorsettensis***	***Inachus thoracicus***	***C. retusa***
References	Oh and Ko (2012)	Marco-Herrero *et al.* (2012)	Ingle (1992)	Ingle (1992)	Ingle (1992)	Guerao *et al.* (2002)	Present study
Carapace							
Ventral margin(s)	8	3	n/d	n/d	n/d	2*	7
Antennule							
Exo terminal (ae/s)	6 /1	6 / 1	8 / 0	n/d	9 / 0	7 / 0	8 / 0
Maxillule							
Cox (pds)	8	7	9	9	9	7	7
Endo (pds)	1, 2+4	0, 3	n/d	n/d	n/d	0, 4	1, 2+4
Maxilla							
Endo (pds)	5	4	n/d	n/d	n/d	4	7
Sca (ps, marginal)	19	18	16	15	19	18	32–34
Maxilliped I							
Bas (pds)	2+2+2+3	2+2+2+3	n/d	n/d	n/d	2+2+2+3	2+2+2+3
Endo (pds)	3, 2, 1, 2, 5	3, 2, 1, 2, 5	n/d	n/d	n/d	3, 2, 1, 1, 5	3, 2, 1, 1, 5
Maxilliped II							
Bas (pds)	3	0	n/d	n/d	n/d	0	3
Endo (pds)	0, 1, 4	0, 0, 4	n/d	n/d	n/d	0, 1, 4	0, 1, 4
Pleon							
Number of somites	6 2	5 0	5 0*	5 n/d	6 0*	5 0*	6 5

Somite I (s)							
Telson							
cleft (pairs)	1 ps + 3 ss	n/d	3ss*	3s*	3ss*	3s*	4

Abbreviations: Sca: scaphognathite; cox: coxa or coxal endite; bas: basis or basial endite; exo: exopod; endo: endopod; seg: segments; sp: spine(s); s: simple setae; ps: plumose setae; pds: plumodenticulate setae; ss: serrated setae; ae: aesthetascs; n/d: not described; (*): based on the figure in Ingle (1992).

Table 2.3. Comparison of major larval morphology of the megalopal larva of species from the family Inachidae.

Species	***Macropodia rostrata***	***Macropodia czernjawskii***	***Dorhynchus thomsoni***	***Inachus dorettensis***	***Inachus thoracicus***	***Camposcia retusa***
References	Ingle (1992)	Marco-Herrero *et al.* (2012)	Ingle (1992)	Ingle (1992)	Guerao *et al.* (2002)	Present study
Antennule						
Exo setation (ae/s)	2, 4/0, 0	1, 4/0, 0	2, 4/0, 0	1, 4/0, 0	1, 4/0, 0	3–4, 4/0, 1
Antenna						
Protopod (s)	1, 0, 1	1, 0, 1	1, 0, 1	1, 0, 1	0, 0, 1	1, 2, 4
Maxillule						
Cox (pds)	7	7	7	7	7	8
Bas (pds)	14	13	14	14	13	18
Maxilla						
Sca (ps, marginal)	19	18 – 20	35	22	20	33–35
Maxilliped III						
Epipod (pds)	n/d	1	n/d	2	2	5
Pereiopod I						
Merus sp	2	1	several	3	3	0
Thoracic sternum						
sp	2	n/d	3	4	n/d	0
s	n/d	n/d	1	n/d	n/d	10
Pleon						
Number of somites	5	5	5	6	5	6
Uropod	absent	present	present	absent	absent	present

Abbreviations: Sca: scaphognathite; cox: coxa or coxal endite; bas: basis or basial endite; exo: exopod; endo: endopod; seg: segments; sp: spine(s); s: simple setae; ps: plumose setae; pds: plumodenticulate setae; ss: serrated setae; ae: aesthetascs; n/d: not described.

For inachid megalopae, the diagnostic features include: 1) the exopod setation of the antennule; 2) the setal formula of antennal protopod; 3) the setation of the coxal and basial endites of the maxillule; and 4) the number of plumose marginal setae on the scaphognathite of the maxilla (Table 2.3). Finally, it is worth noting that according to Clark *et al.* (1998) the number of cincinnuli should be constant for all pleopods in inachid megalopae. However, a variation of cincinnuli number on the endopod of pleopods, either 2 or 3, was observed in this study for *C. retusa*.

2.5.2 Comparison with a previous description of larval morphology

Camposcia retusa was previously described by Gohar & Al-Kholy (1957). These authors described the prezoea, 2 zoeal stages, and the megalopa. The prezoeal stage has been observed in several brachyuran species (Guerao & Abelló 1996; Hong 1988b). However, in more recent studies, the prezoeal stage is considered as the last embryonic rather than the first larval stage due to the lack of functional appendages or other larval organs (Anger 2001; Clark *et al.* 1998; Williamson 1982). Therefore, the present study does not include the description of the prezoeal stage.

Table 2.4 summarises the differences of larval morphology described by Gohar & Al-Kholy (1957) and the present study for *C. retusa*. The description by Gohar & Al-Kholy (1957) differs greatly from the present study, particularly in terms of larval size, segmentation development and setation of appendages. Larval sizes of all stages in Gohar & Al-Kholy (1957) were smaller than the present study and no standard deviation was provided. More importantly, we observed that the ventral margin of the carapace of all zoeal larvae had one plumose ‘anterior seta’; however, this was not described by Gohar & Al-Kholy (1957). As pointed out by Rice (1980) and Clark *et al.* (1998), the appearance of ventral marginal setae on carapace is one of the key characters of majid crab zoeal larvae; therefore, it is worth of noting here.

Table 2.4. Comparison of morphology of larval stages of *Camposcia retusa* described by Gohar & Al-Kholy (1957) and the present study.

Larval morphology	Gohar & Al-Kholy 1957	This study
Zoea I		
Size		
CL	n/d	1.18 ± 0.09 mm
RDL	1.5 mm	1.94 ± 0.10 mm
Carapace		
Ventral margin (ps)	0*	6 ps
Antennule		
Exo (setation)	5	3 ae
Maxillule		
Cox (setation)	7	6 pds
Bas (setation)	10	7 pds
Maxilla		
Cox (setation)	7	9 pds
Bas (setation)	8	9 pds
Endo (setation)	6	8 pds
Sca (setation)	15–20	18–19 ps
Maxilliped I		
Endo (setation)	0, 1, 1, 2, 4	3, 2, 1, 2, 5 (all pds)
Maxilliped II		
Endo (seg/set)	0/4	3/0, 1, 4 (all pds)
Pleon		
Somites/set	5/0	5/4, 2, 2, 2, 2 (all s)
Telson		
Cleft (pairs)	3 ps	3 ss
Zoea II		
Size		
CL	n/d	1.42 ± 0.05 mm
RDL	1.6 mm	2.24 ± 0.06 mm
Carapace		
Ventral margin (ps)	0*	7 ps
Antennule		
Exo (setation)	9	8 ae
Antenna		
Exo (setation)	0	2 ps
Mandible		

Palp (seg/set)	2/0	0/0
Maxillule		
Endo (setation)	0, 8	1, 6 (all pds)
Maxilla		
Bas (setation)	7	9 pds
Endo (setation)	5	8 pds
Sca (setation)	More setae than Zoae I	33–35 pds
Maxilliped I		
Endo (setation)	0, 1, 1, 1, 3	3, 2, 1, 2, 5 (all pld)
Exo (seg/set)	2/0, 6 ps	partly bi-segmented/6 ps
Maxilliped II		
Endo (seg/set)	0/2	3/0, 1, 4 (all pds)
Exo (seg/set)	2/0, 6 (ps)	partly bi-segmented/6 ps
Pleon		
Somites/set	6/0	6/5, 4, 4, 2, 2, 0
Megalopa		
Size		
CL	1.13 mm	1.45 ± 0.15 mm
CW	0.27 mm	0.95 ± 0.09 mm
CL/CW	4.19	1.54 ± 0.23
Antennule		
Exo setation (ae/s)	n/d	3–4, 4/0, 1
Antenna		
Endo (seg/set)	6/0, 0, 0, 0, 0, 3	7/1, 2, 4, 0, 0, 5, 4 (all s)
Mandible		
Palp (seg/set)	3/0, 0, 2	partly bi-segmented/6 pds
Symmetry	n/d	asymmetrical
Maxillule		
Cox (setation)	3	8 pds
Bas (setation)	10	16 pds
Maxilla		
Bas (setation)	10	12 pds
Sca (setation)	More setae than Zoea II	33–35 ps + 2 pds on blade
Maxilliped I		
Cox + bas (setation)	Setose	16 pds
Endo (seg/set)	2/0	0/0
Exo (seg/set)	2/0, 3 ps	2/0, 4 ps
Maxilliped II		
Endo (setation)	0, 0, 2, 2**	0, 1, 2, 5 (all pds)

Exo (seg/set)	4/0, 0, 0, 4*	2/0, 4 ps
Maxilliped III		
Cox + bas (setation)	n/d	10 pds
Endo (setation)	(10–15, 0, 0, 1, 6) *	15, 7, 5, 6, 5 (all pds)
Exo (setation)	0, 3 ps	0, 4 pds
Pleon		
Somites/set	6/0	6/4, 6, 6, 6, 6, 2 (all s)
Pleopods		
1st (cin/ps)	9	3–4/13–15
2nd (cin/ps)	9	3–4/13
3rd (cin/ps)	9	3–4/12–13
4th (cin/ps)	5	3–4/10–11
Uropod		
Setation	n/d	6 ps
Telson		
Setation	5 pairs, plumose	1 pair, simple setae

Abbreviations: Sca: scaphognathite; cox: coxa; bas: basis; exo: exopod; endo: endopod; seg: segments; set: setation; s: simple setae; ps: plumose setae; pds: plumodenticulate setae; ss: serrated setae; ae: aesthetascs; cin: cincinnuli; n/d: not described; (*): based on the figures by Gohar & Al-Kholy (1957).

Aside from differences mentioned above, the segmentation and setation of the antennule, maxillule, maxilla, the first and second maxillipeds, and pleon of the Z_1 illustrated by Gohar & Al-Kholy (1957) also differ from the present study (Table 2.4). For instance, the endopod of the second maxilliped was described as unsegmented by Gohar & Al-Kholy (1957); however, 3 segments were observed in the present study. Since endopod segmentation is common for the second maxilliped in brachyuran zoeae, and 3 segments is the usual number for Inachidae (Clark *et al.* 1998; Clark & Webber 1991; Guerao & Abelló 2007; Ingle 1992; Oh & Ko 2012), the description by Gohar & Al-Kholy (1957) is likely incorrect. In general, the differences in the morphology of Z_1 larvae were relatively minor and mostly related to fine structures (e.g., marginal setae on the ventral margin of the carapace), which may be a result of technical limitations of the time, such as the lower resolution of the microscope.

For the Z_2 and megalopa, major differences were found in various prominent morphological features (Table 2.4). For instance, for the Z_2 larvae, the palp of the mandible and the endopod of the second maxilliped were observed to have 0 and 3 segments, respectively, in the present study, but Gohar & Al-Kholy (1957) reported 2 and 0 segments, respectively, instead. For megalopal larvae, the carapace shape described by Gohar & Al-Kholy (1957) was also notably different from the present study; indeed, the carapace was much narrower based on the description of Gohar & Al-Kholy (1957), which has a CL/CW ratio of 4.19 as compared to only 1.54 ± 0.23 in the present study. In addition, Gohar & Al-Kholy (1957) reported no armature other than the narrow sutures on the dorsal surface of the carapace; however, we observed the dorsal surface of carapace covered by many simple setae with a pair of processes present. Moreover, Gohar & Al-Kholy (1957) described the mandibular palp as a 3-segmented structure with 0, 0, 2 setae on each segment, while in the present study, the same structure presents as partly bi-segmented bearing 6 plumodenticulate setae. Although the small size and strong muscular attachments make mandibles one of the most difficult structures to examine (Clark *et al.* 1998), these differences in segmentation and setation of mandibular palps are unlikely due to technical limitation. Furthermore, we observed that the 1st to 4th pleopods bear 3–4 cincinnuli on their endopods, while their

exopods have 10–15 long plumose setae. Gohar & Al-Kholy (1957) did not report any cincinnuli on the endopods but observed 9 and 5 setae on the exopods of 1st–3rd and 4th pleopods, respectively. In fact, a recent study on the larval morphology of another majid crab, *Menaethius monoceros* (Latreille, 1825) by Colavite *et al.* (2014) also questioned the description by Gohar & Al-Kholy (1957) of this species. It was suggested that it is highly possible that the larval specimens collected from plankton and used by Gohar & Al-Kholy (1957) for morphological description do not represent *M. monoceros* (Colavite *et al.* 2014). Based on substantial differences in morphology of the Z_2 and megalopal stages between the present study and that of Gohar & Al-Kholy (1957), and the fact that the latter also relied on specimens collected from plankton for these two larval stages description, we similarly have our doubts on whether the larvae used by them were those of *C. retusa*.

2.5.3 First juvenile stage

The present study describes the C_1 stage of *C. retusa* for the first time, since this stage was not described by Gohar & Al-Kholy (1957) as they were unable to obtain specimens. Indeed, the description of the C_1 stage of species belonging to family Inachidae is scarce, so far only two species have been described and both of them are from genus *Inachus*, i.e., *Inachus dorsettensis* Pennant, 1777 (viz. Ingle 1977) and *Inachus thoracicus* Roux, 1830 (viz. Guerao *et al.* 2002) (Table 2.5). The most prominent character for the C_1 of *C. retusa* is the presence of numerous long curved simple setae and hooked setae on the carapace, pereiopods, and pleon, which allow the attachment of sponge, seaweed, corals, gravel and other items for decorating (camouflage). This character was also observed in some other majoid crabs, such as *Pyromaia tuberculata* Lockington, 1877 (viz. Luppi & Spivak 2003), *Lissa chiragra* Fabricius, 1775 (viz. Guerao *et al.* 2003), *Pisa tetraodon* Pennant, 1777 (viz. Rodríguez 1997), and *Microphrys bicornutus* Latreille, 1825 (viz. Gore *et al.* 1982). In fact, many species in superfamily Majoidea are known as ‘decorators’, showing distinctive decorating or masking behaviour, by which they attach selected materials collected from the environment to

specialised hooked setae on their body (Hultgren & Stachowicz 2011; Woods & McLay 1994). Nevertheless, adult *C. retusa* are generally more prominent than juveniles in their decoration capacity and have been found with heavy decoration in the wild (Hultgren & Stachowicz 2011). The presence of hooked setae in the first crab stage of *C. retusa* suggests that the acquisition of behaviour associated with camouflage occurs as early as when the larvae settle as first-stage crabs.

In summary, *C. retusa* shares a range of general features of its larval development and morphology with other majoid crabs. The larvae of *C. retusa* can easily be recognised by the setation on the exopod of the antennule and the number of marginal setae on the scaphognathite of the maxilla. For C_1 crabs, however, it is too early to come up with reliable diagnostic characters for species identification within family Inachidae since morphology has only been described for two other species, both from genus *Inachus*. In the present study, the most obvious character observed for C_1 crabs is the abundance of long curved setae and hooked setae covering the carapace, pereiopods and pleon, a morphological character associated with the decorating/camouflage behaviour of the species.

Table 2.5. Comparison of major first crab morphology of species from the family Inachidae.

Species	***Inachus dorsettensis***	***Inachus thoracicus***	***Camposcia retusa***
References	Ingle (1977)	Guerao *et al.* (2002)	Present study
Antennule			
Exo (ae/s)	0, 0, 5/0, 0, 1	0, 1, 4/0, 1, 1	4, 3/0, 1
Maxillule			
Bas (s)	12–13	14	14
Maxilla			
Cox(pds)	5	3	8
Sca (ps, marginal)	22	27–28	41
Maxilliped II			
Endo (pds)	0, 1, 4, 6	2, 1, 5–6, 6	0, 1, 1, 5, 6
Maxilliped III			
Merus sp	1	2	0
Carpus sp	0	1	0
Propodus sp	0	1	0
Pereiopods			
Dactylus P4 sp	0	present	0
Pleon			
Number of somites	5	5	6

Abbreviations: Sca: scaphognathite; cox: coxa or coxal endite; bas: basis or basial endite; exo: exopod; endo: endopod; seg: segments; s: simple setae; ps: plumose setae; pds: plumodenticulate setae; ae: aesthetascs.

Chapter 3. Establishing larval feeding regimens for the decorator crab *Camposcia retusa*: Effects of live prey types and density on larval survival and development

3.1 Abstract

To establish larval feeding regime for the decorator crab *Camposcia retusa*, the two traditional preys, rotifers and *Artemia,* were evaluated for their suitability as larval prey. In the 1st experiment, ss-type rotifers *Brachionus rotundiformis* were fed to newly hatched larvae (Zoea 1 or Z_1) at various densities of 0 (control), 30, 60 and 90 ind./ml. All treatments, including the unfed control, had > 60% larvae survived to the 2nd zoeal stage (Z_2) with no significant difference detected among treatments, suggesting the existence of lecithotrophy in *C. retusa* Z_1 larvae. Despite high survival to Z_2, in all treatments, no Z_2 larvae moulted to megalopae, demonstrating that rotifer is not a suitable feed for *C. retusa* larvae. The 2nd experiment hence examined the suitability and optimal feeding density of *Artemia,* as well as effects of co-feeding copepods with *Artemia* on *C. retusa* larval rearing. Five treatments of *Artemia* nauplii provided at 0 (control), 5, 10, and 15 ind./ml, and a copepod *Pavocalanus crassirostris* co-fed with *Artemia* (5 ind./ml each) were set up. Orange guts observed in larvae fed *Artemia* and high Z_1 survival of the unfed larvae confirm that *C. retusa* Z_1 larvae display facultative lecithotrophy. Larvae fed 10 *Artemia*/ml treatment had the highest survival to the megalopal stage (91.3 ± 3.1%), which was significantly higher than all other treatments ($p < 0.05$). However, mass mortality occurred during megalopal stage in all treatments, resulting in poor and no significant survival to the 1st crab (C_1) stage (1.3–12.5%) among treatments. The shortest zoeal developmental duration was found in the 15 *Artemia*/ml treatment, which was significantly shorter than that of 5 *Artemia*/ml treatment but not significantly different from that of 10 *Artemia*/ml treatment. Co-feeding copepods and *Artemia* not only did not improve larval performance but resulted in significant inferior survival and development to megalopae than those of 10 *Artemia*/ml treatment. The 3rd experiment evaluated the effect of *Artemia* enrichment by feeding larvae either newly hatched *Artemia* nauplii or enriched metanauplii at 5 and 10 ind./ml, respectively. Larvae fed

newly hatched *Artemia* at 10 ind./ml displayed a significantly higher survival than other treatments ($p < 0.05$), while no significant difference was found in larval development. Therefore, *Artemia* enrichment did not appear to improve larval survival or development. Based on the results of the present study, a simple feeding regime of *Artemia* nauplii provided at 10 ind./ml throughout larval development appears to be sufficient for *C. retusa* larval culture.

3.2 Introduction

Establishing an optimal feeding regime is a fundamental step in the development of larval culture techniques for aquaculture species, including crabs (Daly *et al.* 2009; Ruscoe *et al.* 2004). The type of live prey and their feeding density are two key criteria in the crustacean larval culture (Tsuji *et al.* 2015). Rotifers (*Brachionus* spp.) and *Artemia* nauplii are two traditional live prey that are routinely used for brachyuran crab larval rearing (Dan *et al.* 2016a; Holme *et al.* 2009; Oliver *et al.* 2017; Ruscoe *et al.* 2004; Støttrup & McEvoy 2003). The advantages of using these traditional preys include their suitable sizes for the larvae, high productivity, relatively easy and cost-effective to mass produce (Anderson & De Silva 2011; Ferreira *et al.* 2008; Navarro *et al.* 1999; Yoshimura *et al.* 2003). However, rotifers and *Artemia* are both known to lack certain essential nutrients, especially highly unsaturated fatty acids (HUFA), which are crucial for normal larval growth and development (Støttrup & McEvoy 2003; Suprayudi *et al.* 2004b). As the result, various ways of enrichment to improve these live prey nutritional values are commonly practiced in hatcheries, taking advantage of their filter-feeding feature (Ferreira *et al.* 2008; Figueiredo *et al.* 2009; Støttrup & McEvoy 2003).

Artemia enrichment has routinely been reported to significantly improve larval performance in various fish and crustacean species, including crabs (e.g.:Avella *et al.* 2007; Beder *et al.* 2018; Jinbo *et al.* 2013; Suprayudi *et al.* 2004a; Suprayudi *et al.* 2004b). For instance, *Artemia* enrichment was reported to significantly improve larval survival of the mud

crab *Scylla serrata* (Suprayudi *et al.* 2004b), and accelerated the development of the horsehair crab *Erimacrus isenbeckii* larvae (Jinbo *et al.* 2013). Similarly, in the swimming crab *Portunus trituberculatus*, it was reported that larvae fed *Artemia* nauplii without enrichment resulted in the occurrence of morphologically immature megalopae (retain zoeal features, such as dorsal spin and telson folk), leading to mass mortality during metamorphosis (Dan *et al.* 2016b; Dan *et al.* 2016c). Therefore, enriching *Artemia* is generally recommended in hatchery practices for marine crabs (Figueiredo *et al.* 2009; Suprayudi *et al.* 2004b). However, despite *Artemia* enrichment having become a routine practice in crab hatcheries, there are also reports demonstrating the ineffectiveness of *Artemia* enrichment for crab larval rearing. For example, fatty acid enrichment of *Artemia* reportedly did not significantly improve larval survival in various edible crab species, including the common spider crab *Maja brachydactyla* (Andrés *et al.* 2007), the blue swimmer crab *Portunus armatus* (Basford *et al.* 2021), and megalopae of the mud crab *S. serrata* (Williams *et al.* 1999). Similarly, in marine ornamental crabs *Mithraculus sculptus* and *Mithraculus forceps*, *Artemia* enrichment reportedly failed to improve larval survival, growth and development (Rhyne *et al.* 2005). Furthermore, excess HUFA intake was identified as a cause that induced moulting death syndrome (MDS) in the larval culture of several portunid crabs, including *Scylla paramamosain*, *P. trituberculatus* and *S. serrata* (Dan *et al.* 2016c; Hamasaki *et al.* 2002a; Hamasaki *et al.* 2002b).

As natural diets for most marine fish and crustacean larvae, copepods have received increasing attention as the live feed for marine larval rearing in recent years (Fileman *et al.* 2014). Compared with rotifers and *Artemia*, copepods are superior in nutritional value (high protein, rich in HUFA, phospholipids and other micronutrients, e.g., taurine, astaxanthin and zinc) and digestibility (Ajiboye *et al.* 2011; Corner & O'Hara 1986; Dhont *et al.* 2013; Støttrup & McEvoy 2003), hence have a high potential as live prey for marine larval rearing; however, due to the difficulty in intensive culture of copepods, the utilisation of copepods in hatcheries is still relatively limited (Drillet *et al.* 2011; van der Meeren *et al.* 2014). In fact, previous studies on the use of copepods for larval culture were mainly carried out for fish species, which generally obtained very positive results (Barroso *et al.* 2013; Payne &

Rippingale 2000; Zeng *et al.* 2018). However, similar studies for crustacean are very few (Farhadian *et al.* 2009; Tang *et al.* 2020; Waiho *et al.* 2018), which probably is explained by to the fact of crustacean larvae generally being successfully cultured with rotifers and *Artemia* alone. Of these limited studies, some have shown the positive effects of using copepods. For example, the survival and growth rate of black tiger shrimp *Penaeus monodon* postlarvae were significantly higher when a co-feeding regime of cyclopoid copepod *Apocyclops dengizicus* and *Artemia* nauplii was applied (Farhadian *et al.* 2009). Similarly, the megalopae of the Chinese mitten crab *Eriocheir sinensis* showed a significantly higher metamorphosis rate when fed with frozen copepods (*Centropages dorsispinatus* as the major species) (Tang *et al.* 2020). It is therefore interesting to evaluate if co-feeding copepods might improve crab larval rearing success.

The decorator crab *Camposcia retusa* is a popular marine ornamental crab species but has not been previously reported to be bred in captivity (Calado *et al.* 2003a). Only very recently, a complete larval development of *C. retusa* was described by the present authors based on laboratory reared material, which includes two zoeal larval stages and a megalopal stage (Xu *et al.* 2019). Currently, all individuals traded in the global marine aquarium market are sourced from the wild, which can have negative impacts on wild populations. Therefore, the objective of the present study was to establish a reliable larval feeding regime for *C. retusa* with a series of experiments that investigated: 1) the suitability of rotifers as prey for newly hatched larvae of *C. retusa*; 2) the suitability of newly hatched *Artemia* nauplii as prey for *C. retusa* larvae and its optimal feeding density; 3) the effects of calanoid copepods as a supplemental larval diet to *Artemia* on larval performance; and 4) the effects of *Artemia* enrichment on *C. retusa* larvae.

3.3 Materials and Methods

3.3.1 Broodstock maintenance

Broodstocks of the decorator crab *C. retusa* were obtained from Cairns Marine (Cairns Marine Pty Ltd; Queensland, Australia), a commercial marine ornamental collector and whole seller. After being air-freighted to James Cook University, Townsville, the crabs were kept in pairs in separate 50 L tanks connected to a recirculation unit (renewal rate = 90 L/h). Half terracotta pots and rocks were placed in the broodstock tanks as shelters. All crabs were fed *ad libitum* with chopped prawn and mussels, as well as thawed blood worms twice daily. Throughout the experiments, seawater in the broodstock tanks were maintained at temperature 26–28 °C, salinity 34–36, photoperiod L: D = 14 h:10 h, pH = 7.9–8.2, NH_4^+/NH_3 and NO_2^- < 0.25 ppm and NO_3^- < 5.0 ppm.

3.3.2 Live prey culture and preparation

Three different live prey were used in the present study: ss-type rotifer *Brachionus rotundiformis*, Brine shrimp *Artemia* sp. (INVE Thailand, Amphoe Wachiabarami, Thailand), and calanoid copepod *Pavocalanus crassirostris*.

The rotifer, *B. rotundiformis*, were cultured in 100 L conical tanks and fed a commercial concentrated microalgal *Nannochloropsis* paste (RotiGrow® Nanno, Reed Mariculture, USA) daily. The culture temperature was maintained 26–27 °C and the salinity 27–33. Rotifers were harvested daily with density estimated by averaging three 1 ml samples taken from the harvested rotifer stock.

Artemia (INVE Thailand, Amphoe Wachiabarami, Thailand) were hatched and harvested daily. During the hatching period (around 23h), temperature and salinity was maintained at 26–28 °C and 34–35, respectively. Newly hatched *Artemia* nauplii were collected the next morning for either feeding larvae immediately or subsequent enrichment. A commercial emulsion (Selco S.presso®, INVE Aquaculture, UT, USA) was used to enrich *Artemia* nauplii manufacturer's instructions. The enrichment procedure followed the manufacturer's instruction, including the technical card on enrichment protocol (TC-EN-SPRESSO-190509)

by the producer. Briefly, the S.presso was firstly emulsified in freshwater for 3 min, it was then added at a dosage of 0.5 g per litre each time in two times with an Artemia density up to 400 nauplii per ml for enrichment. The enrichment lasted for a period between 18–22 h. Density estimation of *Artemia* was similarly done as for rotifers.

Copepods, *P. crassirostris*, were cultured in a series of 250 L conical tanks using the methods established by our laboratory (Alajmi & Zeng 2014; Alajmi *et al.* 2015). Briefly, the copepods were fed a 1:1 mixed algal diet of *Isochrysis* sp. and *Chaetoceros muelleri* daily and were harvested also daily during Experiment 2 to feed the crab larvae. During harvesting, the culture water was firstly drained through a 150 μm mesh and then through a 25 μm mesh. The 150 μm mesh retained mostly copepodites and adults while the 25 μm mesh collected nauplii. Only copepodites and adults were used to feed crab larvae, while the nauplii collected on 25 μm mesh were returned to the culture tanks. The estimation of copepod density was performed as above mentioned for rotifers.

3.3.3 Larval feeding experiments

3.3.3.1 General procedure

Under the above-mentioned culture conditions, egg incubation generally took 21–25 days. A banjo filter was plugged to the outlet of the aquarium before larval hatching. In the morning of the day of hatching, aeration was first stopped, and the room light was turned off. The positively phototactic newly hatched larvae were attracted to an upper corner of the tank by a torch, then gently scooped out with as little water as possible. The day of larval hatching was defined as day 0.

For all larval experiments, larvae were reared in 600 ml glass beakers with seawater filled to 500 ml, which formed a replicate. The initially stocking density for all experiments was 20 newly hatched larvae per beaker. Throughout the experiment, water temperature was maintained at 27 ± 0.5 °C, salinity 35.3 ± 0.5, and photoperiod was set at L: D = 14 h:10 h.

Once megalopae appeared, any newly metamorphosed megalopae found during daily water exchange were transferred to a separate beaker containing identical live prey to prevent cannibalism. Daily 100% water exchange was conducted by moving live larvae using a broad-mouthed pipette to a new beaker containing fresh seawater and identical feed. Meanwhile, the development stage (based on larval stage description in Chapter 2) and the mortality of larvae was observed and recorded. An experiment was terminated when all larvae had either metamorphosed to become juvenile crabs or died.

3.3.3.2 Experimental design

Experiment 1: Rotifer feeding trial

Aimed at evaluating the suitability of rotifer *B. rotundiformis* as prey for *C. retusa* larval rearing, this experiment consisted of four triplicated feeding treatments in which newly hatched larvae (Z_1) were fed rotifers at 1) 0 (unfed control), 2) 30 ind./ml, 3) 60 ind./ml, and 4) 90 ind./ml, respectively.

Experiment 2: *Artemia* nauplii feeding density and copepod co-feeding

As results of Experiment 1 showed that rotifers were not a suitable feed for *C. retusa* larvae, this experiment was hence designed to test the suitability of *Artemia* nauplii as prey for *C. retusa* larvae and its appropriate feeding density; in addition, any positive effects of co-feeding *Artemia* with copepods *P. crassirostris* was also evaluated. The newly hatched larvae (Z_1) of *C. retusa* were subjected to five treatments: *Artemia* nauplii fed to larvae at different densities of 1) 0 (unfed control), 2) 5 ind./ml, 3) 10 ind./ml, and 4) 15 ind./ml; and finally, the co-feeding treatment 5) 5 *Artemia* + 5 copepods/ml. There were four replicates per treatment for this experiment.

Experiment 3: Effects of *Artemia* enrichment

This experiment was carried out to determine if fatty acid enrichment of *Artemia* improved larval performance. In this experiment, five treatments were set up: unfed control; larvae fed newly hatched *Artemia* nauplii and enriched metanauplii at 5 ind./ml and 10 ind./ml, respectively. Each treatment had four replicates.

3.3.4 Statistical analysis

Data are expressed as mean ± standard error (SE). 'Larval surviving time (LST) of Z_1' and 'LST of Z_2' were calculated for all treatments of the rotifer feeding trial (Experiment 1) and the unfed controls of the other two experiments. LST of Z_1 and LST of Z_2 was defined as the average surviving time (days) from hatching to the death of all Z_1 or Z_2 larvae in a treatment, respectively. LST of Z_1 (Z_2) was calculated using the formula:

$$Larval\ surviving\ time\ (days)\ of\ Z_1\ (Z_2) = \left(\sum_{t=1}^{n} t \times N_t\right) \Big/ \sum_{t=1}^{n} N_t$$

Where N_t is the number of dead Z_1 (Z_2) larvae recorded on day t of an experiment; and n represents the days that dead Z_1 (Z_2) larvae were found.

A Kaplan-Meier test was run to compare the survival trend between the unfed control and different rotifer feeding density treatments in Experiment 1. Differences between survival curves were detected by a pairwise comparison using the log-rank test from the package 'survival' of the R language (Therneau 2020).

Larval survival and average developmental duration of different treatments in Experiment 1 and 2 were analysed using one-way ANOVA followed by Tukey's HSD post hoc test. A two-way ANOVA was performed to analysis data of Experiment 3. The normality of the data and homogeneity of variance were tested prior to the ANOVA analysis. In addition, to compare differences of Z_1 survival between the unfed control and each feeding treatment in Experiment 3, a general linear model was applied. The percentage survival data were transformed using arcsine square root transformation to meet the assumptions of the analysis methods. For all experiments, unfed control was not included for statistical analysis from Z_2 onwards due to no larvae of the controls survived beyond Z_2. A statistical probability of $p <$

0.05 was accepted as significant. All statistics were performed using R language (R 4.0.3, R Core Team, 2020).

3.4 Results

3.4.1 Facultative lecithotrophy of newly hatched larvae and larval quality comparison

Survival of newly hatched Zoea 1 (Z_1) larvae to the next stage (Zoea 2 or Z_2) in the unfed controls of all three experiments was high (≥ 60%), confirming the existence of lecithotrophy in *C. retusa* larvae. When larval survival of unfed controls from the three experiments are compared, Experiment 2 (85.5 ± 2.9%) had significantly higher survival than the other two experiments (65.0 ± 5.0% and 60.0 ± 2.0% for Experiment 1 and 3, respectively; Table 3.1). In addition, although 'Larval surviving time' (LST) of Z_1 larvae from unfed controls of the three experiments were similar (3.2–3.5 days, Table 3.1, $p = 0.771$), LST of Z_2 larvae of the Experiment 2 control was the longest (4.6 ± 0.3 days), and significantly longer than that of Experiment 3 control (3.8 ± 0.1 days, $p = 0.036$). The mean Z_1 duration of the larvae successfully moulted to Z_2 in the three experiments ranged from 1.5 to 1.8 days and the larvae of Experiment 2 again was the fastest in their development (1.5 ± 0.1 days) although no significant difference was detected among three batches of larvae (Table 3.1, $p = 0.483$).

Table 3.1. Comparison of quality of different batches of newly hatched larvae of *Camposcia retusa* from three experiments.

	Mean surviving time of unfed larvae (days)		**Survival to Zoea 2**	**Zoea 1 stage duration (days)**
	Zoea 1	**Zoea 2**		
Exp. 1	3.3 ± 0.4	4.1 ± 0.2^{ab}	65.0 ± 5.0^{b}	1.8 ± 0.2
Exp. 2	3.5 ± 0.3	4.6 ± 0.3^{a}	85.5 ± 2.9^{a}	1.5 ± 0.1
Exp. 3	3.2 ± 0.3	3.8 ± 0.1^{b}	60.0 ± 2.0^{b}	1.7 ± 0.2

Data are presented as mean ± standard error. Different subscript letters within a same column indicate significant differences ($p < 0.05$).

3.4.2 Experiment 1: Rotifer feeding

The daily survival of *C. retusa* larvae under different rotifer density treatments showed a similar trend, and no significant difference was detected among them (Fig. 3.1, Kaplan-Meier test, p = 0.193). Larval survival to Z_2 was relatively high (65.0%–68.3%) in all treatments, including the unfed control, and no significant difference was detected between treatments (Table 3.2, p = 0.502). Likewise, Z_1 duration was similar between treatments (Table 3.2, p = 0.058). Despite high larval survival to Z_2, Z_2 larvae suffered a total mortality in all treatments. The LST of the Z_2 larvae ranged from 3.7 to 4.4 days from different treatments, but no significant difference was detected between any of them (Table 3.2, p = 0.244).

Table 3.2. Survival and development duration of *Camposcia retusa* larvae fed rotifers at different densities.

Rotifer density (ind./ml)	**0**	**30**	**60**	**90**
Survival to Zoea 2 (%)	65.0 ± 5.0	68.3 ± 8.3	56.7 ± 1.7	58.3 ± 6.7
Zoea 1 duration (days)	1.8± 0.2	1.7 ± 0.2	1.8 ± 0.2	1.9 ± 0.1
Survival to megalopa (%)	0	0	0	0
Mean surviving time of Zoea 2 (days)*	4.1 ± 0.2	3.9 ± 0.2	3.7 ± 0.2	4.4 ± 0.3

Data are presented as mean ± standard error. * These surviving times are from hatching to die as Zoea 2 larvae.

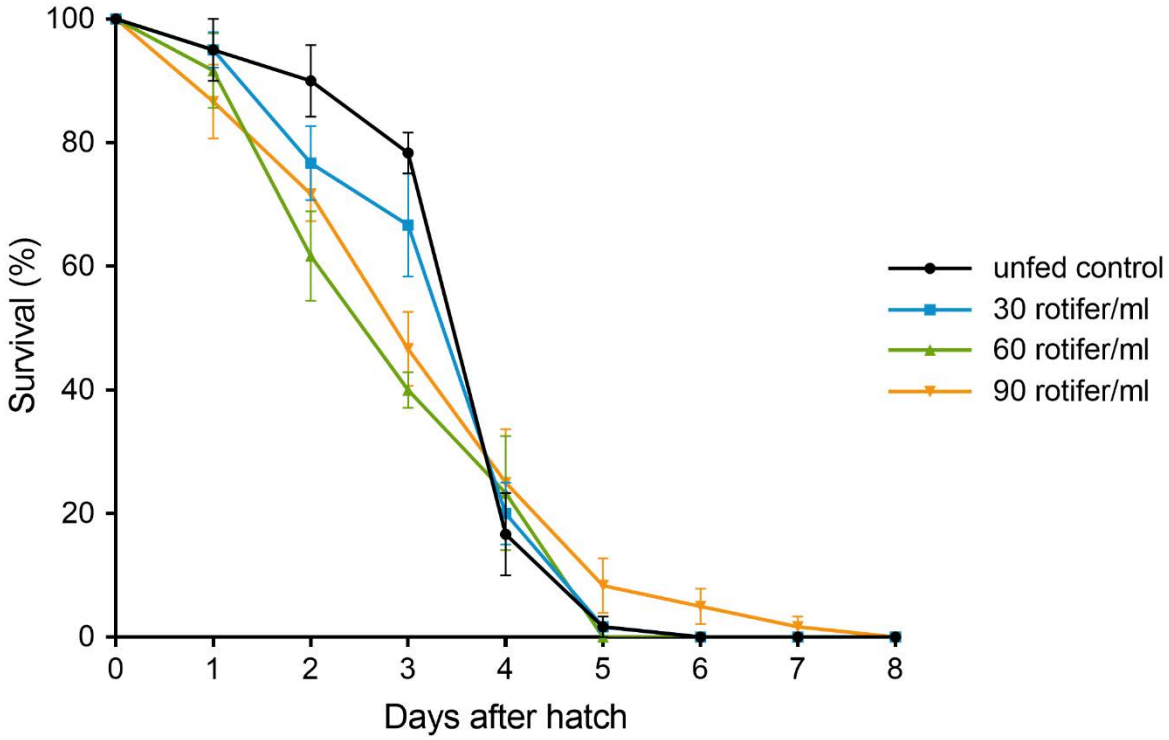

Figure 3.1. Daily larval survival of decorator crab *Camposcia retusa* fed different densities of rotifers. The error bars indicate standard errors.

3.4.3 Experiment 2: Artemia nauplii feeding density and copepod co-feeding trial

Daily larval survival was similar across all treatments during the first two days, however, survival of the unfed control declined rapidly from day 3 onwards with total mortality by day 7, while larval survival of all fed treatments was higher than 20% with the highest survival of 60.0% found the 10 *Artemia*/ml feeding treatment (Fig. 3.2). Indeed, between day 5–10, larvae in the 10 ind./ml *Artemia* feeding treatment showed consistently higher daily survival than all other treatments ($p < 0.05$). However, from day 10 onwards, larval mortality of this treatment also increased and eventually declined to a level only marginally higher than those of other treatments on day 12 when the experiment was terminated ($p > 0.05$; Fig. 3.2 and Table 3.3).

When survival is evaluated as the percentage of larvae successful that moulted to the subsequent larval stages, the survival of Z_1 larvae to the next Z_2 stage was very high (> 80%) across all treatments with the highest survival of 93.8 ± 2.4% being found in the treatment using a density of 10 ind./ml, but no significant difference was detected between treatments, including unfed control (Table 3.3). Z_2 larval survival to the megalopal stage was also high for all treatments (> 70%) except the unfed control, in which total mortality occurred. In the fed treatments, survival of Z_2 larvae in the 10 and 15 *Artemia*/ml feeding treatments were significantly higher than those of the 5 *Artemia*/ml and 5 *Artemia* + 5 copepod/ml treatments ($p < 0.05$; Table 3.3). As the result, the overall zoeal stage survival was high (> 60%) in all fed treatments, the highest survival achieved was again from the treatment in which larvae were fed *Artemia* nauplii at 10 ind./ml (91.3 ± 2.7%), which was significantly higher than all other treatments (Table 3.3). However, despite high zoeal survival, survival of megalopae was poor across all treatments (lower than 20%) and declined particularly sharply when megalopae metamorphosed to juveniles, resulting in a low overall larval survival to the first crab stage (C_1). The best overall larval survival from Z_1 to C_1 was from the 10 *Artemia*/ml feeding treatment (11.3 ± 3.8%), which was not significantly different from that of 15 *Artemia*/ml feeding (7.5 ± 4.8%) and 5 *Artemia* + 5 copepods/ml co-feeding (3.8 ± 2.4%)

treatments, but was significantly higher than 5 *Artemia*/ml treatment (1.3 ± 1.3%). Throughout the experiment, no significant difference was found between the larvae fed 5 *Artemia*/ml and 5 *Artemia* + 5 copepods/ml co-feeding treatment (Table 3.3).

In terms of larval development, Z_1 instar duration was not significantly different among all treatments (Table 3.3). However, for zoeal development time to megalopae (Z_1 to M), the two higher *Artemia* feeding density treatments (10 and 15 ind./ml) were significantly shorter compared to the other two fed treatments (5 *Artemia*/ml and 5 *Artemia* + 5 copepod/ml) ($p < 0.05$; Table 3.3). For the overall larval development duration (Z_1 to C_1), the 5 *Artemia* treatment was excluded from the statistical analysis as only a single larvae survived to C_1. Of the remaining three fed treatments, the overall larval duration was again significantly shorter in the 10 and 15 *Artemia*/ml treatments when compared to that of 5 *Artemia* + 5 copepod/ml treatment (Table 3.3).

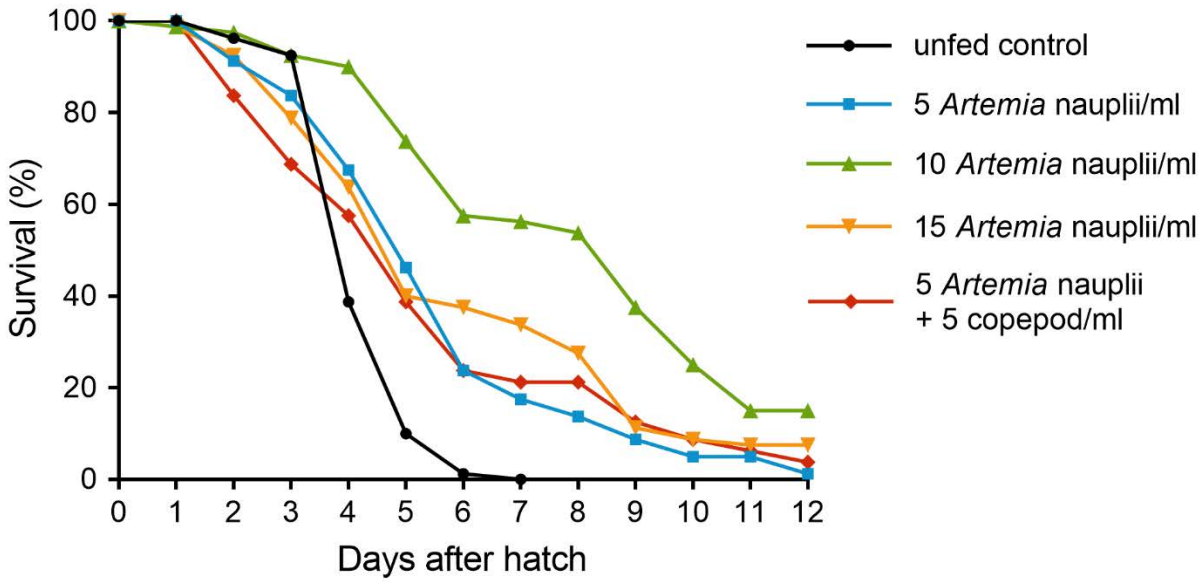

Figure 3.2. Daily larval survival of decorator crab *Camposcia retusa* fed different densities of *Artemia* nauplii and co-fed *Artemia* nauplii with copepods *Pavocalanus crassirostris*. For clarity of the figure, the standard error bars are omitted.

Table 3.3. Survival and development duration of *Camposcia retusa* larvae fed different densities of *Artemia* nauplii and co-fed *Artemia* nauplii with copepods *Pavocalanus crassirostris.*

Treatment		Unfed	5 *Artemia* nauplii/ml	10 *Artemia* nauplii /ml	15 *Artemia* nauplii /ml	5 *Artemia* nauplii + 5 copepods/ml
Survival (%)	**Z_1 to Z_2**	85.5 ± 2.9	88.8 ± 1.3	93.8 ± 2.4	87.5 ± 1.4	87.5 ± 3.2
	Z_2 to M	0	71.8 ± 2.3^{a}	97.4 ± 2.6^{b}	85.9 ± 4.9^{b}	70.2 ± 2.5^{a}
	Z_1 to M	0	63.8 ± 2.4^{bc}	91.3 ± 3.1^{a}	75.0 ± 3.5^{b}	61.3 ± 1.3^{c}
	M to C_1	–	2.1 ± 2.1^{a}	12.7 ± 4.5^{b}	10.1 ± 6.8^{ab}	5.9 ± 3.7^{ab}
	Z_1 to C_1	0	1.3 ± 1.3^{a}	11.3 ± 3.8^{b}	7.5 ± 4.8^{ab}	3.8 ± 2.4^{ab}
Development duration (days)	**Z_1 to Z_2**	1.5 ± 0.2	1.2 ± 0.0	1.4 ± 0.1	1.3 ± 0.0	1.3 ± 0.1
	Z_1 to M	–	4.3 ± 0.1^{a}	3.8 ± 0.1^{b}	3.5 ± 0.1^{b}	4.7 ± 0.1^{c}
	Z_1 to C_1	–	11*	8.8 ± 0.3^{a}	8.3 ± 0.3^{a}	10.3 ± 0.3^{b}

Data are presented as mean ± standard error. Z_1: first stage zoea; Z_2: second stage zoea; M: megalopa; C_1: first stage juvenile. Means with different superscript letters within a same row indicate significant differences (one-way ANOVA; $p < 0.05$).

* Statistical analysis did not include 5 *Artemia*/ml treatment due to only one larva survived to C_1 of the treatment.

3.4.4 Experiment 3: Effects of Artemia enrichment

Daily larval survival showed a very similar pattern during the first 3 days for all treatments, then as in previous experiments, survival in the unfed control plummeted and a 100% mortality occurred on day 6 (Fig. 3.3). Of the fed treatments, between day 4 and 6, larval survival dropped sharply in all treatments except the treatment in which *Artemia* nauplii were fed at 10 ind./ml, resulting in significantly higher survival of that treatment when compared to all other treatments between day 4 and 9 ($p < 0.05$). However, larval mortality of the 10 *Artemia*/ml subsequently also increased and at the end of the experiment, larval survival was not significantly different among all treatments (Fig. 3.3).

In terms of larval survival on an instar basis, survival of Z_1 larvae of the unfed control was the lowest but was not significantly different compared with *Artemia* feeding treatments (Fig. 3.4A). Similar to the results in Experiment 2, among fed treatments, the best survival to Z_2 stage was found in treatments in which larvae were fed *Artemia* at 10 ind./ml, either as unenriched nauplii (76.3 ± 2.4%) or enriched metanauplii (72.5 ± 4.8%; Fig. 3.5A), although the difference was not significant compared with lower *Artemia* feeding density treatments at 5 ind./ml ($F_{1,12} = 0.179$; $p = 0.680$). However, from Z_2 onward, significant differences in larval survival started to emerge: highly significant effects of both *Artemia* density and enrichment on Z_2 larval survival were detected ($p < 0.01$) although no significant interaction of the two was found (Table 3.4). The unenriched *Artemia* nauplii fed at 10 ind./ml yielded the highest Z_2 survival (65.1 ± 6.3%), as well as the overall zoeal survival (i.e. Z_1 to megalopae; 50.0 ± 6.1%), with both survival values being significantly higher than those other treatments (Fig. 3.5 B & C). However, similar to Experiment 2, high mortality occurred during the megalopal stage in all treatments, resulting in the highest survival to C_1 only being at 7.5 ± 2.5% when larvae were fed 10 ind./ml unenriched *Artemia* treatment; two-way ANOVA detected no significant difference in survival to C_1 related to *Artemia* density, enrichment, or their interaction (Table 3.4).

Table 3.4. Results of two-way ANOVA on the survival and development duration of *Camposcia retusa* larvae fed at two densities (5 and 10 ind./ml) in the forms of *Artemia* unenriched nauplii and enriched metanauplii, respectively.

Treatment		**A: enrichment**			**B: *Artemia* density**			**A×B**			**Error**
Source of variation		**df**	**F**	***p***	**df**	**F**	***p***	**df**	**F**	***p***	**df**
Survival (%)	**Z_1 to Z_2**	1	0.974	0.343	1	0.179	0.680	1	0.202	0.890	12
	Z_2 to M	1	28.323	<0.001	1	14.346	0.003	1	1.711	0.215	12
	Z_1 to M	1	20.903	0.001	1	9.290	0.010	1	1.613	0.228	12
	M to C_1	1	1.348	0.268	1	1.348	0.268	1	1.013	0.334	12
	Z_1 to C_1	1	3.953	0.070	1	3.953	0.070	1	3.953	0.070	12
Duration (days)	**Z_1 to Z_2**	1	1.189	0.297	1	2.048	0.178	1	2.860	0.117	12
	Z_1 to M	1	0.024	0.880	1	0.029	0.869	1	0.028	0.869	12

Z_1: first stage zoea; Z_2: second stage zoea; M: megalopa; C_1: 1st juvenile crab. Significant differences (two-way ANOVA: $p < 0.05$) are underlined. ANOVA was not performed for the development duration from Z_1 to C_1 due to insufficient data collected due to low survival in several treatments.

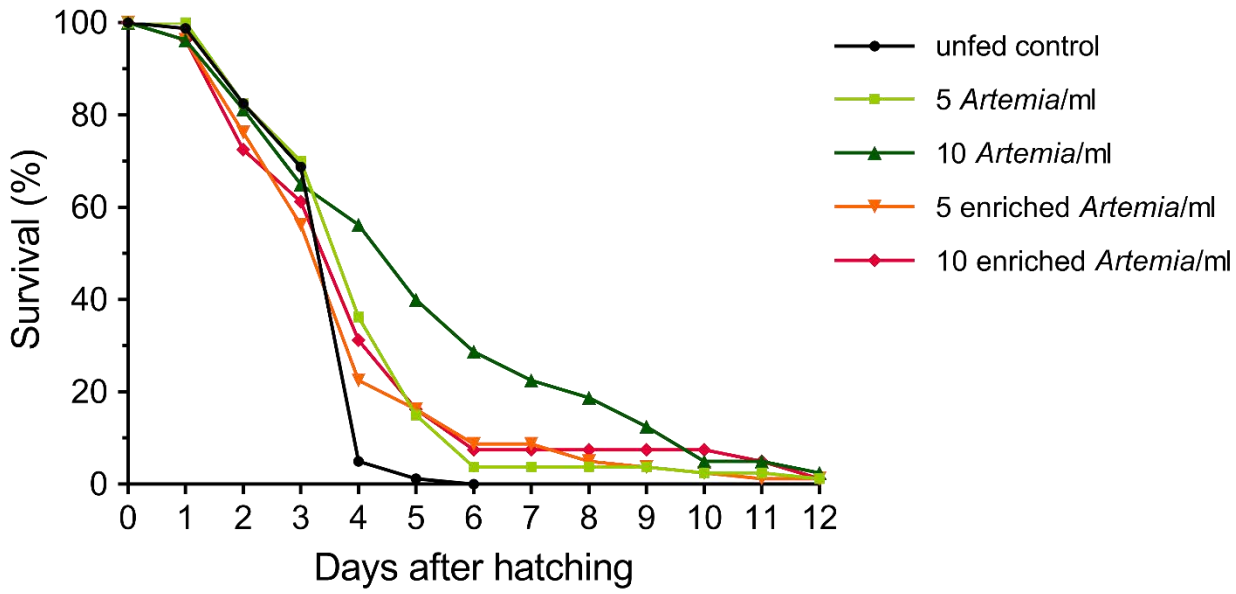

Figure 3.3. Daily larval survival of decorator crab *Camposcia retusa* fed *Artemia* at two densities (5 and 10 ind./ml) in the forms of unenriched nauplii and enriched metanauplii, respectively. For clarity of the figure, the standard error bars are omitted.

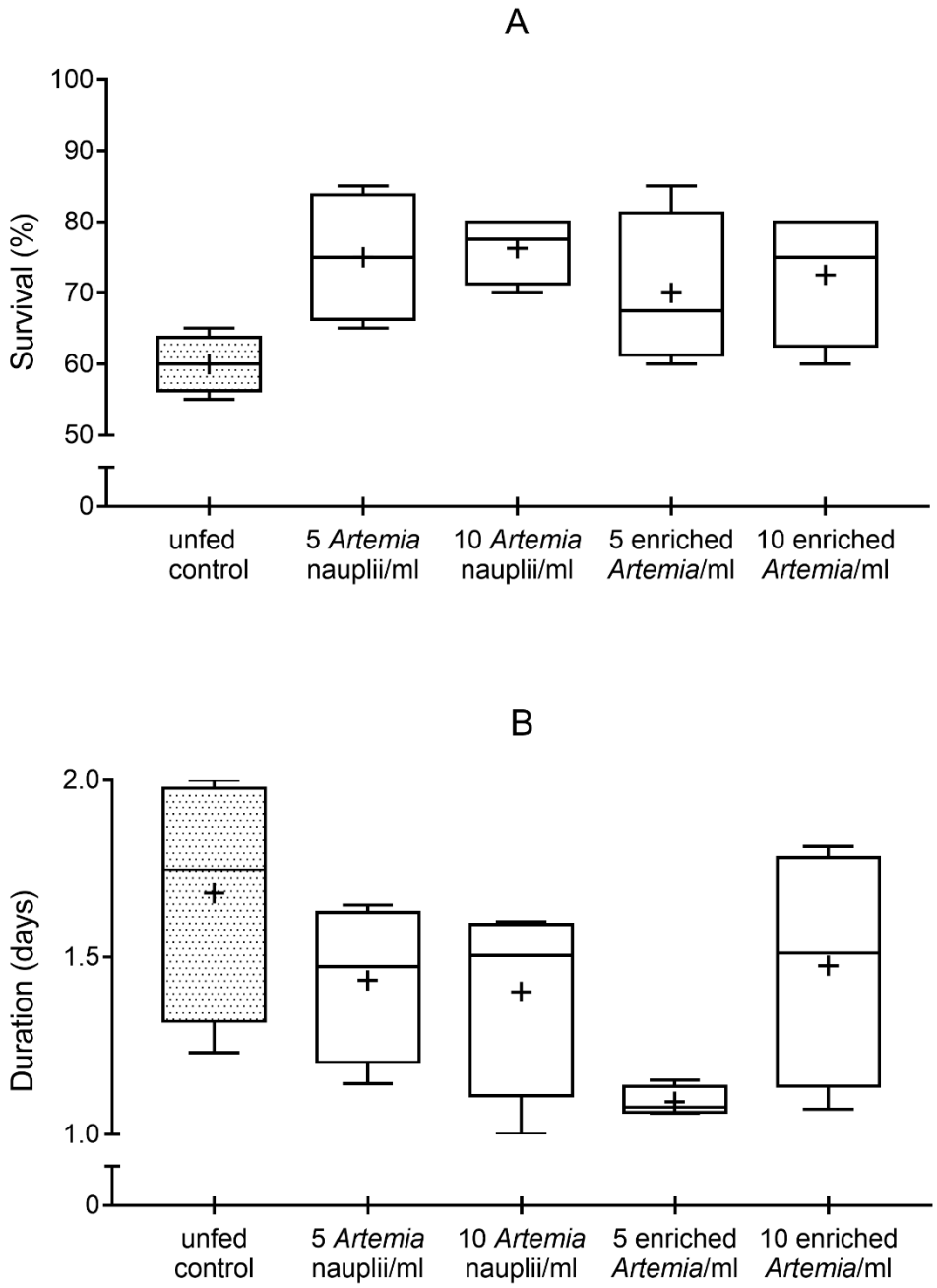

Figure 3.4. Survival (A) and development duration (B) of the first stage zoeal larvae of *Camposcia retusa* from the unfed control and the treatments in which larvae were fed *Artemia* at two densities (5 and 10 ind./ml) in the forms of unenriched nauplii and enriched metanauplii, respectively. Data are presented as general linear model: A line within the box marks the median value; a cross within the box marks the mean value, the box spans the 25% and 75% while the bar represents the minimum and maximum value of a treatment. No significant difference was detected between unfed control and feeding treatments.

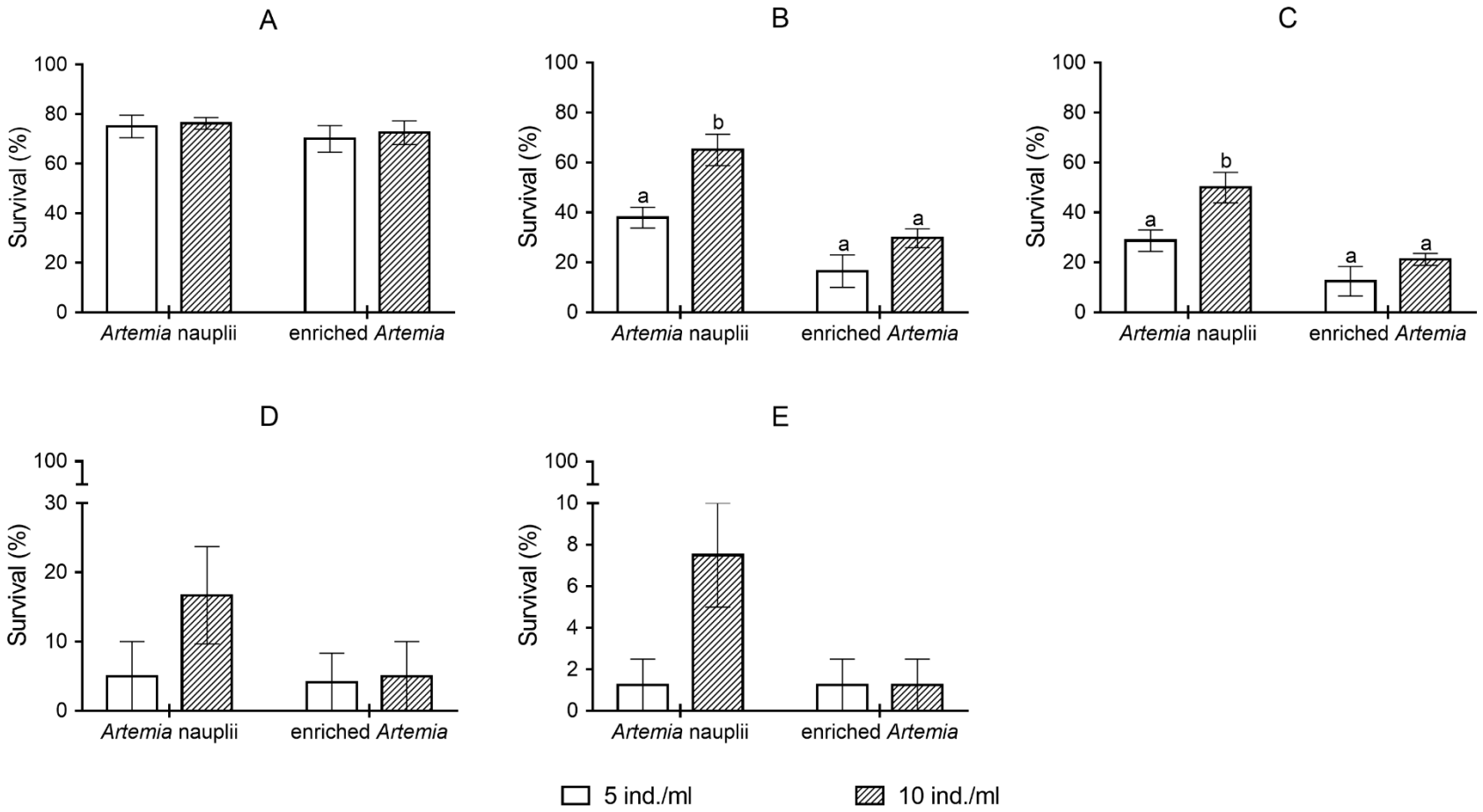

Figure 3.5. Effects of *Artemia* feeding density and enrichment on the survival of *Camposcia retusa* larvae. A: From Zoea 1 (Z_1) to Zoea 2 (Z_2); B: From Z_2 to megalopal stage (M); C: From Z_1 to M; D: From M to 1st crab stage (C_1); E: From Z_1 to 1st crab stage (C_1). Different letters on the tops of bars indicate significant differences (two-way ANOVA; $p < 0.05$).

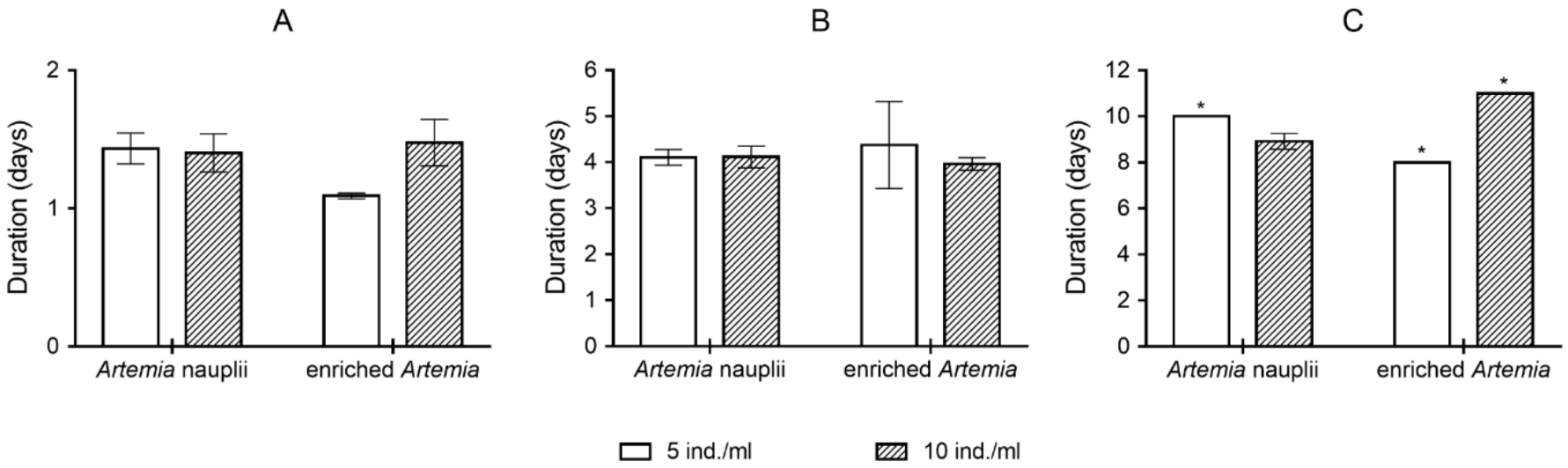

Figure 3.6. Effects of *Artemia* enrichment and feeding density on the larval development of *Camposcia retusa*. A: Zoea 1 (Z_1) duration; B: Zoeal duration (Z_1 to megalopal stage); C: Overall larval duration (Z_1 to 1st crab stage). * Only 1 datum obtained from each of these treatments.

In terms of larval development, two-way ANOVA detected neither significant effect of both *Artemia* density and enrichment, nor the interaction of the two on both Z_1 and Z_2 durations ($p > 0.05$; Table 3.4; Figs. 3.5B, 3.6A & B). For overall larval development from Z_1 to C_1 (Fig. 3.6 C), no statistics was preformed due to only 1 datum being available in several treatments.

3.5 Discussion

The results of the three larval experiments showed that when not fed at all, newly hatched Z_1 larvae of *C. retusa* could still display a survival between 60.0% to 83.8% to the Z_2 stage, confirming that Z_1 larvae are lecithotrophic. There are in fact two types of lecithotrophy, obligatory and facultative lecithotrophy. Obligatory lecithotrophy is defined as larvae being unable to feed and solely relying on yolk reserves to develop into the next stage, while facultative lecithotrophy is defined as larvae being capable of successfully developing to the next stage when food is absence, but being able to feed if food is available (Anger 2001; Zeng *et al.* 2020). Based on such definitions, *C. retusa* Z_1 larvae should be considered as facultative lecithotrophic because in both Experiment 2 and 3, it was noticed that in all *Artemia* feeding treatments, the guts of Z_1 larvae turned orange after a while. Additionally, for both Experiments, Z_1 survival of the unfed control was always lower than any *Artemia* feeding treatments. Furthermore, for larval stages that have been confirmed as primary obligatory lecithotrophy from various decapod species, including *Macrobrachium acanthurus* (Rocha *et al.* 2017), *Macrobrachium jelskii* (Rocha *et al.* 2016), *Lithodes santotta* (McLaughlin *et al.* 2001), *Sesarma windsor* (González-Gordillo *et al.* 2010) and *Metopaulias depressus* (González-Gordillo *et al.* 2010), the mouthparts have been reported as rudimentary, i.e., with simplified mandible, rudimentary maxillule, and setal-reduced maxilla. On the contrary, the mouthparts of Z_1 larvae of *C. retusa* were much better developed (see Chapter 2, Section 2.4.1, Figure 2.1 D–F), thus enabling them to feed if prey is available.

Facultative lecithotrophy is generally considered as an adaptive mechanism evolved to cope with low or unpredictable food availability in the environment where larval development takes place (Anger 2001; Tapella *et al.* 2012; Zeng *et al.* 2020). It is hence more commonly found in food availability poor or unpredictable environments, such as high latitude waters or in freshwater (Anger 2001). The current finding that *C. retusa* Z_1 larvae display facultative lecithotrophy is therefore interesting since *C. retusa* is considered a tropical species (WoRMS 2021). However, environments that are normally considered as highly productive may also experience temporal or spatial variations in plankton availability, leading to transitory or localised food limitation (Constable *et al.* 2003; Mackas *et al.* 1985). A development mode with high level of trophic flexibility, such as facultative lecithotrophy, would clearly benefit larval survival in the wild (Anger 2001; Giménez & Anger 2005) since the ability to cope with short-term food shortage should enhance the chances of survival for *C. retusa* newly hatched larvae.

By comparing survival to Z_2 of unfed newly hatched larvae, and average surviving time of deceased Z_1 larvae among the three batches of larvae used in different experiments, significant differences in both parameters were confirmed. Since the larvae from unfed controls of the three experiments were subjected to nearly identical rearing conditions, the results indicate significant variations in larval quality of the batches. Larval quality variation among different batches is a well-known phenomenon in aquaculture, which can significantly affect larval rearing outcomes (Calado & Leal 2015; Mann *et al.* 1999). The current results suggested that larvae used for Experiment 2 were likely of better quality than the other two batches used in Experiment 1 and 3, and the larvae used in Experiment 3 were probably of the lowest quality of the three. The difference in larval quality between batches is further confirmed by the major difference in larval survival recorded when those larvae were reared under a same feeding regime in the two experiments are compared. For example, when *C. retusa* larvae were fed *Artemia* nauplii at 10 ind./ml, survival to megalopa reached 91.3 ± 3.1% in Experiment 2, but it was only 50.0 ± 6.1% in Experiment 3 (Table 3.3 and Fig. 3.4). The current result also suggests that larval survival to Z_2 can be used as a good indicator for larval quality for *C. retusa.*

While as the result of facultative lecithotrophy, high percentages (> 60%) of Z_1 larvae in the unfed controls of all three experiments successfully moulted to Z_2, none of them survived to the next stage as megalopa. This result showed that from the Z_2 onwards, food availability, as well as their quality and quality become crucial to the survival of the larvae. In Experiment 2, larvae were fed rotifers at a density ranging from 30 to 90 ind./ml, none of the Z_2 larvae in any of the treatments survived to megalopae, with total mortality occurring within 8 days regardless of rotifer feeding density. In addition, Z_1 survival of fed treatments (56.7–68.3%) and the unfed control (65.0%) were very similar (Table 3.2). Despite rotifers have been shown to be an appropriate diet for newly hatched larvae of a range of portunid and spider crabs (Baylon 2009; Kogane *et al.* 2007; Nghia *et al.* 2007; Suprayudi *et al.* 2002; Zmora *et al.* 2005), our results suggest that rotifers are not a suitable diet for *C. retusa* larvae, which is likely due to too small size of the rotifers. In this study, the super-small type or ss-type rotifer *Brachionus rotundiformis* was used, which has a size range of 90–160 μm; such a size range is likely too small for *C. retusa* larvae to either capture or, despite being consumed, the energy budget being poor so that could not sustain successful development to the next stage. Similarly, in the southern rock lobster, *Jasus edwardsii*, phyllosoma larvae showed prolonged development when live prey was too small (Ritar *et al.* 2003). Indeed, the relationship between prey size and larvae is a critical factor to consider in larval feeding, the disproportion of the size between food particles and larval feeding appendages may incapacitate the larvae to effectively capture the prey or despite larvae being able to capture and consume the prey, they would eventually die by being unable to acquire sufficient energy to sustain normal development (Guarizo *et al.* 2020; Ritar *et al.* 2003; Ruscoe *et al.* 2004; Sui *et al.* 2008).

Although copepods have been reported as the natural prey and meet nutritional requirements of decapod larvae (Fileman *et al.* 2014; Waiho *et al.* 2018), few previous research reports have used copepods as the sole prey for decapod larval culture. On the other hand, supplement of copepods with other traditional prey in decapod larval rearing have been trailed and reportedly enhanced survival and growth in several species, including the mud crab *Scylla olivacea* (Jantrarotai *et al.* 2004), the Chinese mitten crab *E. sinensis* (Tang *et al.* 2020), the ornamental shrimp *Lysmata debelius* (Sudharma & Edirsinghe 2016), and the

black tiger prawn *Penaeus monodon* (Farhadian *et al.* 2009). However, in the present study, co-feeding 5 copepods/ml with 5 *Artemia*/ml not only did not produce any clear beneficial effects on larval survival and development when compared to those fed 10 *Artemia*/ml, but both larval survival and development were significantly inferior. In fact, larval survival and development of the copepod co-feeding treatment were similar to the treatment of *Artemia* nauplii supplied at 5 ind./ml (Table 3.3), suggesting that the copepod *P. crassirostris* hardly offered any additional benefits to *C. retusa* larvae. There were two possible explanations for the ineffectiveness of copepod co-feeding, firstly the length of copepodites and adult *P. crassirostris* ranged between 195–445 μm (Alajmi *et al.* 2015); thus, they are far smaller than *Artemia* nauplii (400–500 μm). As mentioned previously, the disproportion of the size of prey and the mouthpart of larvae may render it difficult for the larvae to capture them (Guarizo *et al.* 2020). Secondly, the fast and zigzag spurting swimming behaviour characterised by calanoid copepods is also likely to make it more difficult for the larvae to successfully capture and consume them (Lumasag *et al.* 2007).

In contrast to rotifers and copepods, the results of Experiment 2 showed that in all *Artemia* feeding treatments, larval survival to megalopae were higher than 60%. Thus, it proved that *Artemia* is an appropriate prey for zoeal larval rearing of *C. retusa*. Moreover, larvae fed *Artemia* at 10 ind./ml has significantly better survival and development to the megalopal stage than those fed *Artemia* at lower density of 5 ind./ml, suggesting *Artemia* feeding density is important, which can be explained by the fact that decapod larvae are considered 'inactive predators' and their food consumption often relied on chance encounters with prey (Jeffs & O'Rorke 2020; Tsuji *et al.* 2015; Zeng & Li 1999). Indeed, previous studies have shown positive relationship between ingestion rate and prey density until a saturation level is reached in various decapod larvae, including crabs *E. sinensis* (Sui *et al.* 2009), *Hyas araneus* (Anger & Dietrich 1984), *Ranina ranina* (Minagawa & Murano 1993a), and shrimps *Lysmata wurdemanni* (Zhang *et al.* 1998a), *Penaeus kerathurus* (Yúfera *et al.* 1984), and *Penaeus monodon* (Loya-Javellana 1989). The importance of prey density is probably particularly relevant to early larvae that typically have weak swimming ability (Lumasag *et al.* 2007). Interestingly, in the same experiment, as *Artemia* density further

increased to 15 ind./ml, larval survival decreased significantly. This probably can be explained by the excess number of *Artemia* degrading water quality and/or bacterial explosion (Sui *et al.* 2009; Zhang *et al.* 1998a). Dan *et al.* (2016a) also reported that an excessive high *Artemia* density could induce morphological abnormalities in larvae of the last zoeal stage in the swimming crab *Portunus trituberculatus*, which led to mortalities during metamorphosis. Therefore, based on the results of the current study, an *Artemia* density of 10 ind./ml appears to be appropriate for *C. retusa* larvae.

Although larval survival to the megalopal stage was relatively high in Experiment 2, mass mortality still occurred during this larval stage, particularly during the period when megalopae metamorphosed to first crabs (i.e. days 8–10; Fig. 3.2). Since malnutrition could be a major cause of the mass mortality of megalopae observed, Experiment 3 was designed to test if *Artemia* enrichment could improve megalopal survival. *Artemia* enrichment has been reported to enhance larval survival and development for various decapod species (Suprayudi *et al.* 2004b; Dan *et al.* 2016b; Dan *et al.* 2016c). For example, in larval rearing of swimming crab *Portunus trituberculatus,* it was shown that larvae fed microalgae *Nannochloropsis* enriched *Artemia* achieved a higher survival (40.7%) than those fed unenriched *Artemia* (1.9–3.5%) during the megalopal stage (Dan *et al.* 2016b). Likewise, mud crab *S. serrata* larvae fed unenriched *Artemia* were reported to exhibit reduced survival and prolonged development to C_1 stage (Suprayudi *et al.* 2004b). However, in the present study, *Artemia* enrichment not only failed to improve megalopal survival and development, but led to significant lower zoeal survival when compared to the same density treatments in which larvae were fed unenriched *Artemia* (i.e. zoeal survival almost halved when larvae were fed enriched *Artemia* when compared to those of feeding unenriched nauplii for both 5 and 10 ind/ml density treatments). Therefore, it appears that *C. retusa* larvae should be reared on unenriched *Artemia* nauplii instead of enriched *Artemia*. Interestingly, a recent paper by Basford *et al.* (2021) also reported *Artemia* enriched with the same medium used in this study (Selco S.presso) did not improve zoeal larval survival, growth or development of the blue swimmer crab *P. armatus*. Another paper from the same primary author reported a clear effect of HUFA enrichment using S.presso with fatty acid analysis (Basford *et al*. 2020), which showed that DHA content

increased from 1.76 mg g lipid^{-1} in unenriched *Artemia* to 71.10 mg g lipid^{-1} in enriched *Artemia*, and similarly, EPA content increased from 13.32 to 29.76 mg g lipid^{-1} after enrichment. In fact, an earlier study also indicated that *Artemia* enriched with Frippak booster (a commercial microencapsulate enrichment diet, Frippak, England) did not significantly improve megalopal survival of *S. serrata* (Williams *et al*. 1999). Furthermore, in the larval culture of *M. brachydactyla* and *E. isenbeckii*, *Artemia* enrichment reportedly only enhanced larval development but not survival (Andrés *et al.* 2007; Jinbo *et al.* 2013). Similarly, in the cases of marine ornamental crabs, Rhyne *et al.* (2005) also reported that *Artemia* enrichment failed to improve larval survival, growth and development of *M. sculptus* and *M. forceps*.

It is interesting to note that in this study, *Artemia* enrichment actually led to significant inferior zoeal survival, which was been reported by previous studies. For example, in *S. serrata*, excessive dietary DHA (docosahexaenoic acid) accelerated morphogenesis of the last zoeal stage larvae, which developed inside the exoskeleton enlarged chelipeds that obstructed the moulting process, and led to mass mortality during metamorphosis (Dan & Hamasaki 2011; Hamasaki *et al.* 2002a; Hamasaki *et al.* 2002b). In addition, in the present study, *Artemia* were enriched with S.presso, a commonly used oil emulsion from INVE, which had a high DHA/EPA (eicosapentaenoic acid) ratio (> 7), hence it is possible that accelerated morphogenesis may have occurred in Z_2 larvae fed enriched *Artemia*. Alternatively, the lower survival of the larvae fed enriched *Artemia* might be linked to lower protein and energy contents of enriched *Artemia* metanuaplii as compared to unenriched *Artemia* nauplii. Newly hatched *Artemia* nauplii are lecithotrophic, hence the development to the next metanauplii stage when they start filter feeding and can be enriched means their nutritional and energetic content will reduce substantially (Navarro *et al.* 1999). Indeed, for the GSL *Artemia* strain used in the present study, it was reported that compared to newly hatched nauplii, metanauplii had a 34% reduction in individual dry weight and a 37% reduction in energy content (Vanhaecke *et al.* 1983). Commonly used oil emulsions are aimed at fatty acid enrichment, often do not supplemented with proteins and other nutrients. In fact, enriching GSL *Artemia* with an oil emulsion (DHA Selco, INVE) similar to the one used in this study showed a 19% loss of proteins compared to newly hatched nauplii (Evjemo *et al.* 2001). Proteins are known

as a key nutrient for crustaceans, especially during larval development when rapid tissue synthesis occurs (Anderson & De Silva 2011; Anger 2001). Hence it is possible that in the present study, when *Artemia* metanauplii enriched with S.presso, which is known to be rich in lipids but with limit protein supplement, an unbalanced protein/lipid ratio may have been promoted which ultimately led to an inferior survival in zoeal larvae fed enriched *Artemia*.

Indeed, multiple factors have been reported to cause mortality during the megalopal phase of larval rearing in crab species, which include moult death syndrome, cannibalism, lack of appropriate settlement cues and substrates, and an inappropriate physical culture environment (Beder *et al.* 2018; Dan *et al.* 2016a; Dan *et al.* 2016c; Hamasaki *et al.* 2007; Hamasaki *et al.* 2002b; Jinbo *et al.* 2013). For example, in the present study, it was observed that up to 51% of dead megalopae were missing limbs or body parts, suggesting that cannibalism may play a major role in the megalopal mortality observed. Hence, certain measures to reduce cannibalism during megalopal rearing may improve survival (e.g. reduce stocking density and providing shelters). Additionally, an inappropriate culture environment, such as the lack of suitable substrate and settlement cues, could also negatively affect the success of megalopae metamorphosis (Forward *et al.* 2001). Therefore, following chapters will focus on these areas to improve megalopal survival.

3.6 Conclusion

Through a series of three experiments, this study investigated a suitable larval feeding regime for captive breeding of the decorator crab *C. retusa,* a popular marine ornamental crustacean. This is the first report on the successful rearing of this species from newly hatched larvae to the first juvenile crab stage, achieving a survival of up to 91.3% to the megalopal stage and 11.3% to the first juvenile crab stage. These results suggested that newly hatched *C. retusa* Z_1 larvae are facultative lecithotrophic, being able to develop to the next stage independently of food but can also perform exogenous feeding when food is available. It also reveals that unlike many crab species, rotifers are not an appropriate prey for *C. retusa*

zoeal larvae as it led to total mortality at Z_2 stage. In contrast, *Artemia* nauplii was found to be a good diet for the larvae while the feeding density of *Artemia* was also important. An *Artemia* feeding density of 10 ind./ml was identified as producing the best larval performance. Meanwhile, both co-feeding *Artemia* with copepods and *Artemia* enrichment were not found to improve larval survival and development. Therefore, a feeding regime of newly hatched *Artemia* nauplii at 10 ind./ml appears to be sufficient for *C. retusa* larvae.

Chapter 4. Effects of salinity on survival and development of the decorator crab *Camposcia retusa* zoeal larvae and megalopae and food consumption pattern of megalopae

4.1 Abstract

Effects of salinity on survival and development of zoeal larvae and megalopae of the decorator crab *Camposcia retusa* were investigated by two separate experiments. Firstly, newly hatched zoeae were reared at 7 different salinities of 26, 29, 32, 35, 38, 41 and 44. At salinity 26–32, all larvae died within 3 days, but survival to megalopae was achieved when salinity was 35–44. Zoeal survival was significantly higher at salinity 35 and 38 (87.7% and 78.3%, respectively) when compared to of a salinity 44 (61.7 ± 3.3%; $p < 0.05$). No significant differences were detected in zoeal developmental duration among all salinity treatments. In the subsequent experiment, newly moulted megalopae were subjected to 6 salinity conditions of 26, 29, 32, 35, 38 and 41. Megalopal survival to the first crab stage was the highest when reared at a salinity of 32 (84.0 ± 11.7%), which was significantly higher than salinity 26, 38 and 41 treatments ($p < 0.05$). Meanwhile, megalopal durations were significantly shorter when reared under lower salinities (26–32) than that at higher salinities (35–41; $p < 0.05$). To better understand underlying mechanisms of salinity effects on megalopae, daily ingestion rate was measured throughout megalopal development for each of 30 megalopae cultured individually at one of 3 salinity conditions of low (27), optimal (32) and high salinity (37). The mixed-effect model analysis suggested that salinity significantly affected feed intake of the megalopae, especially during the first half of their intermoult duration ($p < 0.01$). The two-segmented regression of days of megalopal development and the cumulative *Artemia* consumption by individual megalopa showed that at optimal salinity, the breakpoint in daily *Artemia* ingestion rate was at 58.1 ± 3.8% of megalopal duration, while it was significantly later under unfavourable salinities (73.6 ± 3.7% and 71.3 ± 5.4% for salinity

27 and 37, respectively; $p = 0.018$). Results of this study reveal significant effects of salinity on larval survival and development of *C. retusa,* as well as major differences in salinity preference of zoeal larvae and megalopae.

4.2 Introduction

In the marine environment, salinity is one of the most critical environmental factors that can significantly impact larval survival and development of aquatic animals, and brachyuran crabs are no exception (Anger 2001; Anger 2003; Zeng *et al.* 2020). In aquaculture settings, salinity is a physical parameter that can be relatively easy manipulated in hatchery to provide optimal conditions for larval rearing (Nurdiani & Zeng 2007). In order to improve larval rearing success in the hatchery, it is important to identify both salinity tolerance range and optimal salinity for marine larvae of the species being targeted for aquaculture. Since larval salinity preference of saltwater species often varies substantially with ontogenetic development (Anger & Charmantier 2000; Charmantier 1998; Marochi *et al.* 2017), it is also necessary to study each developmental stage. Indeed, past studies have demonstrated that salinity significantly affects larval survival and developmental in various crab species, including the grapsid crab *Armases miersii*, the crucifix crab *Charybdis feriatus*, the snow crab *Chionoecetes opilio*, the Chinese mitten crab *Eriocheir sinensis*, the red frog crab *Ranina ranina*, the mud crab *Scylla serrata* and the common spider crab *Maja brachydactyla* (Anger 2001; Anger *et al.* 2000; Baylon & Suzuki 2007; Baylon 2010; Castejón *et al.* 2015b; Dan & Hamasaki 2011; Minagawa 1992; Nurdiani & Zeng 2007; Yamamoto *et al.* 2015a). In general, results show that the optimal salinity for larvae of a decapod crustacean species is normally related to the salinity in its natural habitat that experienced by larvae (Anger 2003; Zeng *et al.* 2020).

Unfavourable salinity conditions typically lead to supressed larval survival, development and growth (Anger 2001; Zeng *et al.* 2020). It has been reported that reduced feeding under unfavourable salinity conditions could be a major contributor to poor larval performance. For

instance, when zoeal larvae of the red frog crab *R. ranina* was reared at both hypo- and hypersaline conditions, decreased food consumption were observed (Minagawa 1992). Moreover, increased metabolism and energy expenditure required for osmoregulation under unfavourable salinity conditions are also likely to exert some effect on larval development and growth (Dan & Hamasaki 2011; Rey *et al.* 2015). A possible explanation is that besides of increased energy costs for osmoregulation of larvae, their synthetic processes of the biochemical composition, such as lipid and protein, may also be disturbed under such extreme salinity conditions (Anger 2003; Torres *et al.* 2002). Such effect is also expected to be particularly obvious in stenohaline species, for example, as a significant loss of lipid, protein, and biomass was observed in first stage zoeae of the stenohaline crab *Cancer pagurus* when exposed to low salinity (15 or 25) (Torres *et al*. 2002).

Salinity stress may also lead to alterations in larval foraging behaviour, and subsequently food consumption (Minagawa 1992). Larval ingestion rates have been reported for several crab species through laboratory experiments, including the great spider crab *Hyas araneus* (Anger & Dietrich 1984), the shore crab *Carcinus maenas* (Dawirs & Dietrich 1986), the snow crab *Chionoecetes opilio* (Yamamoto *et al.* 2015b), the Florida stone crab *Menippe mercenaria* (Mootz & Epifanio 1974) and the red frog crab *R. ranina* (Minagawa & Murano 1993a; Minagawa & Murano 1993b). These studies showed generally a similar pattern of crab larval food intake during the moulting cycle, i.e., ingestion rate increased sharply soon after moulting, it reached a peak and then plateaus before decreasing to a low level as the larva approached the next moulting (Anger & Dietrich 1984; Dawirs & Dietrich 1986; Mootz & Epifanio 1974; Yamamoto *et al.* 2015b). However, for the best of our knowledge, past studies on ingestion rate of crab larvae were mostly conducted under favourable salinity conditions for the larvae, except for the study by Minagawa (1992) on the red frog crab *R. ranina*. However, that the study only investigated the hourly ingestion of four selected zoeal stages (i.e. Zoea 1, Zoea 3, Zoea 5, and Zoea 7) within a three-hour experimental period, hence the relationship between salinity stress and larval food consumption pattern over moulting cycle of crab larvae is hitherto largely unknown.

The decorator crab *Camposcia retusa* is a popular species in the marine aquarium trade. Attempts have been made recently to captive bred the species in order to reduce collection pressure on its wild populations. With the first successful larval rearing of the species to settlement, it was revealed that larval development of *C. retusa* includes two zoeal instars and a single megalopal instar before settling as a first stage crab (Xu *et al.* 2019). In our previous studies to establish a larval feeding protocol for *C. retusa*, it was found that newly hatched *C. retusa* larvae could be fed *Artemia* nauplii at a density of 10 ind./ml to achieve high survival to megalopal stage (up to 91.3%), however, mass mortality occurred during megalopal stage, leading to low overall survive to the first crab or C_1 stage (2.1–12.7%, Chapter 3).

To date, no information is available on the suitable salinity range for *C. retusa* larval culture. In fact, even the natural distribution of *C. retusa* larvae is largely unknown, therefore, larval salinity preference cannot even be inferred based on their distribution in the wild. The objectives of the present study were hence to investigate the effects of salinity on the survival and development of both zoeal and megalopal larvae of *C. retusa,* and to determine their optimal rearing salinity. Furthermore, to better understand the underlying mechanisms of salinity effects on larval performance, based on the results from the megalopal salinity experiment, daily *Artemia* ingestion rates of *C. retusa* megalopae throughout the full moulting cycle when reared under optimal vs unfavourable salinity conditions was determined and compared to evaluate salinity effects on feeding of the megalopae.

4.3 Materials and methods

4.3.1 Broodstock maintenance

Adult crabs were purchased from Cairns Marine, a commercial collector from Cairns (Queensland, Australia), then transferred to Marine and Aquaculture Research Unit of James Cook University (Townsville, Queensland, Australia). The crabs were kept in pairs in separate 50 L aquaria connected to a recirculation system, with water renewal rate around 90

L/h. Animals were kept under constant conditions with water temperature was maintained at 26–28 °C, salinity at 35–36, photoperiod at L: D = 14 h:10 h, pH = 7.8–8.2, NH_3/NH_4^+ < 0.25 ppm, NO_2^- < 0.25 ppm, and NO_3^- < 5.0 ppm. Chopped prawn, mussels, and thawed blood worms were provided as broodstock feed twice a day. Under this captive condition, the egg incubation time of *C. retusa* was generally between 21 to 25 days.

4.3.2 Experiment 1: Effects of salinity on survival and development of zoeal larvae

Seven salinity treatments at a salinity of 26, 29, 32, 35, 38, 41, and 44 (± 0.5), were selected for this experiment. To start the experiment, on the early morning of the day of larval hatching, the newly hatched Zoea 1 larvae (Z_1) were gently collected from the broodstock tank (salinity 35–36) by firstly attracting them to a bright light source utilizing their strong phototactic behaviour. They were then transferred to several 5 L buckets for acclimation: the larvae were gradually acclimated to the designated salinity condition corresponding to each treatment by a stepwise decrease/increase of salinity to the next level, allowing a 0.5 h acclimation period at each level (i.e. the transfer to the adjacent salinity levels was abrupt). After acclimatisation, 20 vigorously swimming larvae were randomly selected and transferred to each of 600 ml glass beakers filled with 500 ml water with a designated salinity to complete set-up. A fine-tipped glass pipette was placed in each beaker and connected to air supply to provide gentle aeration to each beaker. Each beaker served as a replicate of treatments and for each salinity treatment, three replicates were set up.

Based on the results of the previous larval feeding experiments (Chapter 3), throughout the experiment, larvae were fed newly hatched *Artemia* nauplii (INVE Thailand, Amphoe Wachiabarami, Thailand) daily at a density of 10 ind./ml (Chapter 3). During the experiments, water temperature was maintained at 27 ± 0.5 °C, and the photoperiod was set at L: D = 14 h: 10 h. A 100% water exchange was carried out daily in the morning by moving any surviving larvae to a clean beaker containing fresh seawater with identical salinity during which larval mortality and moulting was monitored and recorded. Dead larvae were

examined under a stereomicroscope (Nikon SMZ645) for their developmental stage and any signs of moult-death syndrome (MDS, i.e., the phenomenon of larval mortality due to the inability to completely shed the old exoskeleton during moulting). This experiment was terminated when all larvae had moulted into megalopae or died.

The lower salinity seawater (salinity 26–32) used in this experiment were made up by daily diluting natural seawater (salinity 35) with de-chlorinated freshwater, while the high salinity seawater (salinity 38–44) was prepared by heating natural seawater to produce a high salinity stock (salinity > 50), which was then diluted with de-chlorinated freshwater to the designated salinities. Salinity was measured using a digital salinity meter with an accuracy of 1 (HI96822, HANNA Instruments, Inc., USA).

4.3.3 Experiment 2: Effects of salinity on survival and development of megalopal larvae

For this experiment, newly hatched Z_1 were mass cultured in 5 L buckets at 27 ± 0.2 °C and salinity 35 ± 0.6 to obtain the newly moulted megalopae for the experiment. The salinity used for mass culture was based on the results of the zoeal experiment (Experiment 1). The initial stocking density for the mass culture was 40 larvae/L and larvae were fed *Artemia* nauplii 10 ind./ml. Water exchange and daily maintenance was carried out daily by moving larvae to a newly prepared bucket containing fresh seawater and feed. When larvae were approaching the time of metamorphosis to megalopal stage, the culture were closely monitored at 8 h interval to identify newly moulted megalopae. Megalopae moulted within 8 h were used for the experiment (i.e. individuals moulted after 11 pm were used for the experiment set up about 7 am in the next morning).

Six treatments with salinity at 26, 29, 32, 35, 38 and 41 (± 0.5) were set up for the experiment. The experimental set up and procedure was similar to that for the zoeal larvae as described above (Section 4.3.2). Due to limited availability of megalopal larvae and their tendency towards cannibalism, five megalopae were stocked into a 600 ml beaker as a

replicate and there were five replicates for each salinity treatment. The experiment was terminated when all megalopae had either metamorphosed into C_1 or died.

4.3.4 Experiment 3: Food consumption pattern of megalopae reared under different salinities

To obtain newly moulted megalopae for the ingestion rate experiment, newly hatched *C. retusa* larvae were firstly mass cultured as described for Experiment 2 (Section 4.3.3). Based on the results of previous experiments, at the time when about 12 h before the second zoeal larvae (Z_2) were estimated to start metamorphosing into megalopae, they were re-stocked into a series of 1 L beakers with 10 larvae per beaker to reduce the chance of cannibalism and for easy observation, these beakers contained clean seawater (salinity 35 ± 0.5) without feed. The beakers were monitored at 8 h interval and megalopae that metamorphosed within 8 hours were used for the experiment. A total of 30 newly moulted megalopae were randomly selected and individually stocked in 600 ml glass beakers containing 200 ml of fresh seawater for the experiment with 10 megalopae for each of three salinity treatments at 27 (low), 32 (optimal), and 37 (high) (± 0.5), respectively. The salinity conditions were chosen based on the results of Experiment 2. Each megalopa was given 200 newly hatched *Artemia* nauplii daily in early morning, the culture water was not aerated as it only contained one larva with a low *Artemia* density. The acclimation of the megalopae to experimental salinity conditions was the same as described for the Experiment 1 (Section 4.3.2), hence a total of 1 h acclimation duration was required before experiment commenced. In addition to the normal replicates with megalopae, three blank replicates under exactly the same conditions but without megalopa were set up for each salinity, these blank controls being used to calibrate the number of *Artemia* remaining after 24 h feeding in the replicates with megalopae.

After allowing 24 h to feed, the megalopae were first pipetted into a petri dish containing fresh seawater of the same salinity to check and record any *Artemia* accidentally picked up in the pipette. The megalopa was then transferred to a newly prepared beaker with fresh seawater of the same salinity and 200 newly hatched *Artemia* nauplii to start the next 24-h

experiment. The content in the original beaker with remaining *Artemia* were poured into a labelled sample bottle, and the beaker was then rinsed, and the water collected in the sample bottle to ensure all *Artemia* and fragments were collected. *Artemia* in replicates of the blank controls were similarly collected daily. Formaldehyde was subsequently added to each sample bottle to preserve *Artemia* at a final concentration of ca. 4%. This procedure was repeated every 24 h until the megalopae either metamorphosed to C_1 crab or died. During the experiment period, all replicates were checked every 8 h and any megalopae found moulted as C_1 crabs were removed immediately from the beaker.

The counting of *Artemia* in the sample bottles was conducted under a stereomicroscope (Nikon SMZ645). Fragments of *Artemia* found were roughly estimated for their equivalent to a full *Artemia* based on their sizes. Numbers of *Artemia* nauplii consumed daily were determined by the difference between the initial and calibrated final number of *Artemia* after 24 h (calibrated with the blank control). The experiment was run until all megalopae had either metamorphosed into C_1 or died.

4.3.5 Data collection and statistical analysis

The carapace size of each megalopal and juvenile sample was measured to the nearest 0.01 mm using a microscope (Nikon YS 100) with ocular and stage micrometres. Carapace length (CL) was measured as the frontal margin to the most posterior margin of carapace and carapace width (CW) was measured as the maximum distance across the carapace. For Experiment 1, five megalopae were randomly selected from each replicate while for Experiment 2, five C_1 crabs were randomly sampled from each treatment for the measurement of CL and CW.

Data are presented as mean ± standard error (SE), and statistical analyses were performed using R (R 4.0.3; R Core Team 2020) with a 5% significance level. For the zoeal larvae salinity experiment (Experiment 1), larval survival (%) was analysed in each stage, as well as

cumulative survival (calculated as the number of larvae that moulted successfully to a particular larval stage divided by the initial number of larvae in each replicate), while zoeal larval development was expressed as the mean cumulative development time from hatching to each subsequent larval stage only. Meanwhile, the ratio of unsuccessful moulting was calculated for each stage by dividing the number of dead larvae showing signs of starting the moulting process by the number of total dead specimens of a particular larval stage assuming all such deaths were due to MDS. The data collected from different salinity treatments were analysed by one-way ANOVA and if significant differences were found ($p < 0.05$), the Tukey's post hoc test was performed to detect specific significant differences among treatments. Normality and homogeneity of the data were checked before performing ANOVA. In addition, the polynomial regression was applied to establish the relationship among the larval survival and salinity using the ordinary least squares.

In Experiment 2, the generalized linear model (GLM) was adopted to evaluate the effect of various salinity treatments on larval survival using the 'glm' function (Everitt & Hothorn 2006; McCullagh & Nelder 1989). The numbers of live and dead individuals during the experimental period were used as the two-vector response variable with the binomial family (logit link) to account for overdispersion of the error distribution. For the analysis of megalopal duration and the size of C_1 juveniles, the Gaussian family (identical link) was applied. The categorical fixed factor "salinity" was used as the explanatory variable in these analyses. The significance of the explanatory variables was evaluated using the 'Anova' function (type II) implemented in the 'car' package (Fox & Weisberg 2019). If any significant difference was identified, differences between salinity treatments were evaluated with the Tukey method using the 'glht' function implemented in the 'multcomp' package (Hothorn *et al.* 2008). In addition, a polynomial regression was also applied to estimate the optimal salinity at which the highest megalopal survival was obtained.

For daily ingestion rate data obtained in Experiment 3, a locally weighted scatterplot smoothing (LOESS) was firstly applied to find out the trend of megalopal food intake pattern in different salinities (Wickham 2016). For comparison daily food consumption patterns at

different phases of megalopal development, the whole experimental period of 9 days was divided into three periods: days 1–3: Early; days 4–6: Middle; and day 7 onward: Late, then average numbers of daily *Artemia* ingested for each of these periods were calculated and compared among different salinity treatments. A mix-effect model was used ('lmer' function in the 'lme4' package), with each megalopa treated as a random effect, while salinity and period being treated as fixed effects (Bates *et al.* 2015; Everitt & Hothorn 2006; Zuur *et al.* 2009). In order to normalise residuals, the number of *Artemia* ingested by each megalopa per day was log-transformed prior to the analysis. In addition, a two-segment linear regression was performed to assess correlation between days of megalopal development and the cumulative number of *Artemia* consumed by the larvae subjected to different salinity conditions (Yamamoto *et al*. 2015b). To estimate the slopes and the possible breakpoint of the regression, the 'segmented' package (Muggeo 2003) was used. In this case, the slope of linear equation suggests the mean ingestion rate over a certain period, while the breakpoint indicates the time of sudden change of mean ingestion rate within the megalopal moulting cycle. The difference of mean daily *Artemia* consumption, megalopal stage duration, the time of breakpoint identified as a percentage of the megalopal moulting cycle, and mean ingestion rate before and after the breakpoint under different salinity conditions were analysed by one-way ANOVA, followed by Tukey's test when significant differences were recorded.

4.4 Results

4.4.1 Effects of salinity on survival and development of zoeal larvae

All Z_1 larvae reared at salinity between 26–32 died within three days (Fig. 4.1) without showing any signs of initiating moulting. However, survival to Z_2 was relatively high within the higher salinity range of 35–41 (> 85%), and the highest survival was obtained at a salinity of 41 (96.7 ± 3.3%) although no significant differences were detected between these treatments ($p > 0.1$). However, as salinity increased to 44, Z_1 larval survival dropped to 71.7%, which was significantly lower than both salinity 38 and 41 treatments (Table 4.1). On

the other hand, larval survival during the Z_2 stage (Z_2 to M) gradually decreased from salinity 35 to 41, with significant differences being detected between salinities 35 and 41 (Table 4.1). Although Z_2 larval survival at salinity 44 was higher than that of 41, no significant differences were found between salinities 38–44 (Table 4.1). In addition, overall zoeal survival (Z_1 to megalopae) showed a decreasing trend with the increase of salinity from 35 to 44, leading to a significantly lower survival at the highest salinity of 44 as compared to other treatments ($p < 0.02$), while no significant differences in survival were detected among salinity 35–41 treatments ($p > 0.5$). Polynomial regression showed that overall zoeal survival would reach the maximum at a salinity of 36.3 (Fig. 4.3A; $R^2 = 0.770$, $p < 0.01$). It was observed that for zoeae metamorphosed to megalopae, the phenomenon of MDS occurred only occasionally with a total of 7 (out of 240) zoeae dying due to MDS, with these being largely distributed randomly among various salinity treatments of 35–44 (Table 4.1).

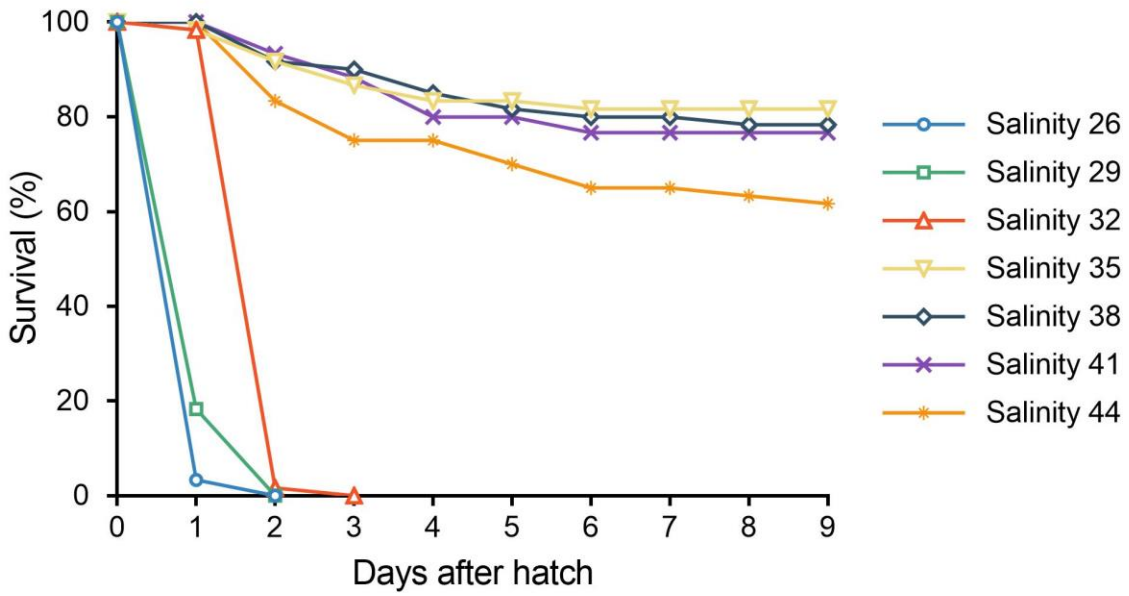

Figure 4.1. Daily survival of *Camposcia retusa* zoeal larvae reared under different salinity conditions. Standard errors were omitted for figure clarity.

Table 4.1. Survival and development of zoeal larvae of the decorator crab *Camposcia retusa* reared under different salinity conditions.

Salinity	Survival (%)			Development duration (days)		Mortalities due to MDS*
	Z_1 to Z_2	Z_2 to M	Z_1 to M	Z_1 to Z_2	Z_1 to M	
26	0	-	-	-	-	0
29	0	-	-	-	-	0
32	0	-	-	-	-	0
35	85.0 ± 2.9^{ac}	96.1 ± 2.0^{a}	81.7 ± 3.3^{a}	1.2 ± 0.1	4.2 ± 0.0	1
38	90.0 ± 2.9^{a}	87.3 ± 4.4^{ab}	78.3 ± 1.7^{a}	1.3 ± 0.1	4.4 ± 0.1	2
41	96.7 ± 3.3^{a}	79.4 ± 2.4^{b}	76.7 ± 1.7^{a}	1.1 ± 0.0	4.4 ± 0.3	3
44	71.7 ± 3.3^{bc}	86.0 ± 0.7^{ab}	61.7 ± 3.3^{b}	1.4 ± 1.1	4.5 ± 0.3	1

Data are presented as mean ± standard error. Values with different superscript letters within a same column are significantly different ($p < 0.05$). *Presented as the total number of cases of a treatment.

Table 4.2. Carapace length (CL), carapace width (CW), and CL/CW ratio of newly moulted decorator crab *Camposcia retusa* megalopae from zoeal larvae reared at different salinity conditions.

Salinity	Carapace Length (CL; mm)	Carapace Width (CW; mm)	CL/CW
35	1.38 ± 0.01	0.91 ± 0.02	1.52 ± 0.02
38	1.40 ± 0.01	0.90 ± 0.01	1.54 ± 0.01
41	1.41 ± 0.00	0.90 ± 0.01	1.56 ± 0.01
44	1.42 ± 0.02	0.91 ± 0.02	1.57 ± 0.01

Data are presented as mean ± standard error.

The mean development duration to Z_2 and to megalopal stage did not differ significantly among salinity treatments, although overall zoeal development duration (Z_1 to megalopae) showed a trend of slightly increases with increasing salinity (Table 4.1).

The CL of the megalopae obtained from different salinity treatments showed an increasing trend with increased salinity, although no statistical differences were detected (Table 4.2). On the other hand, the CW of megalopae were similar across salinity treatments. The CL/CW ratio of megalopae also showed an increasing trend with salinity, i.e. megalopae reared in high salinity environments appeared slimmer (Table 4.2).

4.4.2 Effects of salinity on survival and development of megalopal larvae

Unlike as in the case of the zoeal experiment, the results of the megalopal experiment showed that there were megalopae that successfully moulted to C_1 in all salinity treatments ranging from 26 to 41 (Fig. 4.2). The highest survival was obtained at a salinity of 32 (84.0 ± 11.7%), which was significantly higher than that recorded at a salinity of 26, 38 and 41 ($p < 0.05$), although no significant differences in survival were detected among salinity 29–35 (Table 4.3). Polynomial regression showed that megalopal survival would reach the maximum at the salinity of 32.7 (Fig. 4.3B; $R^2 = 0.395$; $p < 0.01$). During the metamorphosis of megalopae to C_1, the incidents of MDS increased substantially, ranging from 25.0–66.7%. In particular, megalopae reared at the lowest salinity of 26 had the highest ratio of deaths (66.7 ± 11.7%) related to MDS, which was significantly higher than that of salinity 32 (16.7 ± 16.7%, Table 4.3).

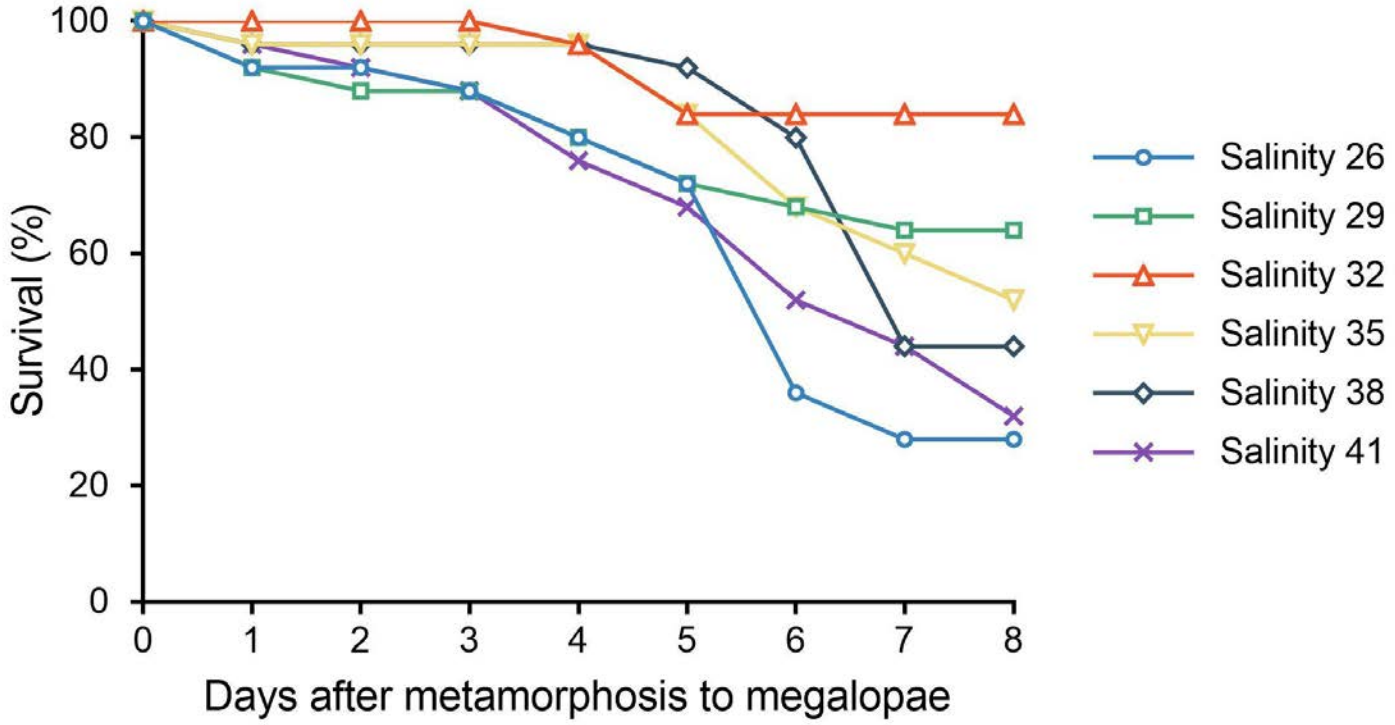

Figure 4.2. Daily survival of *Camposcia retusa* megalopae reared under different salinity conditions. Standard errors were omitted for figure clarity.

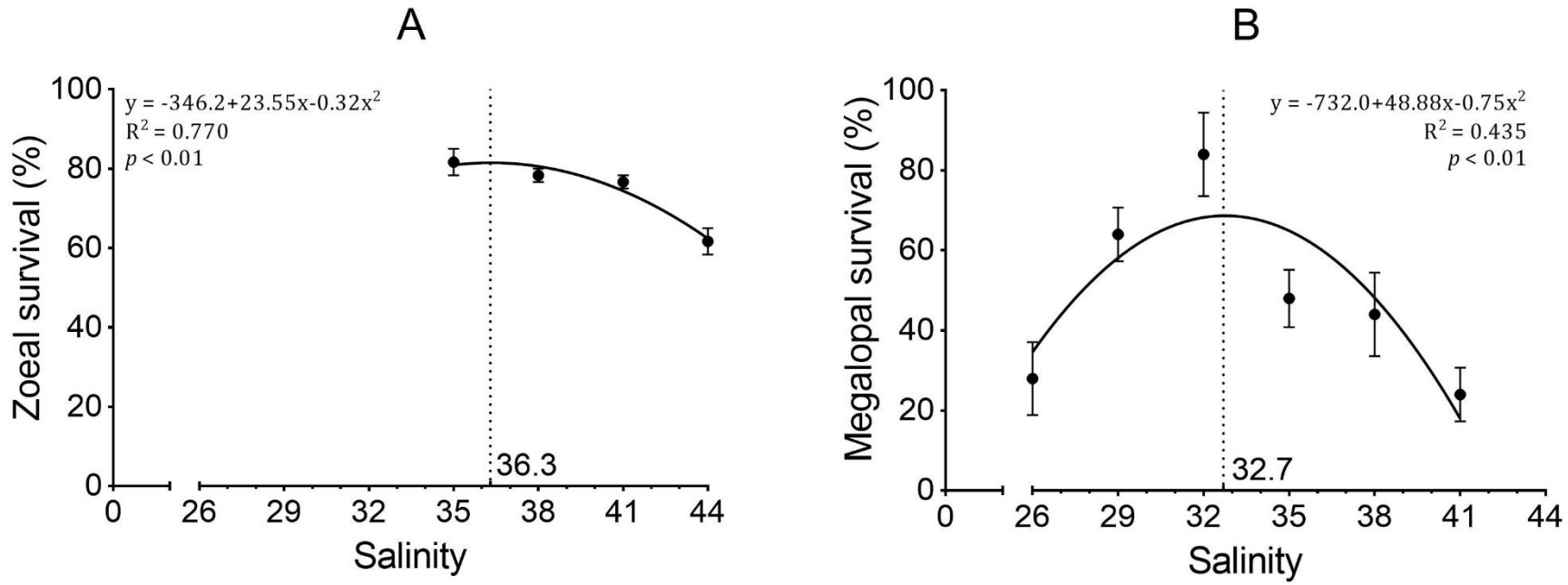

Figure 4.3. Regression of effect of salinity on survival (%) for A) zoeal larvae (from Zoea 1 to megalopal stage) and B) megalopae (to 1st crab stage) of *Camposcia retusa*. y: larval survival for zoeae (A) megalopae (B); x: salinity.

Table 4.3. Survival, development, and percentage of mortality associated with moulting death syndrome (MDS) of megalopae of the decorator crab *Camposcia retusa* reared under different salinities conditions.

Salinity	Survival (%)	Development duration (%)	Percentage mortalities associated with MDS (%)
26	28.0 ± 10.2^{a}	4.3 ± 0.3^{a}	67.7 ± 11.7^{a}
29	64.0 ± 7.5^{bc}	4.2 ± 0.1^{a}	50.0 ± 22.4^{ab}
32	84.0 ± 11.7^{c}	4.3 ± 0.1^{ab}	16.7 ± 16.7^{b}
35	48.0 ± 8.0^{abc}	5.0 ± 0.1^{bc}	25.0 ± 11.2^{ab}
38	44.0 ± 11.7^{ab}	5.5 ± 0.2^{c}	48.3 ± 17.2^{ab}
41	24.0 ± 7.5^{a}	5.1 ± 0.4^{bc}	37.7 ± 10.1^{ab}

Data are presented as mean ± standard error. Values with different superscript letters within a same column are significantly different ($p < 0.05$).

Table 4.4. Carapace length (CL), carapace width (CW), and CL/CW ratio of newly settled decorator crab *Camposcia retusa* first stage crabs from megalopae reared at different salinity conditions.

Salinity	Carapace Length (CL; mm)	Carapace Width (CW; mm)	CL/CW
26	1.57 ± 0.02^{ab}	1.16 ± 0.01^{ab}	1.35 ± 0.02
29	1.56 ± 0.04^{ab}	1.18 ± 0.05^{ab}	1.33 ± 0.07
32	1.67 ± 0.04^{a}	1.19 ± 0.01^{a}	1.41 ± 0.02
35	1.59 ± 0.04^{ab}	1.09 ± 0.02^{b}	1.45 ± 0.02
38	1.61 ± 0.01^{ab}	1.14 ± 0.02^{ab}	1.42 ± 0.02
41	1.54 ± 0.02^{b}	1.10 ± 0.01^{ab}	1.40 ± 0.02

Data are presented as mean ± standard error. Values with different superscript letters within a same column are significantly different ($p < 0.05$).

Salinity also significantly affected megalopae development. In general, megalopae at lower salinities (salinity 26–32; average 4.2–4.3 days) displayed a shorter development time when compared to those reared at higher salinities (salinity 35–41; average 5.0–5.5 days; Table 4.3). In particular, megalopae reared at salinity 38 had a significantly longer development duration than treatments where salinity ranged from 26–32 ($p < 0.001$), and megalopae reared at salinity 35 and 41 also had a significantly longer duration than those of salinity 26 and 29 ($p < 0.05$, Table 4.3). However, no significant differences in megalopal development were detected among salinity 26–35 treatments (Table 4.3).

Finally, both CL and CW of newly moulted C_1 crabs were the largest when megalopae were reared at a salinity of 32 and significant differences were found between salinity 32 and 41 for CL, and salinity 32 and 35 for CW ($p < 0.05$). However, no significant differences were detected for CL/CW among any treatments (Table 4.4).

4.4.3 Effect of salinity on megalopal food consumption pattern

At the end of experiment, 1 deformed crab appeared among 8 and 7 that successfully moulted to C_1 in low (27) and high salinity (37) treatments, respectively, and both died soon after moulting. However, no deformed crabs were found at the optimal salinity (32) treatment. Similar to the results from Experiment 2, megalopa showed a trend of having longer development time with increasing salinity: the mean development duration of megalopae increased as salinity increased, although the difference between treatments was marginally not significant (Table 4.5, p =0.056).

The total number of *Artemia* consumed during the whole megalopal stage increased with salinity: i.e. from 126.8 ± 11.0 at low salinity (27) to 154.1 ± 8.2 at the optimal salinity of 32, and further up to 157.3 ± 6.3 at a higher salinity (37); however, no significant differences were found between the three salinity treatments (Table 4.5, $p = 0.07$). On the other hand,

megalopae reared at the optimal salinity of 32 had a higher mean daily *Artemia* ingestion rate (28.1 ± 1.4 *Artemia* day^{-1}) when compared to that of the low and high salinity treatments (25.9 ± 1.7 and 25.5 ± 1.7 *Artemia* larva^{-1} day^{-1}, respectively). Again, the differences were not statistically significant ($p = 0.371$; Table 4.5). In terms of daily ingestion rate change during the megalopal moulting cycle, the results suggest that most individuals showed a brief peak of feeding that lasted 1–2 days, and such feeding peak tended to occur earlier for megalopae reared at optimal salinity (Fig. 4.4B) than those reared at low or high salinity (Fig. 4.4A & C). The mix-effects model analysis suggests that while the average numbers of *Artemia* ingested daily by megalopae from different salinity treatments are not significantly different ($p = 0.391$), after dividing the megalopal duration into Early, Middle and Late periods with the daily ingestion rates averaged for each period, significantly differences in mean daily ingestion rates for each period were clearly shown among salinity treatments ($p < 0.001$), which are illustrated by the boxplots in Figure 4.5. Moreover, a strong interaction between salinity and the period was also evident ($p < 0.01$).

Two-segmented regressions provided a good fit for the relationship between the cumulative number of *Artemia* consumed and the days of megalopal development for most megalopae that moulted successfully to C_1. The analysis showed that the breakpoint of daily *Artemia* ingestion rate (indicating a sudden change in mean ingestion rate) for the megalopae from the optimal salinity treatment was at 58.1 ± 3.8% of the whole megalopal development duration, which was significantly earlier than that of the low (77.0 ± 4.5%) or high salinity treatment (74.0 ± 5.3%) ($p < 0.05$; Table 4.5). In addition, the mean daily food consumption rate before the breakpoint (45.1 ± 3.4 *Artemia* day^{-1}) for the megalopae reared at the optimal salinity was also significantly higher than those reared at less favourable salinity treatments (33.0 ± 2.6 and 31.5 ± 1.5 *Artemia* day^{-1} for the low and high salinity treatment, respectively; $p < 0.05$; Table 4.5). However, salinity showed no significant effect on the mean food consumption rate after the breakpoint ($p = 0.474$; Table 4.5).

Table 4.5. The cumulative total and daily *Artemia* consumption by megalopae of the decorator crab *Camposcia retusa* reared at low, optimal and high salinity conditions.

Salinity	***Artemia* consumption**		**Development duration (days)**
	Total	**Daily**	
Low – 27	126.8 ± 11.0	25.9 ± 1.7	4.9 ± 0.3
Optimal – 32	154.1 ± 8.2	28.1 ± 1.4	5.5 ± 0.4
High – 37	157.1 ± 6.3	25.5 ± 1.7	6.3 ± 0.4

Data are presented as mean ± standard error.

Table 4.6. The breakpoint of daily ingestion rate during the moulting cycle (%), mean daily *Artemia* ingestion rate before and after the breakpoint by megalopae of the decorator crab *Camposcia retusa* reared at low, optimal and high salinity conditions.

Salinity	**Breakpoint occurred during moulting cycle (%)**	**Mean daily *Artemia* ingestion (*Artemia* larva^{-1} day^{-1})**	
		Prior to breakpoint	**Post breakpoint**
Low – 27	77.0 ± 4.5^{a}	33.0 ± 2.6^{a}	12.9 ± 1.3
Optimal – 32	58.1 ± 3.8^{b}	45.1 ± 3.4^{b}	12.0 ± 1.4
High – 37	74.0 ± 5.3^{a}	31.5 ± 1.5^{a}	10.4 ± 0.9

Data are presented as mean ± standard error. Values with different superscript letters within a same column are significantly different ($p < 0.05$).

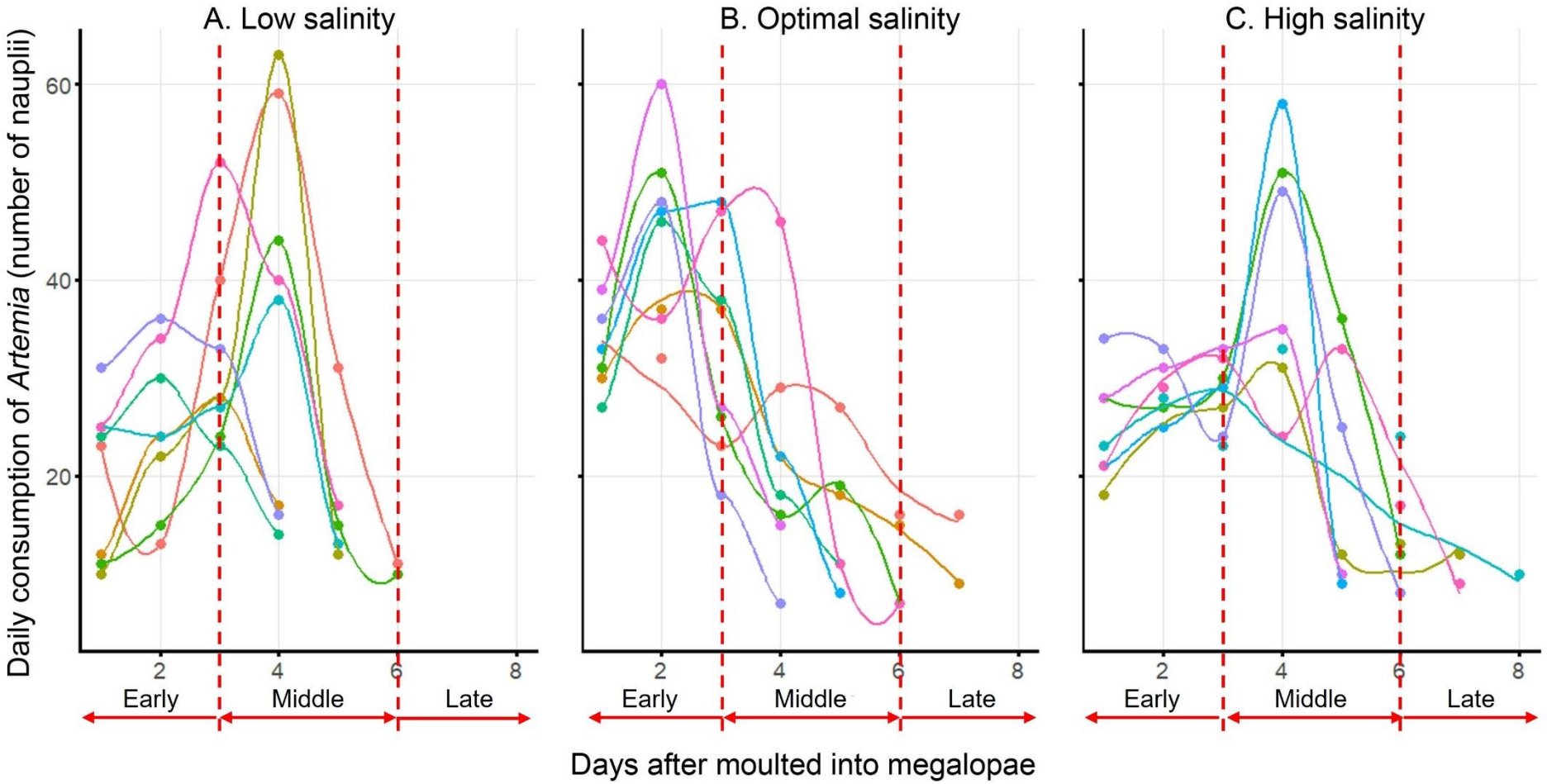

Figure 4.4. Daily *Artemia* nauplii consumption pattern during development by individual megalopa (n = 10) of *Camposcia retusa* reared at one of three different salinity conditions tested; low salinity (salinity 27, A), optimal salinity (salinity 32; B) and high salinity (salinity 37; C). LOESS smoother was applied for data treatment.

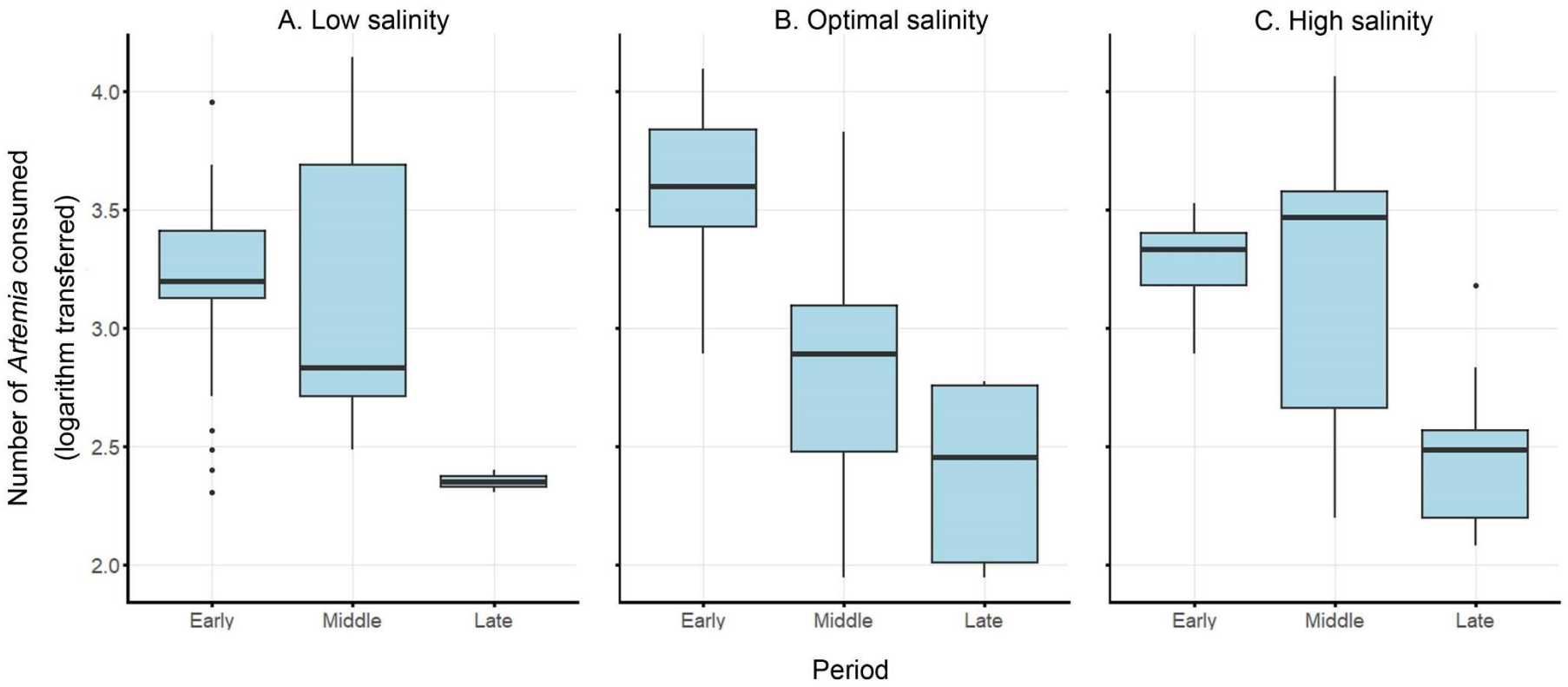

Figure 4.5. The pooled number of *Artemia* consumed by *Camposcia retusa* individual megalopa (n=10) during Early, Middle and Late phase of megalopal development when the megalopae were reared at one of three salinity conditions; low salinity (salinity 27, A), optimal salinity (salinity 32; B) and high salinity (salinity 37; C). The line within each box marks the median value, and the boxes span the 25 and 75% percentiles; the vertical bars represent the minimum and maximum values; and the dots stand for outliners.

4.5 Discussion

All newly hatched zoeal larvae reared at salinity 26–32 died within 3 days, this is surprising considering that these larvae are facultative lecithotrophy (Chapter 3) and salinity 32 is typically considered within the normal range of seawater salinity (Webb 2020). On the other hand, survival of the newly hatched larvae to the megalopal stage was high (> 60%) within higher salinity range 35–44, suggesting that zoeal larvae of *C. retusa* are highly vulnerable to low salinity and their tolerable salinity range is skewed towards high salinity. As larval salinity preference often reflects the condition in their natural habitats (Anger 2001; Anger 2003; Zeng *et al.* 2020), it implies that *C. retusa* release their larvae in offshore waters. It is also worth noting that while Z_1 suffered total mortality at salinity 32, their survival to the next stage increased dramatically to 85% at only a slightly higher salinity of 35, which is very unusual and suggests a lower salinity tolerance threshold exists somewhere within a narrow salinity range of 32–35.

Between salinity 35–41, despite Z_1 larval survival to Z_2 stage showed an increasing trend with salinity, no significant differences were detected. However, once salinity increased further to 44, survival dropped significantly. The result suggests that *C. retusa* zoeae have a relative narrow range of salinity tolerance. Previous studies on osmoregulation of majid crabs showed that they in general are weak osmo-regulators with high sensitivity to salinity fluctuations (Charmantier 1998). For example, a relatively narrow salinity tolerance range was also reported for Z_1 of *Maja brachydactyla* (30–40; Castejón *et al.* 2015b). On the other hand, zoeal larvae of some other brachyuran crabs have been reported to have much broader salinity tolerance ranges, for example, Z_1 larvae of crab *Armases ricordi* reportedly can survive a 5–55 salinity range for 6 days (Diesel & Schuh 1998), while both grapsid crab *Armases miersii* and Xanthid crab *Rhithropanopeus harrisii* can complete their larval development within a wide salinity range of 5–35 (Anger 1996; Forward 2009). As salinity tolerance largely reflects the salinity of natural habitats (Anger 2001), species with a narrow larval salinity tolerance range most likely to inhabit waters with oceanic influence (with the

exception of species that migrate to offshore to spawn), whereas those species with broad salinity tolerance generally dwell in environments with fluctuating salinity conditions, such as intertidal zone, estuaries or rocky pools (Anger 1995; Charmantier & Charmantier-Daures 1995; Forward 2009).

At the megalopal stage, larvae successfully metamorphosed to juvenile crab stage under all tested salinity conditions between 26 to 41, with this range encompassing the salinity conditions that caused total mortality of zoeal larvae (26–32). Interestingly, the highest megalopal survival was actually obtained at the salinity of 32, at which a total mortality of zoeal larvae occurred. Such a result demonstrates significant ontogenetic shifts and a broadening of salinity tolerance at the megalopal stage of *C. retusa*. Larval ontogenetic changes in salinity preference and tolerance are commonly reported among brachyuran species, including the Chinese mitten crab *E. sinensis* (Anger 1991), the red frog crab *R. ranina* (Minagawa 1992), the grapsid crab *A. miersii* (Anger 1996), the mangrove crab *Sesarma curacaoense* (Anger & Charmantier 2000), the crucifix crab *Charybdis feriatus* (Baylon & Suzuki 2007), and the mud crab *Scylla serrata* (Baylon 2010; Dan & Hamasaki 2011). The salinity tolerance of crab larvae is generally related to their osmoregulation capability (Anger 2001; Charmantier 1998). As for other brachyurans (Hong 1988a; Martin *et al.* 2014), a limited osmoregulatory ability of newly hatched larvae of *C. retusa* is expected since the functional gills and other osmoregulatory structures are absent at this larval stage (Xu *et al.* 2019).

Comparing the results of zoeal and megalopal experiments, it shows that the optimal salinity for *C. retusa* larvae decreased with larval ontogenetic development: the optimal salinity for zoeal larvae was estimated at salinity 36.3, but it was at a lower salinity of 32.7 for megalopae. In fact, during the Z_1 stage, larval survival increased as the salinity increase from 35 to 41, while the survival of Z_2 larvae showed a reversed trend. This suggests the change of salinity preference of zoeal larvae may already start at Z_2 stage (Table 4.1). More importantly, the newly hatched zoeal larvae were found not tolerant to salinity of 32, leading to total mortality within 3 days. Such knowledge is clearly highly important for the successful

larval rearing of the species. In fact, it is notable that the highest megalopal survival in the present study reached 84 ± 11.7%, which was a significant improvement when compared with the previous larval feeding experiment (< 15%; Chapter 3) were salinity was set at 35 ± 0.5. Finally, a major shift in salinity preference between zoeal and megalopal larvae of *C. retusa* suggest the natural habitats are likely to be different at the two developmental phases. Unfortunately, the natural habitats of different larval stages of *C. retusa* are largely unknown, hence such a hypothesis is still to be confirmed.

The occurrence of MDS during the first metamorphosis from zoeae to megalopae was not particularly high (7 out 206) and was largely randomly distributed among various salinity treatments, hence the occurrence of MDS is likely not related to salinity stress during the first metamorphosis. On the other hand, the ratio of MDS associated mortalities was considerably higher during the second metamorphosis from megalopae to the first crabs. Additionally, the highest ratio of MDS associated mortalities (66.7 ± 11.7%) was found at the lowest salinity treatment at 26, which was significantly higher than that of salinity 32 (at which the highest megalopal survival achieved), suggesting that salinity stress likely contributed to the high occurrence of MDS under the low salinity condition. During the moulting cycle of crustaceans, the late pre-moult, the ecdysis, and the early post-moult stages are considered highly sensitive periods (Anger 2001) because during these periods around ecdysis, the internal body fluids of the larvae are less protected against external osmotic pressure, hence more likely to lead to physiological damages, and consequent mortality (Anger 2001; Anger 2003).

The results of larval rearing experiments showed that both zoeae and megalopae of *C. retusa* are stenohaline. Based on the results of larval survival, development and occurrence frequency of MDS, the recommended salinity for *C. retusa* larval culture is 35–37 for zoeae and 30–33 for megalopae. It needs to be particularly emphasised that salinity ≤ 32 should be avoided for zoeal rearing since it would lead to complete mortality.

It was observed that swimming activity of the larvae subjected to different salinity

conditions were different during the experiment. In particular, those larvae exposed to low salinities (zoeae subjected to salinity ≤ 32, and megalopae subjected to salinity ≤ 29) substantially reduced their swimming activity and stayed on the bottom most time. A similar behaviour was reported for other brachyuran larvae under salinity stress (Baylon & Suzuki 2007; Castejón *et al.* 2015b). Castejón *et al.* (2015) suggested that in the wild, such a behaviour would bring the larvae to the deeper water where salinity is higher. As for zoeal larvae, however, the low activity in low salinities was more likely due to physiologic damage caused by osmotic pressure since all larvae died within 3 days.

The megalopae obtain at the end of the zoeae rearing experiment at different salinities showed an increasing trend of CL with increasing salinity, but their CW were similar. As the consequence, the CL/CW ratio was higher with higher salinity. Since CL/CW ratio defines the carapace shape, this means that megalopae obtained from zoeae reared under higher salinity had longer and slimmer carapace. For the first stage crabs obtained from megalopae salinity experiment, it was showed that C_1 crabs with the longest CL and broadest CW were obtained at salinity 32, which was significant longer than CL and CW of C_1 crabs obtained at salinities 41 and 35, respectively. These results might be explained by that under a near optimal salinity of 32, larvae probably were able to allocate more energy to growth (Dan & Hamasaki 2011; Rey *et al.* 2015).

Overall, salinity did not appear to exert a significant effect on zoeal larval development as unfavourable salinity conditions did not prolong their development duration. In contrast, megalopal development was significantly affected by salinity with those reared at high salinities (35–41) having on average nearly a one-day delay in their metamorphosis (4.3 vs 5.2 days) when compared with those reared at low salinities (26–32). Delayed metamorphosis by megalopae under unsuitable salinity conditions is commonly observed in brachyurans and has been reported in various species, including *E. sinensis* (Anger 1991), *R. ranina* (Minagawa 1992), *A. miersii* (Anger 1996), *S. curacaoense* (Anger & Charmantier 2000), *S. serrata* (Nurdiani & Zeng 2007), and *C. opilio* (Yamamoto *et al.* 2015a). Reduced salinity accelerates megalopae metamorphosis is typically observed in species with juveniles

occurring in coastal/brackish waters (Forward *et al.* 1994; Forward *et al.* 2001; Nurdiani & Zeng 2007). The fact that low salinity accelerated *C. retusa* megalopae metamorphosis, together with the results that range of salinity tolerant and optimal salinity for *C. retusa* megalopae were lower than those of zoeae, it suggests that the natural habitats of zoaea/spawning adults likely to differ from those of megalopae and juveniles, which probably inhabit lower salinity environments.

Past studies showed that the mean daily ingestion rate by megalopae of the cold-water crab species *H. araneus* was 12.7 *Artemia* (total 546 *Artemia* ingested over 42.9 days; Anger & Dietrich 1984) and 63.0 *Artemia* for *C. opilio* (total 1920 *Artemia* over 30.5 days; Yamamoto *et al.* 2015b), respectively. In the case of *Carcinus maenas,* the ingestion rate was shown to increase with water temperature, being 7.1 *Artemia* daily over 23.9 days at 12 °C but increased to 27.9 *Artemia* daily over a much shorter 7.5 days at 25 °C (Dawirs & Dietrich 1986). Thus, the result showed that the mean daily ingestion of *C. retusa* megalopae between 25.5–28.1 *Artemia* per day was at the medium level when compared to those of other crab species. Despite daily ingestion rate by brachyuran megalopae having been studied in several species, to the best of our knowledge, the present study appears to be the first one that attempted to evaluate effects of salinity on larval food ingestion pattern during moulting cycle of a brachyuran crab. The results showed that during the moulting cycle of *C. retusa* megalopae, the food intake appeared to be more intensive during the Early to Middle phase of the moulting cycle with daily ingestion decreased as they approach moulting. Food ingestion during the megalopal stage had been reported for several other crabs, including the Florida stone crab, *M. mercenaria* (Mootz & Epifanio 1974), the spider crab, *H. araneus* (Anger & Dietrich 1984), the shore crab, *Carcinus maenas* (Dawirs & Dietrich 1986), and the snow crab *C. opilio* (Yamamoto *et al.* 2015b). In those studies, either newly hatched or enriched *Artemia* were used and ingestion rates of the megalopae also decreased when they approach metamorphosis.

In the present study, although the total number of *Artemia* ingested by *C. retusa* megalopae during their development duration was not significantly difference between

salinity treatments, the daily ingestion rates averaged over three phases of megalopal duration, i.e. Early (day 1–3), Middle (day 3–6) and Late period (day 7 onward), showed highly significant differences among salinity treatments ($p < 0.001$). It clearly showed that the megalopae reared under the optimal salinity, the peak daily feed intake period occurred during the Early period, whereas for those megalopae reared at low and high salinities, the peak feed intake period both occurred during the Middle period. Such a result suggests that salinity stress led to major shift in daily food intake pattern of the megalopae over their moulting cycle. In fact, there is a critical point within the moulting cycle that is termed Point of Reserve Saturation or PRS. If the initial feeding period exceed the PRS, after which starvation has no more effect on larval development and metamorphosis (Anger 1987). While the physiological consequences of such shift in daily food consumption is unknown, it could result in the megalopae under the salinity stress had less time to prepare or being ready for all critical metamorphosis, and hence lower chance of success. That is, megalopae under the optimal salinity may be able to reach the PRS and acquired enough energy to successfully metamorphose earlier, compared with conspecifics under suboptimal salinity conditions.

The results of two-segmented regression of accumulative *Artemia* consumption by *C. retusa* megalopae further confirm the shift in daily food consumption pattern over megalopae duration when subjected to salinity stress. While the slope of daily ingestion rate prior to the breakpoint was larger than after the breakpoint for all treatments, which suggests that daily feed intake was always tailed off toward the end of moulting cycle regardless of salinity condition. The breakpoint for the megalopae reared at the optimal salinity occurred at 58.1 ± 3.8% of their whole megalopal duration, corresponding to the premoult stage of moulting cycle (Anger 2001). However, for megalopae subjected to the low and high salinity, the occurrence of the breakpoint was significantly delayed (77.0 ± 4.5% and 74.0 ± 5.3% of megalopal duration, respectively; $p = 0.018$). This might suggest that the progress of *C. retusa* megalopal moulting cycle was delayed under unfavourable salinity conditions. Within the moulting cycle of decapod larvae, the intermoult stage, the stage prior to the premoult stage, is believed to be flexible (i.e. a larva could be ‘arrested’ at this stage under unsuitable environment); in contrary, once larval development has advanced into the premoult stage, the

progress typically becomes inflexible, and the larva would either successfully complete moulting or die due to moulting failure within a short timeframe (Anger 2001).

In addition, for the megalopae reared at the optimal salinity, their mean daily feed intake before the breakpoint was significantly higher than those megalopae reared at the low or high salinities. However, once the breakpoint was achieved, salinity appeared to have very little effect on the daily food consumption of the megalopae ($p = 0.474$), suggesting that at this point megalopae may have reached their PRS. In another word, salinity affected larval feeding predominately occurred during the early phase, probably prior to the premoult stage, of their moulting cycle. Decapod crustaceans are known to show decreased feeding activity after entering the premoult stage (Anger 2001). Similarly, in megalopae of several decapod crustaceans, it has been reported that starvation during the late phase of the moulting cycle did not affect their survival and success moult into the crab stage (e.g. Figueiredo *et al.* 2008). Such an ability has been suggested to have adaptive significant as it allows megalopae that have reached the PRS and entered the premoult stage. This mechanism ensures that megalopae have reserved enough energy to metamorphose, then they are able to concentrate on finding appropriate settlement locations and habitats to ensure better chance of survival of newly settled juvenile crabs (Anger 2001).

4.6 Conclusion

The present study revealed that both zoeal larvae and megalopae of *C. retusa* are stenohaline, each with a relatively narrow range of salinity tolerance. For example, it was shown that salinity ≤ 32 caused total mortality of newly hatched zoeae within 3 days; while at slightly higher salinity of 35, survival of Z_1 larvae showed a dramatic improvement to 85%. This unusual result suggests the lower threshold of salinity tolerance of *C. retusa* zoeal larvae is in-between a narrow salinity range of 32–35. With ontogenetic larval development from zoeae to megalopae, larval tolerance to lower salinity conditions increased substantially and optimal salinity for *C. retusa* larvae shifted towards a lower value. Based on the results of this

study, the recommended salinity for *C. retusa* larval culture is 35–37 for zoeal larvae, but 30–33 for megalopae. Hence during larval rearing of *C. retusa*, salinity lower than 35 should be avoided for zoeae while at megalopal stage, salinity should be reduced to improve their survival. By providing megalopae with a reduced salinity at 32, megalopal survival of *C. retusa* has improved dramatically from below 15% from previous rearing trials to over 80%. Such a high megalopal survival could be a result of less energy being consumed for osmoregulation and more active swimming/foraging activity that resulted in higher food consumption during the early phase of megalopal development under such favourable salinity condition.

Chapter 5. Survival, development and metamorphosis of decorator crab *Camposcia retusa* megalopae: effects of stocking density and basal area of culture vessel, conspecific exudates and artificial substrates

5.1 Abstract

Previous experiments have shown that survival of decorator crab *Camposcia retusa* megalopae was consistently low despite being fed different live prey and their combination. To improve survival and better understand settlement and metamorphosis requirements of *C. retusa* megalopae, a series of four experiments were conducted to investigate effects of stocking density and basal area of culture vessel, the presence of conspecific exudates and artificial substrates (50–800 μm nylon nets) on survival, development, and metamorphosis of *C. retusa* megalopae. The results of the 1st experiment showed that survival of *C. retusa* megalopae improved significantly with reduced stocking density and increased basal area of cultural vessel, and the highest survival of 53.3 ± 5.4% was achieved at a stocking density of 10 megalopae/L and 260.2 cm^2 basal area of culture vessel; however, megalopal development was not significantly different among all treatments. The subsequent two experiments assessed effects of conspecific exudates on megalopal survival and development using natural and artificial seawater, respectively. The results of the two experiments showed an overall similar trend: the presence of adult exudates improved megalopal survival (by up to 18%), while early juvenile exudates did not significantly influence survival when compared to that of the control. In terms of larval development, the time to metamorphosis (TTM) of megalopae showed a trend of being shorter with the presence of adult exudates while juvenile exudates prolonged TTM; as the result, TTM of the megalopae reared with early juvenile exudates was significantly longer than those reared with adult-exudates in both natural and artificial seawater ($p < 0.05$). The final experiment examined the effects of nylon nets of various mesh sizes as substrates, with megalopal survival showing a clear trend of gradually

increasing with increasing mesh size of nylon net from 50 μm to 800 μm. However, significant differences were only found between the control (no nylon net provided) and the treatment using 800 μm mesh net as substrates (40.6 ± 4.0% vs. 59.4 ± 6.0%). TTM of megalopae was not found significantly influenced by the presence of nylon nets. The results of the present study suggest that in *C. retusa* larval culture, once zoeal larvae had moulted into megalopae, stocking density should be reduced while culture vessels with larger bottom areas should be used to prepare for metamorphosis to juvenile crabs; providing 800 μm nylon net as substrate should also help to improve survival. In addition, the presence of adult conspecific exudates will also likely enhance the development and metamorphosis of *C. retusa* megalopae.

5.2 Introduction

The previous larval feeding experiments (Chapter 3) have established a feeding regime for *C. retusa* zoeal larvae: i.e. *Artemia* nauplii at the density of 10 ind./ml can be used to feed *C. retusa* zoeal larvae from hatching to megalopal stage with high survival (up to 91.3 ± 2.7%), however, high mortality (> 80%) persisted during megalopal stage. To improve megalopal survival, copepod co-feeding with *Artemia* nauplii and feeding larvae with enriched *Artemia* were also tested and compared; however, neither of them produced significant positive effect on megalopal survival. Therefore, it was speculated that the low survival of *C. retusa* may not be linked to their feeding, but other inappropriate culture conditions.

In fact, in the previous experiments, it was observed that up to 50% of dead larvae had missing appendages or body parts (Chapter 3), suggesting likely high rate of cannibalism among the megalopae. For decapod larvae, cannibalism is a common phenomenon observed in both natural and captive conditions, particularly during postlarval stages (Bromilow & Lipcius 2017; Chen *et al.* 2014; Romano & Zeng 2017; Tapella *et al.* 2012; van den Bosch *et al.* 1988; Zmora *et al.* 2005). In natural environment, cannibalism may function as an

ecologically important process for larvae as it can serve as 'lifeboat mechanism' when feed is unpredictable (Anger 2001; van den Bosch *et al.* 1988). That is, during periods of food scarcity, younger/weaker larvae may become a food source for older/stronger conspecifics; therefore, intraspecific predation becomes a nutritional buffer to ensure larval survival to juvenile stage (Anger 2001). However, under aquaculture settings, cannibalism is an unwanted behaviour since it can lead to substantial losses to the industry (Romano & Zeng 2017). Therefore, various measures have been adopted in aquaculture to reduce cannibalism, which include the use of a lower stocking density, enlarging the settlement area and providing shelters or substrates to mitigate the physical confrontation of individuals (see review by Romano & Zeng 2017).

Past studies have also shown that various chemical and physical cues can play an important role in settlement and metamorphosis of brachyuran crab megalopae (see review by Forward *et al.* 2001 and Gebauer *et al.* 2020). Additionally, for species such as most brachyuran crabs whose life cycle displays two phases, it is important to understand requirements when they undertake the transition from a pelagic to a benthic environment (Anger 2001; Gebauer *et al.* 2020). Settlement is a complex process that requires finding a suitable location for morphogenetic changes and subsequent benthic life (Gebauer *et al.* 2020). Recognising appropriate settlement sites for many marine decapods, including crabs, requires chemical and/or physical cues that facilitate colonisation of favourable habitats and stimulate metamorphosis (Anger 2001; Forward *et al.* 2001; Gebauer *et al.* 2003; Gebauer *et al.* 2020).

Settlement and metamorphosis cues could be physical and chemical that relate to postlarval habitats (Forward *et al.* 2001; Gebauer *et al.* 2020). Chemical cues have been shown to include exudates (odours) of conspecific adults or juveniles, preys and predators, as well as compounds from settlement sites; while physical cues can include the texture of tridimensional structures (rocks, sands, etc.), salinity, as well as sounds from habitats (Anderson *et al.* 2010; Anger 2003; Forward *et al.* 1996; Forward *et al.* 1997; Gebauer *et al.* 1998; Geburzi *et al.* 2018; Stanley *et al.* 2012; Steinberg *et al.* 2008). The absence of

settlement cues may delay the settlement and metamorphosis of megalopae from days to nearly a month; and in severe cases, even led to mortalities (Forward *et al.* 2001; Gebauer *et al.* 2003). Such delay increases the costs and risks in hatchery production and could also have carry-over effects on subsequent juvenile performance (Carvalho & Calado 2018; Diele & Simith 2007; Rhyne & Lin 2004; Simith *et al.* 2013b).

Among various chemical cues, conspecific adult exudates have been recognised as one of the most important stimuli that induced metamorphosis of megalopae of brachyuran crabs (Gebauer et al. 2020). For example, in crab species of *Hemigrapsus sanguineus*, *Panopeus herbstii*, *Rhithropanopeus harrisii*, and *Uca* spp., the presence of conspecific adult exudates has been shown to shorten the average time required to reach metamorphosis by megalopae (Andrews *et al.* 2001; Fitzgerald *et al.* 1998; Forward *et al.* 2001; Geburzi *et al.* 2018; Simith *et al.* 2017; Simith *et al.* 2010; Welch *et al.* 2016). However, a lack of stimulus effect of adult exudates has also been reported in other species, such as *Callinectes sapidus*, *Carcinus maenas*, *Maja brachydactyla*, and *Menippe mercenaria* (Castejón *et al.* 2019; Forward *et al.* 1994; Krimsky & Epifanio 2008; Zeng *et al.* 1997). On the other hand, effects of conspecific juvenile exudates as settlement cues for megalopae are far less studied (Anderson *et al.* 2010; Geburzi *et al.* 2018), probably due to difficulties in acquiring suitable juveniles for the experiments.

Physical cues may also influence metamorphosis of brachyuran megalopae. For example, substrates that provide appropriate texture can induce metamorphosis of megalopae (O'Connor 2007; Steinberg *et al.* 2008; Weber & Epifanio 1996). Nylon net is one of such substrates that has been tested previously, which was reported to reduce megalopal duration of the Asian shore crab *H. sanguineus*, and such effect was further enhanced by introducing biofilms from the habitat of crab species (Anderson & Epifanio 2009; O'Connor 2007; Steinberg *et al.* 2008). However, for some other crabs, structure cues were found to be ineffective in influencing settlement or metamorphosis of megalopae. For instance, artificial structures that mimic the natural substrates, but are free from chemical cues, such as nylon net, glass beads, ribbons, and plastic strips were reported to be ineffective in shortening

megalopal duration of crab *Neohelice granulata* (previously known as *Chasmagnathus granulata*, Gebauer *et al*. 1998), *Uca pagnax* (O'Connor & Gregg 1998), *C. sapidus* (Forward *et al*. 1996), and *P. herbstii* (Weber & Epifanio 1996), respectively. Despite of that, many of these artificial substrates have complex structures, hence can become effective shelters for settling megalopae/early juveniles, leading to reduced aggressive behaviour and cannibalism (Romano & Zeng 2017).

As multiple chemical and physical environmental factors could impact survival, development and metamorphosis of brachyuran megalopae, a better understanding of their effects should allow optimising the culture environment, while also facilitate the design of efficient culture systems in crab hatchery (Hamasaki *et al.* 2011; Silva *et al.* 2012; Zmora *et al.* 2005). To this end, this study was designed and conducted to evaluate effects of stocking density and basal area of culture vessel, the conspecific exudates (both adult and juvenile), and artificial substrates (nylon meshes of different mesh sizes) on survival, development, and settlement of decorator crab *C. retusa* megalopae.

5.3 Materials and methods

5.3.1 Source of broodstock and their maintenance

Broodstock of decorator crab *C. retusa* were purchased from a commercial marine ornamental collector and wholesaler, Cairns Marine (Cairns, Queensland, Australia). After the crabs being airfreighted to Townsville, they were transported immediately to the Marine and Aquaculture Research Facility Units (MARFU) of James Cook University. After a brief acclimation period, they were paired, and each pair was kept in a of a series of 50 L acrylic tanks connected to a large recirculation system with temperature and salinity control that maintained the water temperature at 26–27 °C and salinity 34–35 throughout the experiments. The photoperiod of laboratory was set at L: D = 14 h: 10 h. The broodstock were fed daily with chopped mussels and prawns, as well as thawed blood worms, and checked for

spawning regularly. When a spawning was found, the female was closely monitored during embryonic development until hatching. Under the above-described condition, the egg incubation duration typically lasted between 21 to 25 days.

When it was judged that a batch of eggs were about to hatch, a banjo filter was plugged on the outlet of the broodstock tank late afternoon to prevent the newly hatched larvae being flushed out. The newly hatched larvae were collected by attracting them toward a light source placed near the upper corner of the tank. Once the larvae gathered by their positive phototoxic, they were gently scooped out by a small beaker with as little water as possible.

5.3.2 Mass culture of zoeal larvae

For all experiments in this chapter, newly hatched larvae were firstly mass cultured until they moulted into megalopae when they were used for different experiments. Based on the feeding regime established through previous experiments (Chapter 3), zoeal larvae were fed newly hatched *Artemia* nauplii at 10 ind./L throughout their development. The initial stocking density for the mass culture was 40–50 larvae/L. During the mass culture period, *Artemia* were hatched and harvested daily, and their density were estimated by averaging three 1 ml samples taken from harvested *Artemia* stock. Throughout mass culture, culture water was exchanged 100% each morning by moving live larvae to another container containing fresh seawater and *Artemia*. During the culture, water temperature was maintained at 27 ± 0.5 °C, salinity 36 ± 0.5, and photoperiod set at L: D = 14 h: 10 h.

5.3.3 Experimental design and setup

5.3.3.1 General experimental procedures

For all following megalopal experiments, culture condition was maintained the same as

for zoeal culture (including feeding *Artemia* nauplii at 10 ind./L) except salinity was reduced to 33 ± 0.5 based on the results of salinity experiment for megalopae (Chapter 4). Megalopae used for each experiment originated from different batches. During the experiment, surviving megalopae in each replicate were transferred by a wide mouth pipette each morning to a new container containing fresh seawater and identical treatment condition. During the experiment, larvae were checked every 12 h for any mortalities and newly appeared first stage crabs (C_1). Any dead megalopae were checked under a stereomicroscope (Nikon, SMZ645) to assess damage to their appendages and body parts as an indicator for cannibalism. Each experiment was run until all megalopae in all replicates were either metamorphosed to C_1 or died.

5.3.3.2 Experiment 1: Stocking density and basal area of culture vessel

For this experiment, two types of culture vessels with same shape but different basal areas (BA) were used, i.e. 1 L plastic beaker (BA: 86.6 cm^2) and 5 L plastic bucket (BA: 260.2 cm^2), to provide a difference in basal area of approximately 3 folds; however, the total water volume used for megalopal culture for either vessel was the same at 1 L. Such two culture vessels in combination with two stocking density of 10 and 30 megalopae/L resulted in 4 treatments with different stocking density and culture vessel basal area combinations. Each treatment had 3 replicates. To minimize potential difference that might be caused by using different sized vessels due to different light distribution within the vessels, both culture vessels used was made of similar materials and semi-opaque, and each of the 1 L beaker was placed in the middle of an empty 5 L bucket during the experiment. A fine-tipped glass pipette was placed in the middle of each container to provide gentle aeration. During the final days of mass rearing of zoeal larvae when they were expected to moult to megalopae, the culture was checked every 8 h to collect newly appeared megalopae. The megalopae that moulted within 8 h were then evenly and randomly distributed into each treatment.

5.3.3.3 Experiment 2 & 3: Conspecific exudates

Experiment 2 and 3 had the same design but employed wither natural or artificial seawater for the experiments, respectively. For both experiments, there were 3 treatments: 1) control: megalopae reared in conspecific exudate-free seawater; 2) adult exudates treatment: megalopae reared in seawater containing adult exudates; and 3) juvenile exudates treatment: megalopae reared in seawater containing juvenile exudates. For both Experiment 2 and 3, megalopae were cultured in 5 L buckets filled with 2 L seawater, however, for Experiment 2, the initial stocking density was 20 megalopae/bucket and there were 3 replicates (buckets) per treatment, while for Experiment 3, the initial stocking density was 24 megalopae/bucket and there were 4 replicates per treatment.

Experiment 2 was conducted first, which showed significant effects of adult exudates on both survival and development of *C. retusa* megalopae when natural seawater was used. Considering that natural seawater may contain unknown organic cues that could compound the results, Experiment 3 was conducted using artificial seawater to further verify the results. Artificial seawater was prepared by dissolving seawater salt (Red Sea Salt, Red Sea Fish Pharm Ltd., Israel) in dechlorinated freshwater to obtain the desired salinity of 33 ± 0.5 for the experiment. The reason of use dechlorinated freshwater rather than tap water was that in our pre-experiments that attempt to rear larvae with artificial seawater, larval survival was generally had better and more stable when dechlorinated freshwater was used. Since broodstock and newly hatched larvae were originally from natural seawater, an acclimation period was allowed before the larvae were reared in full artificial seawater: newly hatched first stage zoeal larvae were mass reared in 100% natural seawater for the first day, but from the next day onward, larval rearing water was increasingly replaced by artificial seawater at a rate of 1/3 daily, hence by the fourth day, the larvae were reared in 100% artificial seawater.

To prepare seawater that contained conspecific exudates, three batches of *C. retusa* larvae were mass cultured prior to the experiments to produce juveniles (4–6 weeks) and adults (> 1 year old) that were used to produce conspecific exudate-solute seawater. For

obtaining the seawater containing conspecific exudates, the crabs were soaked in seawater to be used for larval rearing for 24 h. During the soaking period, the crabs were not fed but gentle aeration was provided. After the soaking period, the crabs were returned to their original tanks and were not used again for producing exudates at least for the next 48 h. The conspecific exudate-solute seawater was prepared daily and were again sieved through a 25 µm mesh before being used for the experiment at daily water exchange (100%) in the morning. The procedure of exudate-solute seawater preparation for Experiment 2 and 3 was largely the same, except that in Experiment 2, the crabs were soaked in filtered natural seawater; while in Experiment 3, artificial seawater was used. In the present study, the age of *C. retusa* juveniles used for producing exudate-solute seawater were at crab stage 2–4 (C_2–C_4). Based on the previous studies on crab species, the detectable threshold of conspecific adult and juvenile exudates by megalopae could be as low as 0.6g/L and 0.0115g/L, respectively (Anderson *et al.* 2010; Simith *et al.* 2013a). On this base and depending on availability of crabs, the crab density used for Experiment 2 ranged between 1.79–1.99 g/L for adults and 0.552–0.802 g/L for juveniles, and for experiment 3, it ranged between 1.38–2.66 g/L for adults and 0.389–0.417 g/L for juveniles.

5.3.3.4 Experiment 4: Artificial substrates

White nylon nets of various mesh sizes were used as benthic substrate for this experiment. There were 4 treatments: 1) control: no substrate; and nylon net with mesh size of 2) 50 µm; 3) 200 µm; 4) 800 µm provided as the substrate during megalopal rearing. Except for mesh size, the nylon nets were identical being made of the same material, with the same shape (round), size (diameter: 14 cm) and structure. The nylon nets were also similarly arranged by laying them largely flat on the bottom of the culture vessels. Prior the experiment, all nylon nets were disinfected in a chlorine bath, rinsed repeatedly with tap water and then hung to dry. Similar to conspecific exudate experiments, 5 L buckets (filled with 2 L natural seawater) were used as cultural vessels, and the initial stocking density was

16 megalopae/bucket, with 4 replicates (buckets) per treatment. The nylon nets were minimally manipulated during daily water exchange to avoid stress to the megalopae. No attempt was made to detach megalopae found attached to the nylon net; instead, the number of megalopae were counted before they were quickly transferred over to the new bucket together with the nylon net they attached to.

5.3.4 Data and statistical analysis

Data are presented as mean ± standard error (SE). Megalopal development was defined as time to metamorphosis (TTM), while the day a zoeal larva moulted to megalopa was designated as day 0 after moult. Megalopal survival and TTM data were analysed by one-way ANOVA except for Experiment 1 (stocking density and basal area of cultural vessel experiment: two-way ANOVA was used to analyse the data). If significant differences were found, Tukey's test was performed to identify treatments with significant differences. All data were tested for the normality and homogeneity prior to ANOVA procedures, and arcsine or square root transformation was performed where needed. The critical level (α) to reject the null hypothesis was set at $p < 0.05$. All statistics were conducted using R 3.2.0 (R Core Team, 2020).

5.4 Results

5.4.1 Experiment 1: Stocking density and basal area of culture vessel

Two-way ANOVA analysis showed that megalopal survival was significantly affected by both stocking density ($F_{1,8} = 12.663$, $p = 0.007$) and basal area of rearing vessel ($F_{1,8} = 6.151$, $p = 0.038$); however, no significant interaction was detected between the two factors ($F_{1,8} = 4.198$, $p = 0.075$; Table 5.1). The results showed that both lower stocking density and larger basal area of rearing vessel significantly improved megalopal survival to C_1 (Fig. 5.1).

Indeed, the highest survival (53.3 ± 6.7%) was obtained from the treatment combining the low stocking density (10 megalopae/L) and large basal area of culture vessel (260.2 cm^2), which was significantly higher than all other treatments. On the other hand, megalopae reared in vessels with low basal area (86.6 cm^2) and high stocking density (30 megalopae/L) had the lowest survival (22.2 ± 4.8%), which was less than half of the highest survival obtained (Fig. 5.1).

Microscopic examination of dead megalopae collected at every 12 h interval revealed that in many cases, the megalopae had missing body parts. The percentage of damaged bodies of the dead megalopae was highly correlated to both stocking density ($F_{1,8} = 325.415$, $p < 0.001$) and basal area of rearing vessel ($F_{1,8} = 493.224$, $p < 0.001$), but no significant interaction between the two factors was detected ($F_{1,8} = 0.729$, $p = 0.418$; Table 5.1). It was clear that the percentage of dead megalopae with damaged body parts increased with stocking density but decreased with the increase of basal area of rearing vessel (Fig. 5.2). Indeed, the highest percentage of the dead megalopae with damaged body parts was found to occur in the treatment combing high stocking density (30 megalopae/L) and small basal area (260.2 cm^2) of culture vessel (40.2 ± 1.4%), whereas the megalopae reared at low stocking density of 10 megalopae/L in culture vessels with larger basal area (5 L buckets; BA: 254.5 cm^2), none of the dead megalopae was found with body parts missing (Fig. 5.2).

The mean developmental duration or TTM of the megalopae from different treatments ranged from 5.2 ± 0.1 to 5.6 ± 0.1 days. No significant effect of either stocking density ($F_{1,8} = 3.497$, $p = 0.104$), or basal area of rearing vessel ($F_{1,8} = 5.147$, $p = 0.058$), or their interaction was detected ($F_{1,8} = 4.481$, $p = 0.072$; Table 5.1).

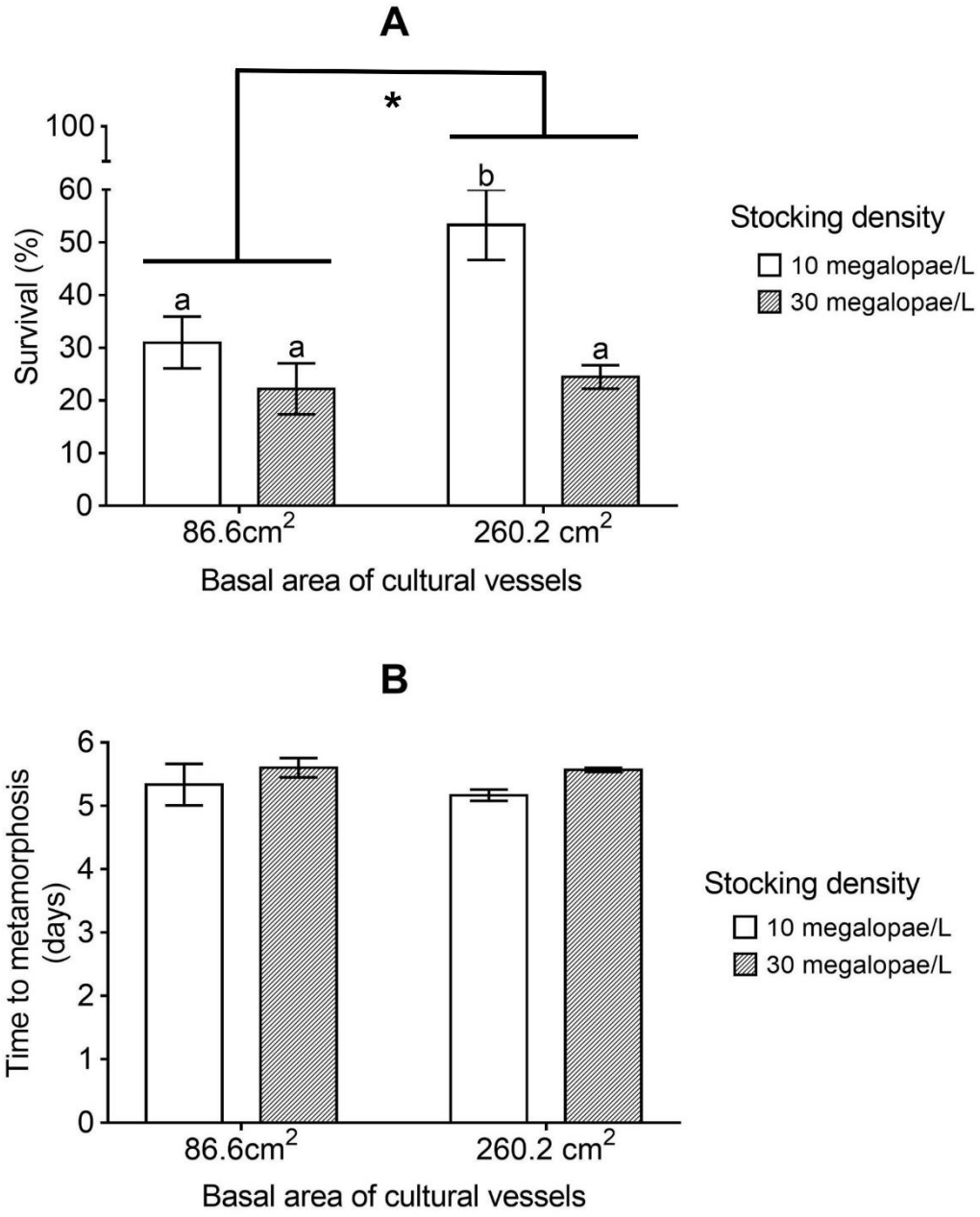

Figure 5.1. Effects of various combinations of stocking density and basal area of culture vessel on the survival and development of the megalopae of *Camposcia retusa* (analysed by two-way ANOVA). A: megalopal survival; B: time to metamorphosis (TTM). The asterisk indicates significant differences in megalopal survival of individuals reared in different basal areas of cultural vessels ($p < 0.05$). Different letters on the tops of bars indicate significant differences among two stocking densities ($p < 0.05$).

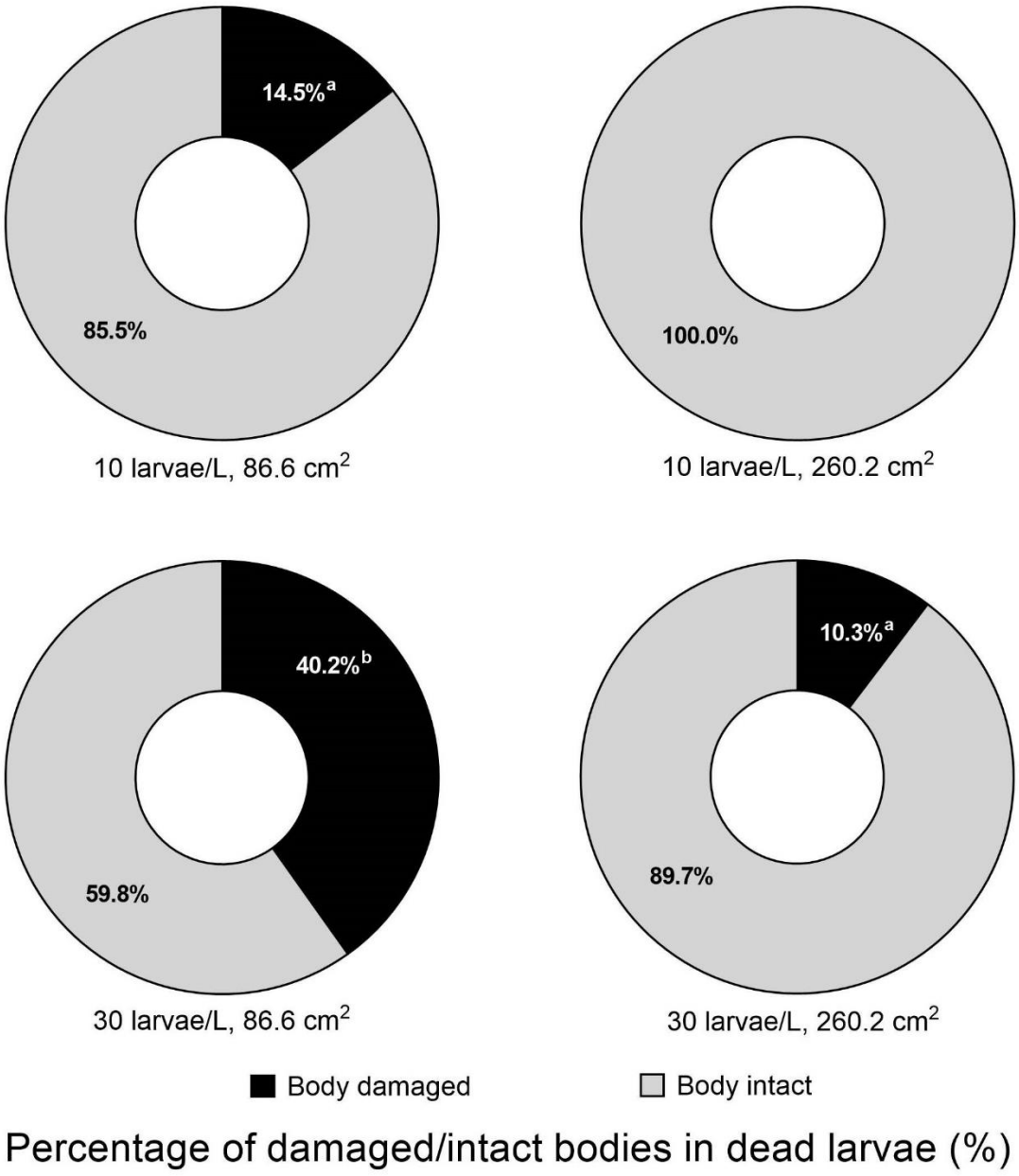

Figure 5.2. Percentage of dead megalopae with damaged body parts when *Camposcia retusa* megalopae were reared with different combinations of stocking density and basal area of culture vessel. Different letters on the tops of bars indicate significant differences among treatments ($p < 0.05$). *: No damaged body was found in the treatment of stocking density at 10 larvae/L and basal area at 260.2 cm^2.

Table 5.1. Results of two-way ANOVA analysis on survival and development (time to metamorphosis or TTM) of *Camposcia retusa* megalopae reared at various combinations of stocking densities and basal areas of culture vessel.

	Source of variation	df	*F*	*p*
Survival (%)	Basal area	1	6.151	0.038*
	Stocking density	1	12.663	0.007*
	Basal area × stocking density	1	4.198	0.075
TTM (days)	Basal area	1	5.147	0.058
	Stocking density	1	3.497	0.104
	Basal area × stocking density	1	4.481	0.072
Percentage of dead	Basal area	1	473.224	< 0.001*
megalopae with damaged	Stocking density	1	325.415	< 0.001*
body parts (%)	Basal area × stocking density	1	0.729	0.418

* indicates significant differences ($p < 0.05$).

5.4.2 Experiment 2 & 3: Conspecific exudates

The results of Experiment 2 demonstrated that when the megalopae were reared in natural seawater, conspecific adult exudates significantly affected survival of *C. retusa* megalopae ($p = 0.040$). Megalopal survival was the highest when adult crab exudates were presented (78.3 ± 4.4%), which was followed by the treatment with juvenile exudates (70.0 ± 0.0%), while the exudates-free control had the lowest survival (60.0 ± 5.0%). The megalopal survival of the adult exudate treatment was significantly higher than that of the control ($p < 0.05$), but no significant difference was detected between other treatments (Table 5.2). The TTM of *C. retusa* megalopae also varied significantly ($p < 0.032$) among treatments. The shortest TTM was obtained from the megalopae reared with adult exudates (5.0 ± 0.1 days), which was significantly shorter than that of juvenile exudates treatment (5.7 ± 0.3 days), but it was not significantly different from TTM of the control (5.4 ± 0.1 days; Table 5.2).

Experiment 3 used artificial seawater instead for the experiment. Although overall megalopal survival was lower than Experiment 2 (e.g. survival of the control: 37.5 ± 1.7% vs.

60.0 ± 5.0%), similarly adult exudates appeared to enhance megalopal survival as the treatment had the highest survival (42.7 ± 2.0%), which was significantly higher than that of the juvenile exudate treatment (30.2 ± 2.0%). However, no significant difference in survival was detected between either juvenile or adult exudate treatment and the exudate-free control (Table 5.2). In the case of megalopal development, the results showed a same trend as Experiment 2: adult exudate treatment had the shortest TTM (4.4 ± 0.1 days), which was significantly shorter than that of juvenile exudate treatment (5.1 ± 0.1 days; $p = 0.010$). However, no significant difference in TTM was detected when compared to that of the control, as well as between juvenile exudate treatment and the control (Table 5.2).

Table 5.2. Survival and development (time to metamorphosis or TTM) of *Camposcia retusa* megalopae reared with the presence of conspecific adult or juvenile exudates in natural and artificial seawater, respectively.

		Exudate-free control	**With adult exudates**	**With juvenile exudates**
Natural seawater	**Survival (%)**	60.0 ± 5.0^{a}	78.3 ± 4.4^{b}	70.0 ± 0.0^{ab}
	TTM (days)	5.4 ± 0.2^{ab}	5.0 ± 0.1^{a}	5.7 ± 0.3^{b}
Artificial seawater	**Survival (%)**	37.5 ± 1.7^{ab}	42.7 ± 2.0^{a}	30.2 ± 2.0^{b}
	TTM (days)	4.9 ± 0.1^{ab}	4.4 ± 0.1^{a}	5.1 ± 0.1^{b}

Data are presented as mean ± standard error. Means with different superscript letters within a same row indicate significant differences ($p < 0.05$).

5.4.4 Experiment 4: Artificial substrate

Survival of *C. retusa* megalopae showed a trend of increasing with the presence of nylon net as substrates, as well as increasing mesh size of the nylon nets used: it ranged from the lowest value of 40.6 ± 4.0% from the control without nylon net to the highest value of 59.4 ± 6.0% of the treatment in which nylon net with the biggest mesh size (800 µm) was used as substrate (Table 5.3). However, significant differences were only detected between the no substrate control and the 800 µm mesh sized nylon net treatment ($p = 0.027$). On the other hand, TTM of megalopae were not differ significantly between any treatments ($p = 0.141$; Table 5.3).

Table 5.3. Survival and development (time to metamorphosis or TTM) of *Camposcia retusa* megalopae reared in natural seawater with nylon nets of different mesh sizes provided as substrates.

	No substrates	**50 µm net**	**200 µm net**	**800 µm net**
Survival (%)	40.6 ± 4.0^{a}	51.6 ± 1.6^{ab}	54.7 ± 3.0^{ab}	59.4 ± 6.0^{b}
TTM (days)	6.4 ± 0.1	6.7 ± 0.1	6.6 ± 0.1	6.2 ± 0.2

Data are presented as mean ± standard error. Means with different superscript letters within a same row indicate significant differences ($p < 0.05$).

5.5 Discussion

The results of current study showed that both lower stocking density and large basal area of culture vessel significantly improved *C. retusa* megalopal survival, stocking density, in particular, exerted highly significant effects on megalopal survival ($p = 0.007$). Such a result is supported by some of previous studies showing similar stocking density effects on larvae of other brachyuran crabs (Penha-Lopes *et al.* 2005). For brachyuran crabs, it is well known that typically once zoeal larvae moult to megalopae with two newly developed large claws, cannibalism is significantly intensified (Romano & Zeng 2017).

Indeed, cannibalism has long been recognised as a major cause of megalopal mortality during brachyuran larval culture (Romano & Zeng 2017; Zhang *et al.* 2018). In this study, when dead megalopae were examined microscopically, many of them were found to have damaged bodies. Since during the experiment, all culture vessels were checked every 12 h for any mortalities of megalopae, which were immediately removed, hence the dead megalopae had never remained in the culture vessels longer than 12 h, therefore, the missing body parts of dead megalopae was unlikely due to natural decomposition. The occurrence of such damaged bodies clearly correlated to high stocking density and small basal area of culture vessel similarly suggested that it was not due to decomposition. Furthermore, during the experiment, it was sometimes observed that megalopae hold on to each other. Therefore, all above evidence suggest that cannibalism likely was an important contributor to megalopal mortality. That said, the percentage of dead megalopae with missing parts from different treatments may not accurately reflect the contribution of cannibalism to megalopal mortality because firstly, the damaged bodies may also be due to necrophagy, a phenomenon previously reported for majid species (Andrés *et al.* 2007; Anger & Nair 1979). Furthermore, megalopae aggressive behaviour might cause small but fatal injuries that were difficult to identify under a microscope. In other words, dead megalopae with seemingly intact bodies cannot be excluded to have experienced cannibalistic interactions.

Since the ultimate goal of aquaculture hatcheries is to produce a large number of seedlings for either grow-out or stock enhancement, intensive production with high stocking density is commonly practiced in hatchery larval rearing. The effects of stocking density on larval rearing have been studied for some crustacean species, including crabs (Dawirs 1982; Fernandez *et al.* 1994; Penha-Lopes *et al.* 2005; Zmora *et al.* 2005). Indeed, for many brachyuran species, including *C. sapidus*, *Metacarcinus magister* (previously known as *Cancer magister*) and *C. maenas*, it has been reported that high density communal culture of megalopae led to mass mortality due to high cannibalism rates (Dawirs 1982; Fernandez *et al.* 1994; Forward *et al.* 1996; Zmora *et al.* 2005). Therefore, very low stocking density (e.g. 0.5 ind./L) for megalopae were normally recommended for their communal culture (Dawirs 1982; Fernandez *et al.* 1994; Forward *et al.* 1996; Forward *et al.* 2001). In this study, a relatively high survival rate (> 50%) was achieved when *C. retusa* megalopae were cultured at 10 ind./L, suggesting that *C. retusa* megalopae probably are less cannibalistic than those of aggressive species mentioned above.

Previous studies on effects of basal area of culture vessel on survival of crabs were mostly focused on juvenile stages (Gil *et al.* 2019; Penha-Lopes *et al.* 2006; Sotelano *et al.* 2016). In general, larger basal area or less individuals per unit area led to increased juvenile survival (Daly *et al.* 2009; Sotelano *et al.* 2016). The present study showed similar results with *C. retusa* megalopae. This probably can be explained by the observation that swimming behaviour of late megalopae is typically reduced, and they are often found settled on the bottom of culture vessels. Therefore, the small basal area of culture vessel likely promoted a higher encounter rate and aggressive behaviour, which led to higher mortality.

In previous studies on the captive rearing of anomuran and brachyuran megalopae, high stocking density reportedly could delay their metamorphosis (Dawirs 1982; Fernandez *et al.* 1994; Forward *et al.* 1996). It was suggested that the prolonged moulting interval under high stocking density could be due to higher level of exudates released by higher number of settled juveniles, which inhibited the progress of megalopal moulting cycle due to high cannibalism

pressure (Fernandez *et al.* 1994). On the other hand, Castejón *et al.* (2019) reported that TTM of the spider crab *M. brachydactyla* megalopae accelerated under communal culture conditions and suggested that accelerated TTM might be due to conspecific hormones released in the culture medium. Different from the studies mentioned above, the current study showed that TTM of *C. retusa* megalopae was not affected by the stocking densities tested. Similar results were also reported for the marine ornamental crab *Mithraculus forceps* (Penha-Lopes *et al.* 2005), suggesting highly species-specific and diverse responses.

It is well known that megalopae of many marine and estuarine brachyuran species respond to various chemical and/or physical cues indicating whether habitats are suitable for settlement (reviewed by Forward *et al.* 2001). However, hitherto no such study has been conducted with the decorator crab *C. retusa*. The effects of conspecific exudates on megalopal survival and development of *C. retusa* were investigated in this study via two experiments of similar design but using natural and artificial seawater, respectively. When megalopal survival of similar treatments from the two experiments were compared, it was clear that megalopae reared in natural seawater were substantially higher, in fact nearly double that of the megalopae reared in artificial seawater. This could be due to different quality of larvae used, but more likely it is linked to ionic differences between natural and artificial seawater. The ionic composition of the artificial seawater may not be ideal for megalopae and their metamorphosis, hence led to reduced survival. For the experiment using artificial seawater, cultural media was gradually transited from natural to artificial seawater over a 3-day period soon after hatching; despite the acclimatization step taken to reduce impacts, the transition likely had negative effects on larval fitness. In spite of the differences in absolute values of megalopal survival, results from the two experiments showed largely similar trend of effects of conspecific exudates on megalopal survival and development. For example, both experiments showed that conspecific adult exudates generally enhanced survival while accelerated TTM of *C. retusa* megalopae, other the other hand, exudates of early juveniles prolonged TTM.

Effects of conspecific exudates on metamorphosis of crab megalopae is a well-studied

area, and the accelerating effect of conspecific adult exudates on metamorphosis has been widely reported for various brachyuran crab species, including *Armass roberti* (Anger *et al.* 2006), *N. granulata* (previously known as *C. granulata*, Gebauer *et al*. 1998), *Hamigrapsus takanoi* (Geburzi *et al.* 2018), *Hamigrapsus sanguineus* (Geburzi *et al.* 2018; O'Connor 2007), *Panopeus herbstii* (Andrews *et al.* 2001; Rodríguez & Epifanio 2000), *Rhithropanopeus harrisii* (Fitzgerald *et al.* 1998), and *Ucides cordatus* (Diele & Simith 2007; Simith *et al.* 2017); although no clear stimulus effect has been reported in other species, such as *C. maenas, M. brachydactyla* and *M. mercenaria* (Castejón *et al.* 2019; Krimsky & Epifanio 2008; Zeng *et al.* 1997). Compared to adult exudates, the effect of juvenile exudates on TTM was less studied and its effects appear to vary among species. For example, conspecific juvenile exudates reportedly accelerated TTM of megalopae of the crabs *H. sanguineus* (Anderson *et al.* 2010) and *U. cordatus* (Simith *et al.* 2013a), and it was suggested that such an effect could be explained by the habitats supporting viable older juvenile population likely would also provide sufficient resources for growth and development of newly settled juveniles (Anderson *et al.* 2010), although an important precondition is that the megalopae and newly moulted juveniles of these species are not cannibalistic (Simith *et al.* 2013a). On the other hand, metamorphosis of common shore crab *C. maenas* megalopae was found postponed by conspecific juvenile exudates (Geburzi *et al.* 2018). The delayed metamorphosis by conspecific juvenile exudates was explained as a mechanism to avoid settling in locations with high density of older conspecific juveniles that are known to be cannibalistic (Geburzi *et al.* 2018). This latter explanation would suit *C. retusa* as high cannibalism rate were observed in communal cultivation of captive bred *C. retusa* juveniles.

The experiment using nylon nets with various mesh sizes as artificial substrate for *C. retusa* megalopae showed a clear trend of increased megalopa survival with increasing mesh size of nylon nets and significant differences were found between the no nylon net control and the treatment using 800 μm nylon net as substrate. It was observed that many late megalopae of *C. retusa* crawled on the nylon nets, which probably were more favourable than the flat bottom of the culture vessels for megalopae to cling to; the nylon nets likely also

serve as shelters to lower encounter rate and hence cannibalism between megalopae (Gebauer *et al.* 2002). The effects of mesh size of nylon nets probably can be explained by that nylon net with larger mesh size being more favourable/comfortable by *C. retusa* megalopae to settle and hold on to. During Experiment 4, *C. retusa* megalopae were observed to settle and hold tightly on both 200 and 800 μm nets, but seldom found on the 50 μm nets; nylon nets with larger mesh size hence provided better shelter effect. Likewise, in a study on spider crab *M. brachydactyla*, megalopae were reported to crawl on nylon nets with mesh sizes of both 500 and 1000 μm nets, but not on 100 μm net (Castejón *et al.* 2019). The provision of nylon net, regardless of their mesh size, was found not significantly affecting TTM in this study, with similar results being reported in previous studies investigating the effects of different sizes of sands and nylon nets on megalopal metamorphosis of crabs *N. granulata* (previously known as *C. granulata*) and *M. brachydactyla* (Castejón *et al.* 2019; Gebauer *et al.* 1998). In terms of megalopal survival, the size of artificial substrates (i.e. sands and nylon mesh), showed no significant effect on neither *N. granulata* nor *M. brachydactyla* (Castejón *et al.* 2019; Gebauer *et al.* 1998). Indeed, there were several research using artificial materials free from chemical cues as substrates, including glass beads (*Uca pagnax*, O'Conner & Gregg 1998), plastic strips (*Panopeus herbstii*, Weber & Epifanio 1996), and ribbons (*C. sapidus*, Forward *et al.*, 1996). These studies generally showed to be ineffective in affecting TTM of crab megalopae. Therefore, in comparison to tactile cues, it appears that chemical cues play a more important role in regulating metamorphosis of crab megalopae.

5.6 Conclusion

This study investigated a range of factors influencing megalopal survival and metamorphosis of the ornamental decorator crab, *C. retusa*, providing important knowledge and insight that help to improve the culture of megalopae of this species, a larval stage that often suffers high mortality. It was shown that a lower stocking density and increased basal area of cultural vessel helped to enhance megalopal survival, probably by reducing

interaction and cannibalism between megalopae. Conspecific adult exudates were found to not only improve survival but also to accelerate metamorphosis of megalopae, while juvenile exudates postponed metamorphosis. As such, it is recommended that when rearing *C. retusa* megalopae, adult-conditioned seawater should be used whenever possible, while newly moulted juveniles should be removed as soon as possible to enhance megalopal survival and avoid delayed metamorphosis. Finally, providing nylon nets of appropriate mesh size as substrates should also help to improve megalopal survival and juvenile production.

Chapter 6. Growth, development and sexual dimorphism of the decorator crab *Camposcia retusa* (Latreille, 1829) reared under individual culture system

6.1 Abstract

Captive bred first stage crabs (C_1) of the decorator crab, *Camposcia retusa,* a marine ornamental species, were reared in the laboratory to reach their market size (i.e. the tenth crab stage or C_{10}) to assess their development and growth pattern, as well as the time and the morphological features of the first appearance of sex dimorphism. During *C. retusa* juvenile development, the intermoult duration increased exponentially from 5.3 ± 1.2 days for C_1 to 47.5 ± 16.5 days for C_{10}. From C_1 to C_{10}, the relationship between development time 'y' and juvenile stage 'x' could be defined as $y = 4.910 \times e^{0.231x}$. The absolute increment of both carapace length and carapace width of juvenile crabs with progressive moulting also followed an exponential trend. Sexual dimorphism of *C. retusa* was first observed when the crabs moulted into the C_5 stage, with the main morphological difference being the appearance of 4 pairs of pleopods in females but only two in males. The survival of juvenile crabs showed variations at different stages: i.e. high survival at the C_1 stage (97.7%), but decreasing at the following C_2–C_4 stages (59.3–70.6%) before improving and stabilising for the subsequent stages up to C_{10} (83.3–93.8%). Based on results from this study, it is estimated that growout of newly settled C_1 to the market size could be achieved in about 7 months.

6.2 Introduction

The decorator crab, *Camposcia retusa,* is a popular marine ornamental crustacean species in the global marine aquarium trade (Calado *et al.* 2003a) due to their fascinating decorating

behaviour. As hitherto no successful captive breeding of the species has been reported, the current aquarium trade of the species is completely relying on the collection of specimens from the wild. In the attempt to captive breed *C. retusa* with the aim to reduce the reliance of wild collection to supply the aquarium trade, a series of studies were conducted focusing on improving larval rearing success of the species and the results are reported in Chapter 2–5. With the optimization of feeding regime and culture conditions, larval survival could reach up to 91.3% for zoeal stage and 84.0% for megalopal stage (Chapters 3–5). However, to achieve the aim of supplying the aquarium trade with marketable size *C. retusa*, the growout and development pattern of *C. retusa* juveniles also need to be investigated.

The life-history of most brachyurans can be divided into two distinct phases, i.e. the planktonic phase and the benthic phase (Anger 2001). The planktonic phase typically involves larval developmental stages; while during the benthic phase, the developmental stage prior to the pubertal moult is defined as the juvenile period (Hartnoll 1982). The juvenile development of brachyuran crabs typically consists of one or more sexual undifferentiated stages (i.e. external morphology shows no sexual differentiation), as well as one or more sexual differentiated but immature stages, during which, the sex of the juveniles can be distinguished by dimorphism of the pleopods (Hartnoll 1982).

Among Brachyurans, the number of sexually undifferentiated stages differ greatly between species. Some species, such as *Carcinus maenas* (Shen 1935) and *Liocarcinus depurator* (Guerao & Abelló 2011), only have one sexual undifferentiated stage, while in extreme cases, such as *Sesarma rectum,* juvenile development undergoes eleven sexual undifferentiated stages (Fransozo 1987). After the pre-pubertal moulting, the sexual undifferentiated stages end while a sexual differentiated but immature period starts, which also has a highly variable number of stages among species (Hartnoll 1982).

Study on juvenile development and growth, as well as associated morphological changes for new crab species for aquaculture is necessary because it provides crucial information for precise estimation of time required, costs involved and viability of growout hatchery

produced crablets to market size (Guerao & Rotllant 2009a; Guerao & Rotllant 2009b; Guerao & Rotllant 2010; Ingle & Rice 1984). Additionally, such information also assists in the identification of juvenile stages in aquaculture practice and other fields of studies (Ingle & Rice 1984), such as ecological, phylogenetic and phylogeographic studies (Bolla *et al.* 2014; Guerao & Rotllant 2009a). Indeed, as morphology of crab juveniles typically only undergoes minor changes, such information is often difficult to come by in field studies, but only possible when development and associated morphological changes are tracked with individually reared juveniles in the laboratory.

The pioneer study on brachyuran juvenile development and sexual dimorphism with the description of the common shore crab, *C. maenus,* to the ninth crab stage was conducted as early as in 1935 (Shen 1935). Hitherto, for the superfamily Majoidea, juvenile development has only been described for a very small number of species (Flores *et al.* 2002; Guerao & Rotllant 2009a; Guerao & Rotllant 2009b; Guerao & Rotllant 2010; Ingle 1977). As for family Inachidae, to which *C. retusa* belongs, only one species, i.e. *Inachus dorsettensis,* has been described up to the 9th crab stage (Ingle 1977). Rice and Hartnoll (1983) also studied the early juvenile development of *Dorhynchus thomsoni*, but only with a superficial description and estimation that sexual dimorphism might occur at the 3rd crab (C_3) stage. The scarcity of such information across all families of spider crabs makes the study of development and growth pattern, as well as the main morphological characteristics related to sexual dimorphism of *C. retusa* significant. The aim of this chapter hence was to describe and provide such information from newly settled first stage crabs up to the 10th crab stage using individually reared specimens.

6.3 Materials and methods

6.3.1 Broodstock management and larval culture

Broodstock crabs of *C. retusa* were purchased from Cairns Marine (Cairns, Queensland,

Australia), a commercial collector and whole seller of marine ornamentals. After being transported into the laboratory, the crabs were kept in pairs in 50 L acrylic tanks which connect with a recirculating system (water renewal rate at 90 L/min). The broodstock tanks were maintained constantly at water temperature 26–28 °C, salinity 34–36, photoperiod L: D = 14 h: 10 h, pH = 7.9–8.2, NH_4^+/NH_3 < 0.25 ppm, NO_2^- < 0.25 ppm and NO_3^- < 5.0 ppm. Chopped mussels and prawns, as well as thawed blood worms were provided twice a day as broodstock feed. Generally, under the aforementioned conditions, the egg incubation period lasted 21–25 days.

To collect the newly hatched larvae, a banjo filter was plugged in the outlet of broodstock tank at the night before larval hatching to prevent the larvae to be flushed out. The newly hatched zoeal larvae (Z_1) were collected via attracting to a light source then scooping out with as little water as possible, and then mass cultured followed by the protocol developed in previous chapters (Chapter 3–5). Briefly, the larvae were fed newly hatched *Artemia* nauplii at 10 ind./ml throughout the larval development. Water temperature was maintained at 27 ± 0.5 °C throughout larval culture but the salinity was set at 35 ± 0.5 for zoeal rearing but then adjusted to 33 ± 0.5 for megalopal rearing. The initial stocking density was set at 40 larvae/L but reduced to ca. 10 megalopae/L during the rearing of megalopae.

6.3.2 Live feed preparation

Newly hatched *Artemia* nauplii (INVE Thailand, Amphoe Wachiabarami, Thailand) were hatched and harvested daily. During the hatching period (about 23 h), water temperature and salinity was maintained at 26–28 °C and 34–35, respectively. Newly hatched *Artemia* nauplii were harvested the next morning for either feeding larvae immediately or subsequent enrichment. *Artemia* enrichment was carried out using S.presso (Selco S.presso®, INVE Aquaculture, UT, USA) enrich emulsion following the manufacture's instruction. Following enrichment, enriched *Artemia* were collected and thoroughly rinsed before being fed to the juvenile crabs. The density of *Artemia* was estimated using a Sedgewick-Rafter counting

chamber under the microscope (Leica LME).

6.3.3 Juvenile cultivation, development and growth measurement and morphology observation

Newly settled first stage crabs (C_1) found in the mass larval rearing vessels during daily routine checking in the morning were immediately collected and transferred to 300 ml plastic containers (diameter: 6.5 cm; high: 10 cm) to be cultured individually. Individual culture of juvenile crabs is necessary to enable a precise tracking of their development and growth, as well as morphological changes during ontogeny. A total of 130 C_1 crabs were used for this study, with these being initially cultured in one hundred thirty 300 ml vessels hanged along the walls of 50 L aquariums connected to the same recirculating system. The seawater circulating rate to each aquarium was approximately 40 L/h and a total of 107 small holes (diameter: 1 mm) were drilled on the wall and bottom of each container to allow water exchange. As young crabs grew bigger, the containers were replaced by larger circular cages (diameter: 8 cm, height: 15 cm) to ensure sufficient space for their movement. Juvenile crabs were initially fed *ad libitum* on enriched *Artemia* metanauplii, but from C_3 onwards, thawed commercially available adult *Artemia* and mysis (Hikari Bio-pure®, Hikari, USA) were also added as feeds. Once juveniles had moulted into C_6, they were fed thawed adult *Artemia* and mysis only. Uneaten *Artemia* was removed by syphoning each morning before adding new feed, while the uneaten thawed feed was removed about 5–6 hours after it was offered to juvenile crabs. Throughout juvenile crab cultivation, water parameters were maintained at 26–28 °C, salinity 34–36; photoperiod L: D = 14 h: 10 h, pH 7.9–8.2, NH_4^+/NH_3 and NO_2^- < 0.25 ppm, and NO_3^- < 5.0 ppm.

Every morning, any new moulting and mortality of crabs were checked and recorded, with data collected being then used for calculating the intermoult duration and survival (Sur%) of each crab stage. Exuviae were also collected for measurement of growth (carapace size) and morphology observation. For early-stage crabs (C_1–C_5), carapace length (CL) and

carapace width (CW) were measured under a microscope (Leica LME), but for large later stage crabs (C_6–C_{10}), the measurements were carried out using a digital vernier calliper (IP67 1199W-616, United Precision Machine Inc., China). The measurements of pleon width (PW) were undertaken using a microscope (Leica LME) until C_8, but a digital vernier calliper was used for C_9 and C_{10} crabs. CL was defined as the distance between the rostral margin and the posterior margin of the carapace, while CW was the greatest distance across the carapace. PW was measured as the widest distance of the 6th somite of pleon of the crabs (Guerao & Rotllant 2009a). Percentage increment of carapace length (% increment in CL) at each moult, as compared to that of the pre-moult stage, was calculated using the following formula:

$$\%\ increment\ in\ CL = \frac{CL\ post\ moult - \ CL\ pre\ moult}{CL\ pre\ moult} \times 100$$

Since decorating behaviour was observed as early as at C_1 stage of *C. retusa* (Chapter 2), once the megalopae had metamorphosed to C_1, typically their exoskeleton is quickly covered by various decorative materials, which are almost impossible to remove without hurting the delicate early juveniles. Hence, weight measurement is highly inaccurate for the species. For this reason, the weight of juvenile crabs was not measured in this study. Due to the fragile nature of exuviae and the fact that *C. retusa* juveniles often re-use the decorating materials on their exuviae, and sometimes even the exuviae, for decoration, it was difficult to obtain the same number of intact specimens for each juvenile stage, especially from C_6 stage onwards. Thus, the number of specimens used for morphological observation and description at each juvenile stage was different (Table 6.1).

Dissection of juvenile exuviae and observation of morphological changes was undertaken under a stereomicroscope (Nikon SMZ256). As the morphological characters of *C. retusa* first crab stage have been described in detail in Chapter 2 (Section 2.4, Figs. 2.5–2.6); in this chapter, the morphological description will therefore focus on the development of sex dimorphism (i.e., morphological changes in pleon and pleopods) during the ontogeny from C_2 to C_{10} stage.

6.3.4 Statistical analysis

Develop (intermoult duration) and growth data (CL and CW) were analysed using non-linear regression to determine their pattern during juvenile crab ontogeny. From the stage when external sexual dimorphism was observed, carapace size (CL and CW) and shape (CL/CW), PW, as well as the ratio of PW/CW were also compared between males and females using a general linear model ($\alpha = 0.05$). The data of size measurements were log-transformed to normalizes the residuals prior to the analysis. All analysis was carried out using R language (R 4.0.3, R Core Team, 2020).

6.4 Results

6.4.1 Juvenile development and growth pattern

Since there were no significant differences between males and females in both development and growth indices measured in this study, the growth data of both sexes were pooled for various calculations (Table 6.1).

The mean intermoult duration of each juvenile crab stage from C_1 to C_{10}, which represents the period in days between two consecutive moults, is shown in Table 6.1. On average, the development time from C_1 to C_{10} was 7 months. The mean intermoult duration increased substantially from 5.3 ± 0.1 days at C_1 stage to 47.5 ± 4.4 days for C_{10} stage (Fig. 6.1). Non-linear regression of the data showed that the relationship of the intermoult duration increase with juvenile ontogenetic development can be described by an exponential model: $y = 4.910 \times e^{0.231x}$ (R^2 =0.748), where y represents the juvenile development time (days) and x represents the juvenile stage. It was also observed that for early crab stages, moulting was substantially more synchronised, for example, 87.4% of C_1 crabs (i.e. 111 out of 127) moulted to C_2 stage within 3 consecutive days. On the other hand, variations in the intermoult duration increased greatly after C_4, and particularly for C_7–C_{10}, when the difference of

intermoult duration between different individuals for a same crab stage could be more than 50 days

Figure 6.2 illustrates the trajectory of growth from C_1 to C_{10} and regression results of *C. retusa* juveniles in terms of carapace length (CL) and carapace width (CW). Since no significant difference was detected for both CL and CW between males and females (p = 0.654 and 0.924 for CL and CW, respectively), CL and CW were calculated by pooling data of both sexes together. The result showed that CL of *C. retusa* juveniles increased from 1.58 mm at C_1 to 10.26 mm at C_{10}, which represented a 549% increase over the development period and can be described by an exponential function: $y = 1.073 \times e^{0.225x}$ where y represents the juvenile development time (days) and x represents the juvenile stage (R^2 = 0.936; Fig. 6.2A). Meanwhile, CW increased from 1.15 ± 0.02 mm at C_1 to 7.12 ± 0.34 mm at C_{10}, which likewise can be described by an exponential function: $y = 0.825 \times e^{0.214x}$ (R^2 = 0.921; Fig. 6.2B) and represented a 519% increase. It is apparent that at each juvenile stage, the ratio between CW and CL or CW/CL largely remained constant (i.e. 1.38 ± 0.03 to 1.45 ± 0.03; Table 6.1). Indeed, CW/CL value at each juvenile stage can be expressed by a linear equation: $y = 1.440x - 0.123$, where y represents the juvenile development time (days) and x represents the juvenile stage (R^2 = 0.995; Fig. 6.3). Throughout juvenile development, the relative increment of carapace length at each moult (% increment in CL) as compared to the previous crab stage appeared to be generally bigger from C_7 onwards (ranging from 15.7 to 23.1% for C_1–C_6, but was 27.7%, 43.2%, 18.1% and 26.7% for C_7, C_8, C_9, and C_{10}, respectively) with the biggest increase occurred at C_8 stage (43.2%) while the smallest increment (15.7%) happened at C_6 stage (Table 6.1). Figure 6.4 illustrates changes in both carapace size and shape throughout *C. retusa* juvenile stage sequence from C_1 to C_{10} and absolute values of CL, CW and CL/CW and% increment in CL of each juvenile stage are shown in Table 6.1.

Table 6.1. Intermoult duration, carapace length (CL), carapace width (CW), CL/CW ratio, relative carapace length increment at each moult (% increment in CL) and survival at each juvenile crab stage of decorator crab *Camposcia retusa*.

Juvenile stage	Intermoult duration (days)	Survival at each crab stage (%)	Number of specimens for growth (CL & CW) measurements	CL (mm)	CW (mm)	CL/CW	% increment in CL
C_1	5.2 ± 0.1	97.7	10	1.58 ± 0.02	1.15 ± 0.02	1.38 ± 0.02	
C_2	7.0 ± 0.2	67.7	10	1.86 ± 0.01	1.33 ± 0.01	1.40 ± 0.01	17.7
C_3	9.8 ± 0.4	59.3	10	2.29 ± 0.02	1.67 ± 0.02	1.37 ± 0.02	23.1
C_4	11.8 ± 0.6	70.6	10	2.72 ± 0.05	2.04 ± 0.05	1.34 ± 0.03	18.8
C_5	16.7 ± 0.8	83.3	10	3.24 ± 0.05	2.50 ± 0.05	1.30 ± 0.02	19.1
C_6	23.5 ± 1.3	83.3	9	3.75 ± 0.11	2.59 ± 0.10	1.45 ± 0.03	15.7
C_7	25.9 ± 3.1	84.0	10	4.79 ± 0.21	3.46 ± 0.17	1.39 ± 0.02	27.7
C_8	33.2 ± 2.6	85.7	7	6.86 ± 0.48	4.93 ± 0.37	1.40 ± 0.03	43.2
C_9	38.1 ± 4.1	88.9	6	8.10 ± 0.52	5.61 ± 0.37	1.45 ± 0.03	18.1
C_{10}	47.5 ± 4.4	93.8	7	10.26 ± 0.42	7.12 ± 0.34	1.45 ± 0.02	26.7

Data are shown as mean ± SE. C_1–C_{10}: the first to tenth juvenile crab stages.

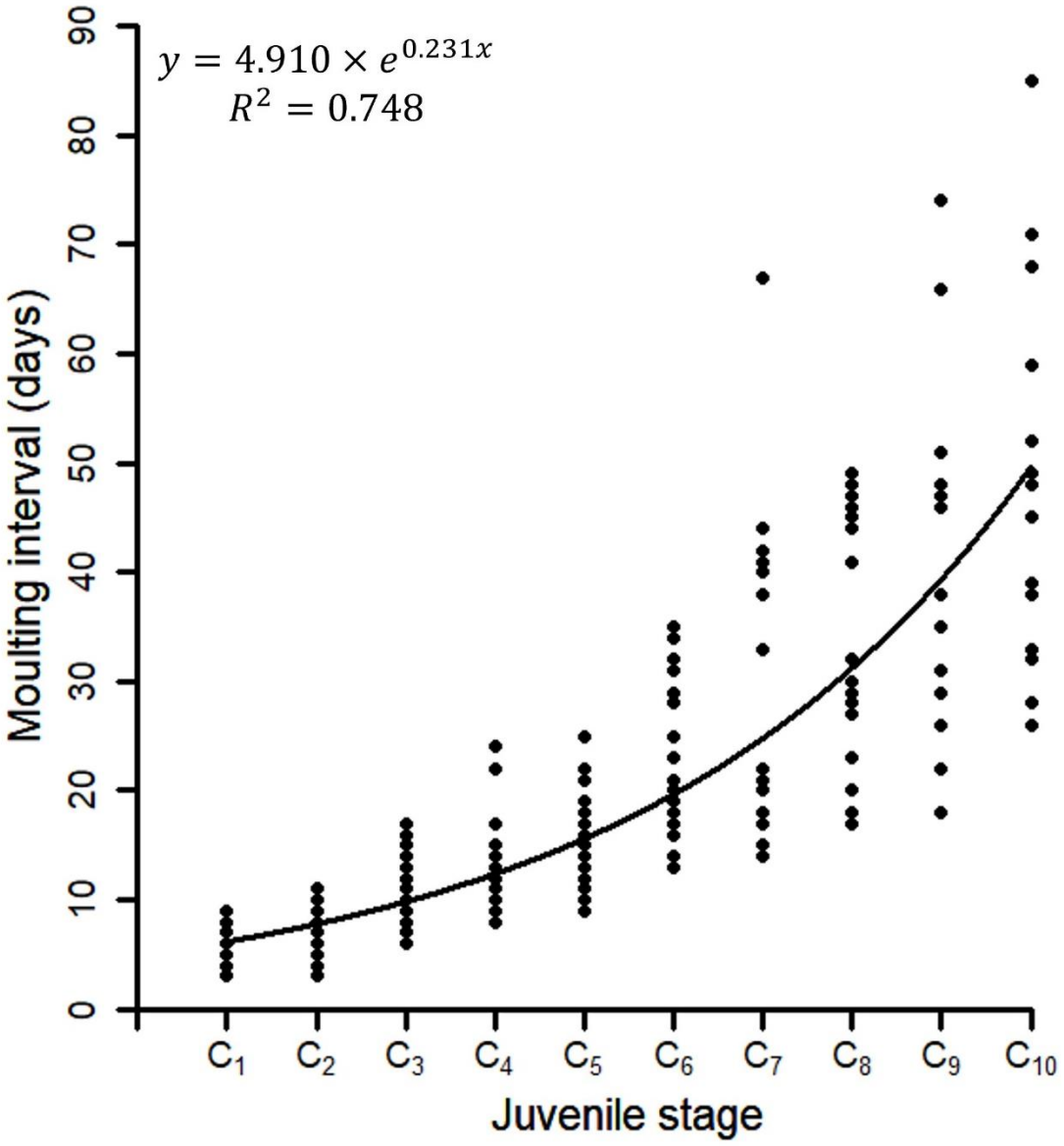

Figure 6.1. The intermoult duration of *Camposcia retusa* juveniles from the first crab (C_1) stage to the tenth crab (C_{10}) stage. y: cumulative development time to a juvenile crab stage (days); x: juvenile crab stage. Please note that some data points are overlapped.

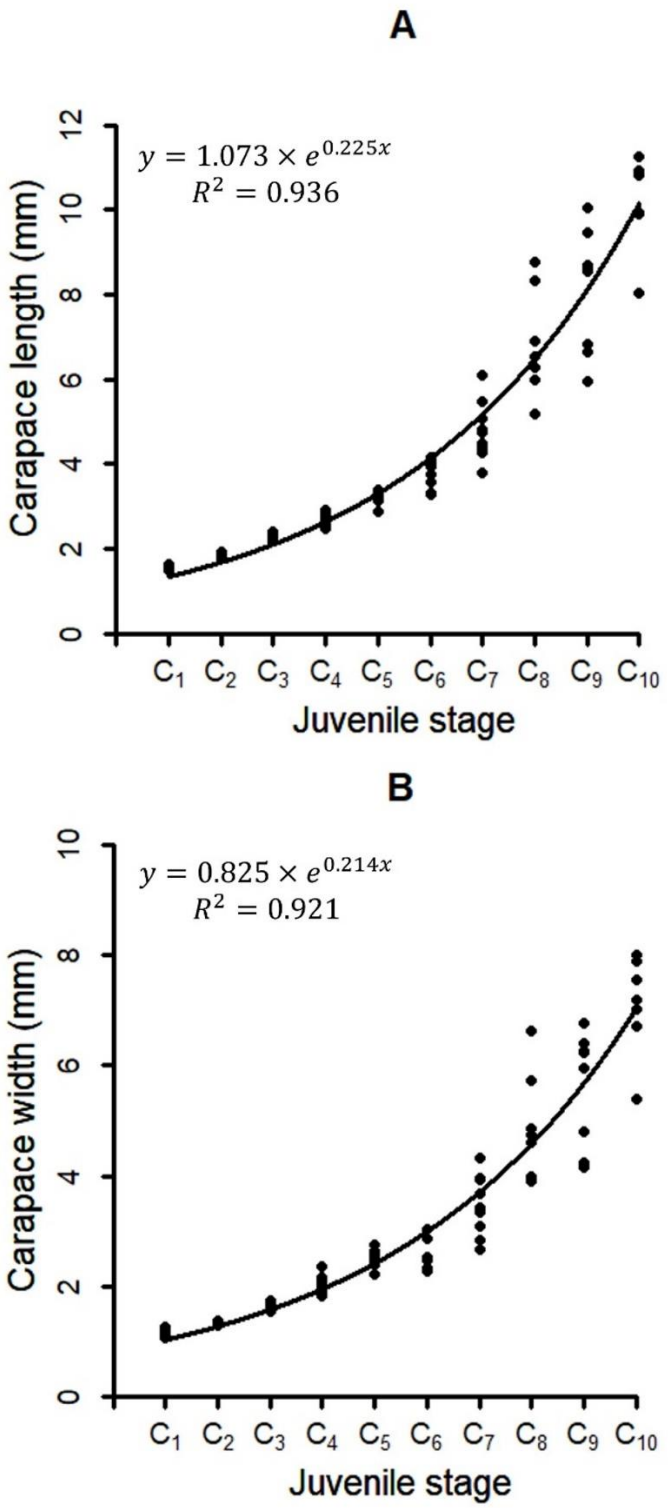

Figure 6.2. Growth trajectory and result of regression in term of carapace length (A) and carapace width (B) of *Camposcia retusa* juveniles from the first crab (C_1) stage to the tenth crab (C_{10}) stage. y: carapace length or width (mm); x: juvenile stage. Please note that some data points are overlapped.

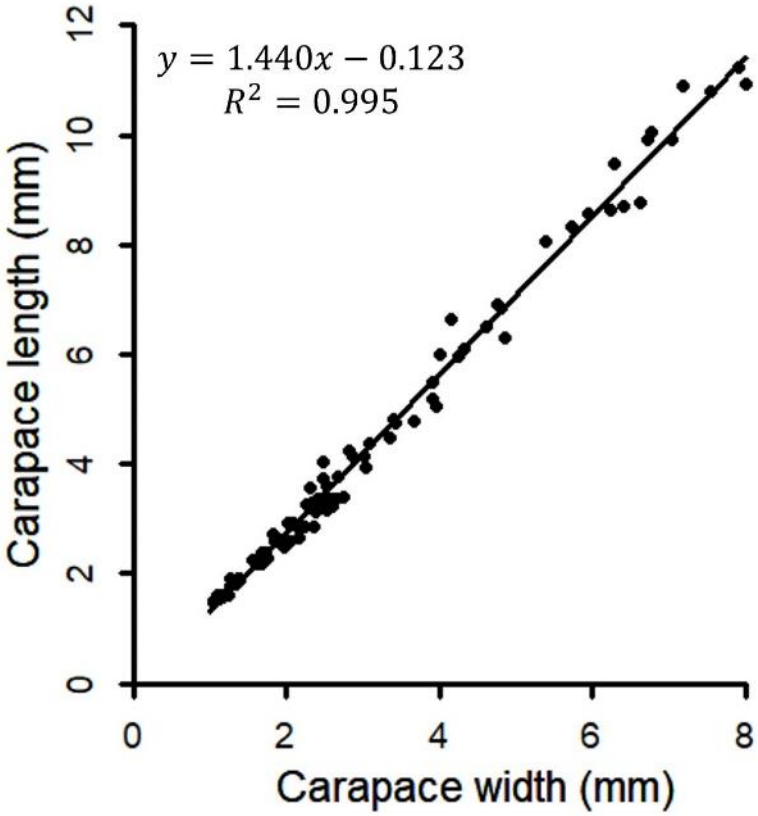

Figure 6.3. Relationship between carapace length and width of *Camposcia retusa* juveniles from the first crab (C_1) stage to the tenth crab (C_{10}) stage. y: carapace length (mm); x: carapace width (mm). Please note that some data points are overlapped.

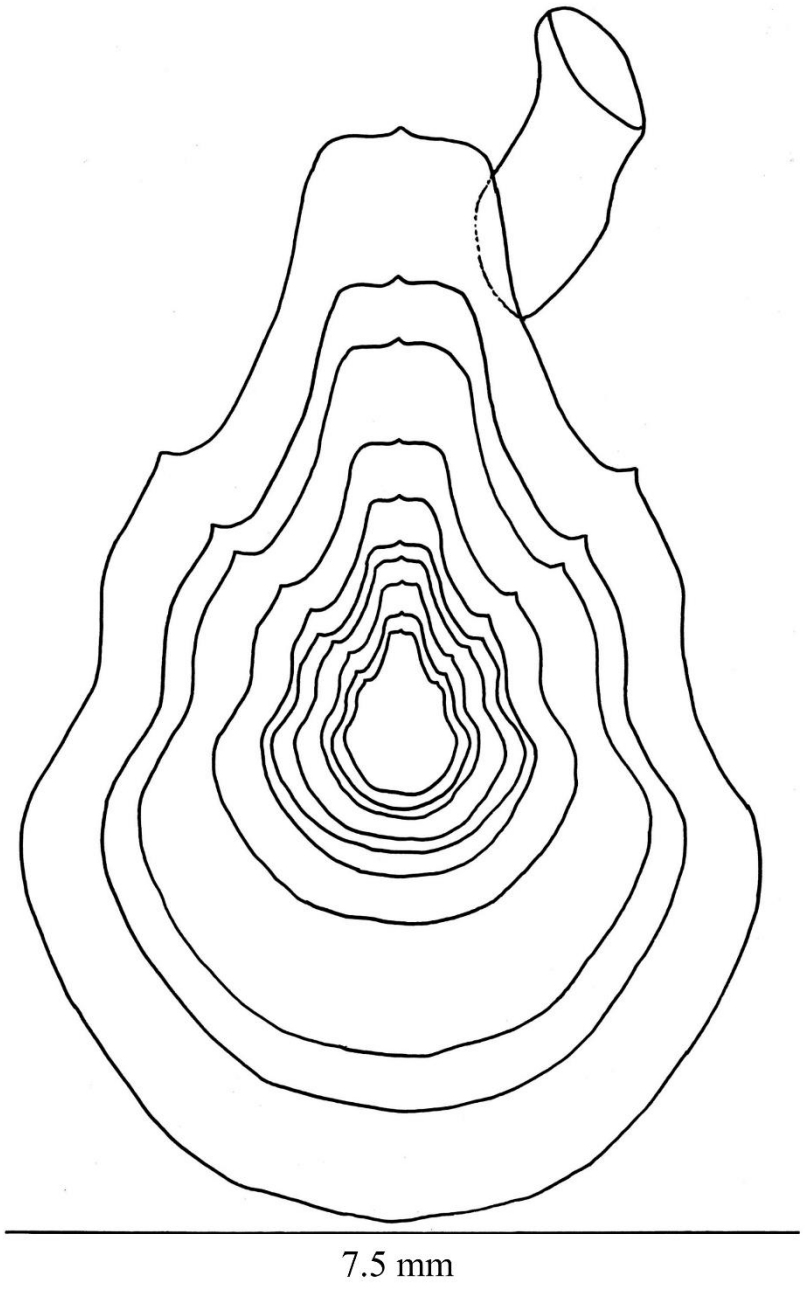

Figure 6.4. *Camposcia retusa*. Dorsal view of the change of carapace shape and size from the first crab (C_1) stage to the tenth crab (C_{10}) stage. Scale bar = 7.5 mm.

Since all juvenile crabs were cultured individually, survival data was calculated by pooling all cultures together. The result showed large variation in *C. retusa* juvenile survival at different developmental stages: C_1 stage had a very high survival (97.7%), but lower survival was observed in the following C_2 –C_4 stages (59.3–70.6%) with the lowest survival (59.3%) being found at C_3 stage. The juvenile survival subsequently improved and stabilised at > 83.4% between C_5–C_{10} stages (83.3-93.8%; Table 6.1).

6.4.2 Development of sexual dimorphism

The major morphological changes during *C. retusa* juvenile development from C_1 to C_{10} are illustrated in Figs. 6.4–6.8. As described in Chapter 2, even at C_1 stage, the surface of carapace and pleon of *C. retusa* are already covered by thick setae (Figs. 2.5A & 2.6I). It was observed that both the number and length of the setae increased dramatically during juvenile ontogeny. For clarity of the illustration, setae on carapace and pleon are not shown on these figures.

Although no significant difference was found in both carapace shape and size of males and females during *C. retusa* development from C_1 to C_{10}, sexual dimorphism started to appear at C_5 stage, mainly as morphological differences of pleopods (Figs. 6.5–6.8). When *C. retusa* first settled as C_1 crabs, they possessed four pairs of pleopods and one pair of uropods (Fig. 6.6). However, from C_2 stage onward, uropods became absent. While the 4 pairs of pleopods were still present at both C_2 and C_3 stages, their sizes reduced with each moult. When *C. retusa* entered C_4 stage, for both males and females, only one pair of pleopods remained (Fig. 6.6) and no morphological difference was observed between males and females. However, once moulted to C_5 stage, there were four pairs of pleopods in females appearing on the 2^{nd}–5^{th} somites of pleon, while in males, only two pairs of pleopods are present (Fig. 6.8). During the subsequent juvenile development, in females, all four pairs of pleopods increased in length at successive crab stages, and all of them became biramous from

C_7 stage onward, while setae appeared on pleopods from C_8 onward (Fig. 6.8). On the other hand, in males, mainly the first pair of pleopods increased in length at successive crab stages with longitudinal groove setae showing up at C_8 stage. The second pair of pleopods only slightly increased in size from C_8 to C_{10} stage and no setation was observed up to C_{10} stage (Fig. 6.8). The shape of the pleon between males and females was similar throughout the crab stages examined from C_1 to C_{10}. Despite the 5^{th}–6^{th} somites of the pleon being slightly wider in females than in males from C_5 onward (Fig. 6.7), the difference of PW ($p = 0.903$) and PW/CW ($p = 0.079$) was not statistically significant (Table 6.2).

Table 6.2. Comparison of pleon width (PW) and the ratio of PW and carapace width (CW) of female and male *Camposcia retusa* juveniles from crab stage 5 to 10 (C_5–C_{10}). Statistical analysis using general linear model ($\alpha = 0.05$).

Juvenile stage	**PW (mm)** $p = 0.903$		**PW/CW** $p = 0.079$	
	Female	**Male**	**Female**	**Male**
C_5	0.90 ± 0.01	0.84 ± 0.02	0.35 ± 0.01	0.34 ± 0.00
C_6	1.07 ± 0.04	0.97 ± 0.04	0.42 ± 0.01	0.36 ± 0.01
C_7	1.51 ± 0.10	1.20 ± 0.05	0.31 ± 0.06	0.38 ± 0.01
C_8	2.42 ± 0.26	1.69 ± 0.05	0.40 ± 0.02	0.39 ± 0.00
C_9	2.41 ± 0.30	2.64 ± 0.17	0.46 ± 0.01	0.40 ± 0.02
C_{10}	4.78 ± 0.73	3.34 ± 0.13	0.53 ± 0.05	0.47 ± 0.00

Data are shown as mean ± SE. C_5–C_{10}: the fifth to tenth juvenile crab stages.

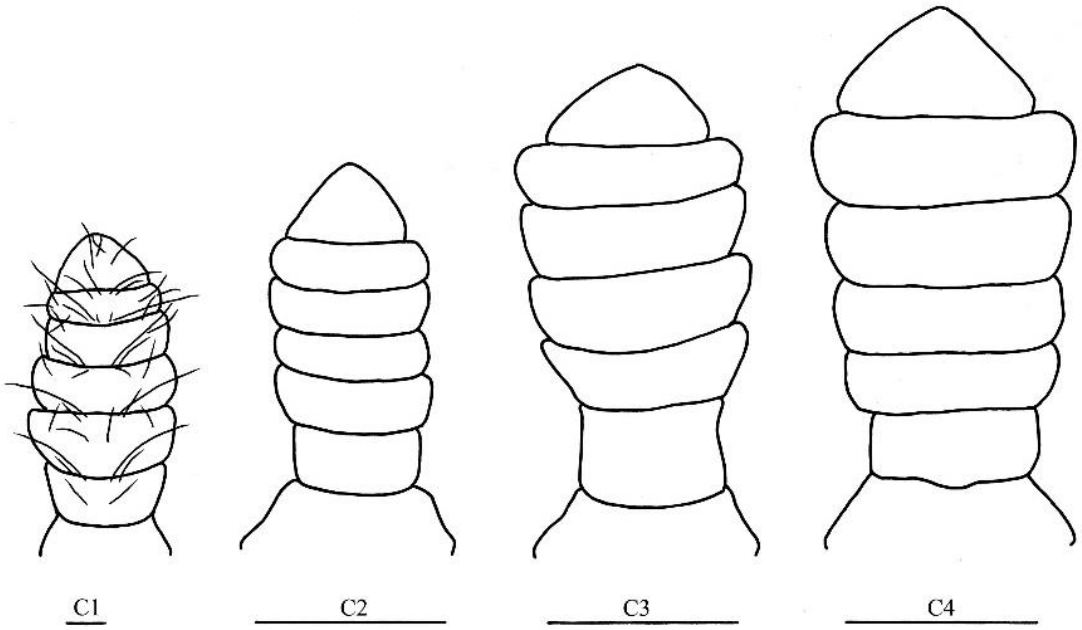

Figure 6.5. *Camposcia retusa*. Pleon of juvenile crab stages 1–4 (C_1–C_4). Scale bars: C_1 = 0.15 mm; C_2–C_4 = 1 mm.

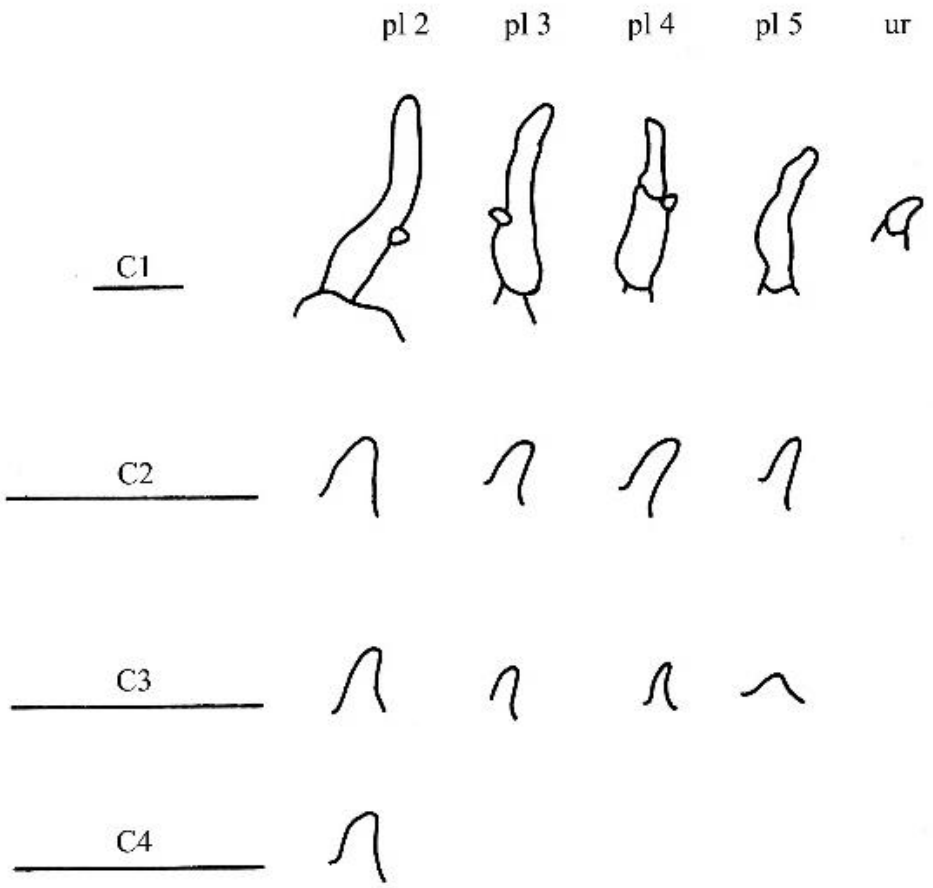

Figure 6.6. *Camposcia retusa.* Pleopods of juvenile crab stages 1–4 (C_1–C_4). Scale bars: C_1 = 0.15 mm; C_2–C_4 = 1 mm. pl 2, pl 3, pl 4 and pl 5 stands for pleopods present on somites 2–5, respectively; ur stands for uropods.

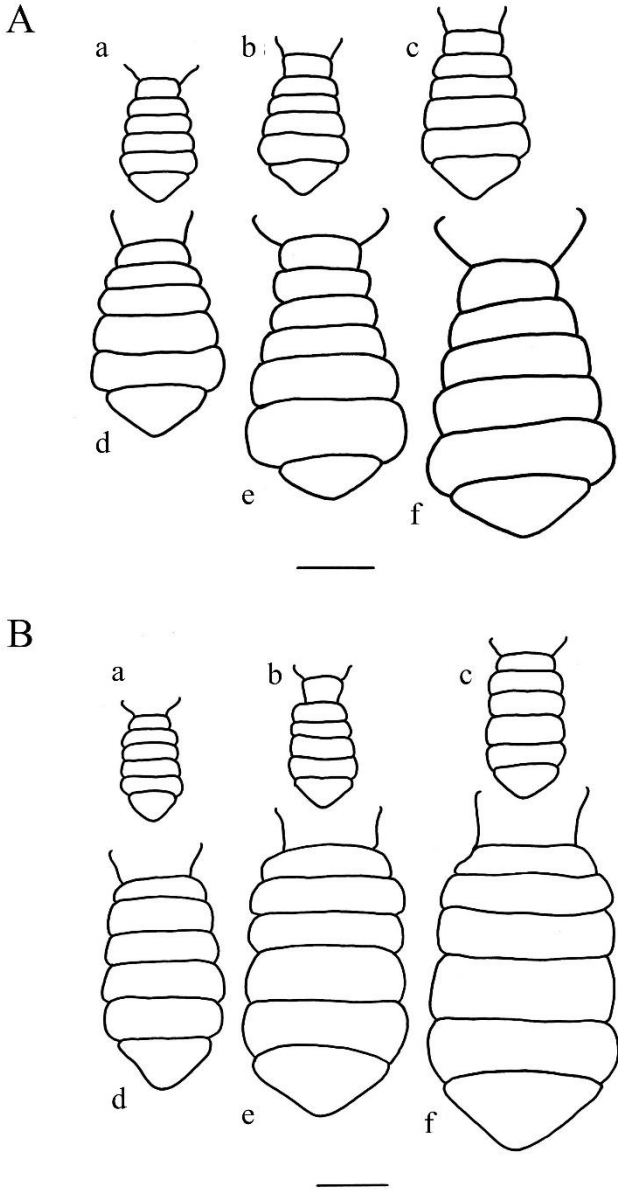

Figure 6.7. Pleon of female (A) and male (B) *Camposcia retusa* juvenile from crab stage 5 to 10 (C_5–C_{10}). a: C_5; b: C_6; c: C_7; d: C_8; e: C_9; and f: C_{10}. Scale bars = 1mm.

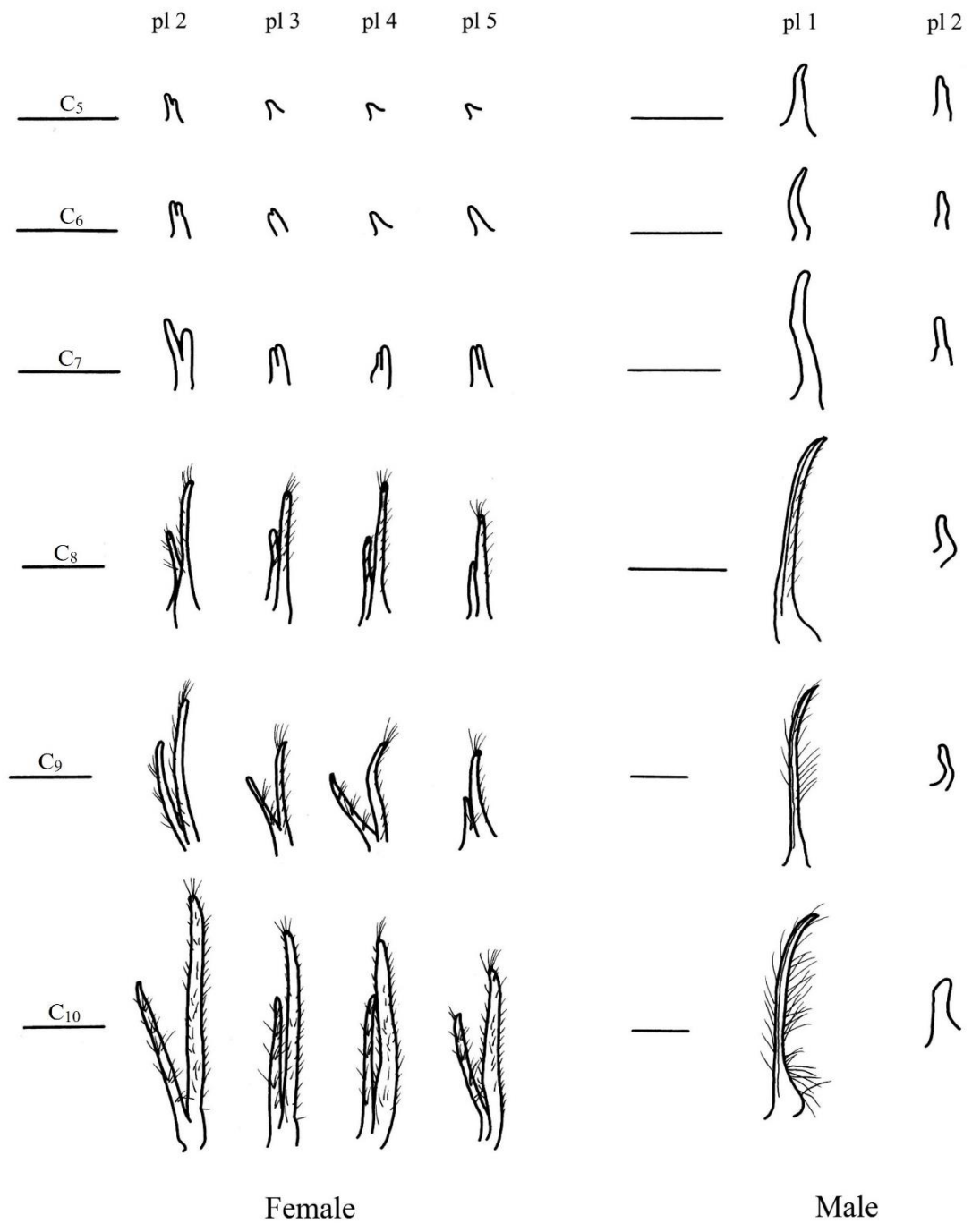

Figure 6.8. Comparison of pleopod morphology of female and male juvenile of *Camposcia retusa* from crab stage 5 to10 (C_5–C_{10}). Scale bars: C_5–C_7 = 0.5 mm; C_8–C_{10} = 1 mm. pl 1, pl 2, pl 3, pl 4 and pl 5 stands for pleopods present on somites 1–5, respectively.

6.5 Discussion

Our result showed that during the ontogeny from C_1 to C_{10}, the increase in intermoult duration of *C. retusa* at successive crab stages followed an exponential model, which is typical for decapods (heterochronal development) (Anger 1984; Guerao & Rotllant 2010). The average cumulative development time from C_1 to C_{10} was 219 days or about 7 months, and the mean carapace length of C_{10} was 10.26 ± 0.42 mm. Since with their elongated appendages, total length of *C. retusa* juveniles typical are about 4 to 6 time of that the carapace length, *C. retusa* C_{10} crabs are considered having reached market size. Such a result suggests that the timeframe for growout of newly settled C_1 crabs to market size is around 7 months, with a price tag around US$ 40–50 for *C. retusa* on international market (based on website of LiveAquaria - https://www.liveaquaria.com/product/627/?pcatid=627, accessed 4/11/2021), this shows a good potential for commercial culture. While outside the scope of this work, with further studies to optimise the culture conditions and feeds used, such a growout timeframe can indeed be further shortened.

It is worth noting that based on the result of this study, asynchronous moulting among individuals commonly existed during *C. retusa* juvenile development, particularly from C_7 onward, which could lead to a higher risk of cannibalism. However, Guerao and Rotllant (2009b) suggested that when proper feeds were provided, cannibalism was not a major issue for juveniles of another spider crab *M. brachydactyla,* even during their moulting. Similarly, moulting intracohort of *Chionocetes opilio*, another majoid crab, was reported as not being particularly vulnerable to cannibalism (Sainte-Marie & Lafrance 2002). As for *C. retusa* juveniles, the possibility of communal rearing and associated cannibalism risk is not known, which warrants further study.

The increment of CL and CW of *C. retusa* juveniles during successive moults followed an exponential model. Similar to intermoult duration, variations of both CL and CW among individual crabs increased significantly from C_7 onward. The carapace length increment at each moult (% increment in CL) from C_1 to C_5 were generally lower than those from C_6

onward, such a trend is somewhat different from other species from superfamily Majoidea, such as *M. brachydactyla* and *M. squinado*, which tended to have a lower moult increment at older juvenile stages (C_5–C_8; Guerao & Rotllant 2009b; Guerao & Rotllant 2010). Such a difference could be explained by a species-specific growth pattern but could also be related to the fact that in this study, juveniles were transferred to larger cages after C_6 stage, which enhanced size increment at each moult. Prior to the transfer, the moult increment could be constrained due to the small space that young crabs were reared in. Hartnoll (1982) suggested that moult increment in crustaceans can be significantly influenced by captive conditions they were kept in; and in many cases, the increment in captivity is different from, and more likely smaller, to that recorded in the wild.

As in this study all juvenile crabs were cultured individually, survival data were calculated by pooling all crabs cultured together, hence without replicates. The survival at each crab stage showed substantial differences: C_1 had nearly 100% survival (97.7%) while good survival ($\geq$ 83.4%) was also found for older crab stages from C_5–C_{10}; however, high mortality occurred between C_2 to C_4, which accounted for around 70% of total mortality from C_1 to C_{10}. The cumulative survival of *C. retusa* from C_1 to C_{10} was at 12.3%; compared to previous reports rearing juvenile crabs of other species in similar individual rearing systems, such a survival is substantially lower than that of another spider crab *Maja brachydactyla* (43.75% to C_8, Guerao & Rotllant 2009b) and the Chinese mitten crab *Eriocheir sinensis* (38% to C_{10}, Yang *et al.* 2018), but much higher than that of *Maja squinado* (5.8% to C_8, Guerao & Rotllant, 2010). Unlike *M. brachydactyla* and *E. sinensis*, for which juvenile rearing techniques have been studied in detail (Alaminos & Domingues 2008; Wen *et al.* 2006; Yuan *et al.* 2017), no prior knowledge exists on appropriate feeds and suitable environmental conditions for *C. retusa* juveniles. In the present study, the high mortality recorded could be caused by excessive handling during culture. It was reported that early juveniles of *M. squinado* were very delicate and handling stress was the major cause of their mortality (Guerao & Rotllant 2010), which can also be the case for juvenile *C. retusa*, in particularly during the C_2–C_4 stages. In this study, such handling stress could occur by daily siphoning of leftover feeds, as well as exuviae collection (for C_2–C_3 juveniles, the small and

tall 300 ml culture vessels used made exuviae collection difficult). Due to the frail nature of these young juveniles, regular daily handling likely impacted their survival more severely. As juveniles grew larger, their tolerance to handling stress increased, which likely contribute to an improved survival of over 83% from C_5 onward. In addition, it was observed that *C. retusa* started their characteristic decorating behaviour right after metamorphosing from megalopae to C_1. Since in this study no shelter or decorating materials was provided in the individual rearing unit used for juvenile rearing, some crabs were found decorating themselves with rotten uneaten food, which could lead to degrading water quality within the culture units. Moreover, the feeds used for feeding the crabs in the present trial probably are not optimal in term of size and nutritional contents, and nutrition deficiencies is known to result in poor condition of crabs with low resistance to stresses, which can lead to high mortality (Hamasaki *et al.* 2002b; Wen *et al.* 2006).

Sexual dimorphism in *C. retusa* was found to first appear at C_5 stage with morphological differences in pleopods between males and females. In other majoid crabs, external sexual differentiation generally appears earlier than in *C. retusa* during juvenile ontogeny as summarised in Table 6.3. For example, in *Pyromaia tuberculata*, pleopod morphology differentiation in males and females occurred as early as the C_2 stage (Flores *et al.* 2002). For the species previously referred, there were no pleopods at C_1, while in the crab stage of *C. retusa* just before sexual dimorphism appearance (C_4), pleopods were also highly vestigial, with only one pair of pleopods present existed. In *I. dorsettensis*, sex could be distinguished at the C_3 stage (Ingle 1977), similarly, pleopods on somites 2–5 were reduced to minute buds at C_2 stage. For two large spider crab species belonging to genus *Maja*, *M. brachydactyla* and *M. squinado*, sex can be distinguished at C_4 stage, and one stage before the appearance of external sexual differentiation, pleopods at C_3 were also minute with uropods absent (Guerao & Rotllant 2009a; Guerao & Rotllant 2010).

Table 6.3. Species of superfamily Majoidae with at least one juvenile crab stage described.

Family	Species	Crab stages described	Crab stage at which sexual dimorphism first appears	Reference
Inachidae	*Camposcia retusa*	C_1–C_{10}	C_5	This study
	Inachus dorsettensis	C_1–C_9	C_3*	Ingle (1977)
	Inachus leptochirus	C_1	?	Clark (1983)
	Inachus phalangium	C_1	?	Clark (1983)
	Inachus thoracicus	C_1	?	Guerao *et al.* (2002)
	Dorhynchus thomsoni	–	C_3**	Rice and Hartnoll (1983)
Inachoididae	*Pyromaia tuberculata*	C_1–C_9	C_2*	Flores *et al.* (2002)
Majidae	*Maja brachydactyla*	C_1–C_8	C_4*	Guerao and Rotllant (2009a)
	Maja squinado	C_1–C_8	C_4*	Guerao and Rotllant (2010)
Pisinae	*Lissa chiragra*	C_1	?	Guerao *et al.* (2003)
	Pisa armata	C_1	?	Ingle and Clark (1980)
	Pisa tetraodon	C_1	?	Rodríguez (1997)

*: The crab stage of sexual dimorphism appearance was identified via pleopod morphological differences; **: the crab stage of sexual dimorphism appearance was estimated by juvenile size difference; ?: the crab stage at which sexual dimorphism first appears was not identified.

Similar to the majoid species, sexual dimorphism of brachyuran species belonging to other superfamilies (e.g., Grapsidae, Portunidae, Xanthoidea) is also largely based on pleopod morphology. It was reported that sex can be distinguished at C_2 stage for *Carcinus maenas* (Shen 1935), *Liocarcinus depurator* (Guerao & Abelló 2011), *Pachygrapsus transversus* (Flores *et al.* 1998) and *Pachygrapsus gracilis* (Arruda & Abrunhosa 2011), at C_3 stage for *Achelous spinimanus* (Bolla & Fransozo 2016), *Eriocheir japonicus* (Lee *et al.* 1994) and *Uca cumulanta* (Hirose *et al.* 2010), and at C_4 stage for *Callinectes danae* (Bolla *et al.* 2014), *Callinectes ornatus* (Bolla *et al.* 2008), *Eurytium limosum* (Guimarães & Negreiros-Fransozo 2005) and *Leptodius exaratus* (Lwin *et al.* 2007). The appearance of sex dimorphism in species from family Sesarmidae appears to be generally later, for example, at C_5 stage for *Armases rubripes* and *Chiromantes ortmanni* (Guerao *et al.* 2012; Negreiros-Fransozo *et al.* 2011), and up to C_{12} stage for *Sesarma rectum* (Fransozo 1987).

Pleon shape and size is another secondary sexual characteristic of brachyurans, which is related to different functions of pleopods in males and females (Hartnoll 1974). That is, pleopods of males become intromittent organs that are covered and supported by the pleon, while in females, pleopods are the structures that developing eggs attach to while pleon serves as an incubatory chamber (Hartnoll 1974). As the result, the pleon of females is generally larger than that of males (Marochi *et al.* 2019). However, the difference of PW/CW ratio between males and females was not evident up to the oldest juvenile stage (C_{10}; p = 0.079) examined in this study. Previous reported sexual differentiation in pleon was first observed at C_9, C_{10}, C_{11}, C_{12} stage for *A. spinimanus*, *A. rebripes*, *C. ornatus*, and *C. danae*, respectively, but pleopod sex dimorphism was observed between C_3–C_5 stages for these species (Bolla & Fransozo 2016; Bolla *et al.* 2014; Bolla *et al.* 2008; Negreiros-Fransozo *et al.* 2011). Likewise, in *P. gracilis* and *U. cumulanta*, morphological differentiation in the pleon between males and females was observed at C_6 and C_7 stage, respectively, which was 4 stages after pleopod sexual dimorphism being observed for both species (Arruda & Abrunhosa 2011; Hirose *et al.* 2010). Thus, it appears that sexual dimorphism of the pleon generally occurs later than that of pleopods in brachyurans.

6.6 Conclusion

Both development and growth of *C. retusa* juveniles showed an exponential pattern, based on the results obtained, it is estimated that on average, newly settled first stage crabs can be reared to reach market size around 7 months, when they reach the10^{th} carb stage. The survival of juvenile crabs showed substantial variations at different crab stages with low survival occurring at C_2–C_4 stages, revealing that further research is required to identify optimal feed and improve culture environment and handling of young crabs. Sexual dimorphism in *C. retusa* first occurred at C_5 stage, as evidenced by morphological differences in pleopods, which is generally later than other majoid species. Hitherto, post-larval development and growth pattern, as well as sexual dimorphism in decapod crustaceans have only been studied in relatively few species. Such information is important for estimating time required for growout, as well as aquaculture potential for new target species; moreover, this information can also be useful for their fishery management.

Chapter 7. General discussion and conclusions

With the aim of developing effective captive breeding techniques for the decorator crab *Camposcia retusa*, this thesis covered a broad range of relevant topics, including complete larval morphology description of the species for the first time, larval feeding regimes from newly hatched larvae to first stage crabs, a range of environmental requirements for larval rearing, and juvenile development to market size. This thesis established a reliable and effective larval culture protocol for the popular marine ornamental crustacean that has never be bred before. The practical and economical application of commonly used hatchery live prey for larval culture and the identification of appropriate culture conditions were the prime focus of the study, which should allow to be easily employed for commercial production. The major outcomes of the thesis are summarised below, including their broader implications for developing captive breeding techniques for marine ornamental crabs where applicable.

Chapter 2. Morphological descriptions of the larval and first juvenile stages of the decorator crab *Camposcia retusa* (Latreille, 1829) from laboratory-reared material

The complete development and morphology of all larval stages and first crab stage of *C. retusa* was described for the first time based on laboratory reared materials. The larval development of *C. retusa* includes two zoeal and one megalopal stage. When compared with other species from family Inachidae, although larval development and morphology of *C. retusa* follows general patterns for majoid crab, some major differences were observed and summarised (Table 2.1–2.3). A previous report on the larval morphology of *C. retusa* (Gohar & Al-Kholy 1957) is insufficient in details and accuracy, and does not conform to the current standard of crab larval morphological description. There are major differences, including larval size, segmentation and setation, between the two studies (Table 2.4), considering the fact that Gohar and Al-Kholy (1957) failed to rear the larvae to pass the Z_1 stage, and their description of Z_2 and megalopal larvae were based on wild-collected plankton, it is possible

that the specimens used by their study may not be those of *C. retusa*. This chapter also included the morphological description of *C. retusa* first crab stage, which was not reported by Gohar and Al-Kholy (1957). A prominent feature of the first crab (C_1) was that there are long and curved simple setae, as well as hooked setae densely covering the carapace, pereiopods and pleon, suggesting that their ability to decorate can occur as early as at the beginning of their juvenile life.

This chapter also provides summary of major larval morphological differences of species from different taxonomic groups of family Inachidae. For the first crab stage, however, due to a scarcity of information (only two species have been described), similar comparisons were not possible.

Chapter 3. Establishing larval feeding regimens for the decorator crab *Camposcia retusa*: Effects of live prey types and density on larval survival and development

The newly hatched Zoea 1 larvae of *C. retusa* were defined to be facultative lecithotrophic since they can develop to the next stage independently of food availability, although larvae will feed when food is available to them.

Rotifers, a live prey routinely used for rearing early zoeal larvae of crabs, was found unsuitable for rearing *C. retusa* newly hatched larvae. On the other hand, *Artemia* nauplii were found to be a good diet throughout *C. retusa* larval development from newly hatched to the first crab stage. The optimal feeding density of *Artemia* was identified as 10 ind./ml. *Artemia* enrichment and copepod co-feeding neither significantly improved survival of zoeae nor megalopae, suggesting high mortality observed during megalopal stage might relate to inappropriate environmental conditions used for their culture.

The outcomes presented in this chapter have several significant implications for practical larval rearing of *C. retusa*. Firstly, the facultative lecithotrophic nature of Z_1 larvae suggests that they are able to develop to the next stage relying on the yolk reservation and resilient to a short food shortage period after hatching. In another word, Z_1 larvae of *C. retusa* do not need

to be fed right after hatching, which often is a requirement for other marine crab species as delay in feeding can negatively affect larval survival. Secondly, *Artemia* nauplii alone is sufficient for the larval rearing of *C. retusa* throughout to the first crab stage, no enrichment or prey transition is required, which making the larval rearing of *C. retusa* simple and low cost. Such advantages in combination with relative short larval duration of *C. retusa* suggests that commercial scale larval rearing of *C. retusa* is highly practical and has high potential.

Chapter 4. Effects of salinity on survival and development of the decorator crab *Camposcia retusa* zoeal larvae and megalopae and food consumption pattern of megalopae

Zoeal and megalopal larvae showed substantial differences in salinity tolerate and preference: Zoeae preferred higher salinities and were unable survive if salinity ≤ 32; while megalopae appeared to prefer lower salinity than zoeae and had a prolonged duration when salinity was higher than 35. It is worth noting that at a salinity of 32, all zoeae died within 3 days, but megalopae showed their highest survival at this salinity, demonstrating a significant shift in salinity preference during larval ontogenetic development. Under unfavourable salinity levels, megalopae showed reduced feeding activity during the early phase of the moulting cycle although total *Artemia* consumption during the whole megalopal duration were not significantly different from those reared under an optimal salinity. Therefore, unfavourable salinities appear to mainly impact food consumption pattern related to the moulting cycle of megalopae rather than their total food consumption.

The key applications of the outcomes of this chapter are two-folds: firstly, the larvae of *C. retusa,* particularly zoeae, are stenohaline with a narrower salinity tolerate range, hence salinity is a crucial consideration for larval rearing. Based on current results, the suitable salinity range is 35–37 for zoeae and 30–33 for megalopae. Such narrow but different ranges of salinity for zoeal and megalopal larval requires different water sources to be made available for use in the hatchery. Secondly, during *C. retusa* larval culture, salinity need to be closely monitored and adjusted if needed, in particular, salinity ≤ 32 should be avoided for

zoeal larval rearing as it led to total mortality.

Chapter 5. Survival, development and metamorphosis of decorator crab *Camposcia retusa* megalopae: effects of stocking density and basal area of culture vessel, conspecific exudates and artificial substrates

It was revealed that both stocking density and basal area of culture vessel could affect survival of *C. retusa* megalopae. The results showed that megalopae reared at a low density in combination with a large basal area of culture vessel (10 megalopae/L and 260.2 cm^2 basal area) obtained the highest survival. Meanwhile, two separate experiments examined the effects of conspecific exudates on megalopal survival and development using natural and artificial seawater, respectively, showed a similar trend: i.e. conspecific adult exudates generally increased survival and accelerate development of *C. retusa* megalopae, while the presence of juvenile exudates prolonged the time to metamorphosis of the megalopae. Finally, it was also found that nylon nets as artificial substrates improved megalopal survival while mesh size of nylon nets also played a role with higher survival associated with increased mesh size. Significant difference in megalopal survival was found between the control (no nylon net provided) and the treatment with 800 μm mesh sized nylon nets supplied as the substrates.

There are three applicable outcomes from this chapter. Firstly, to ensure good survival of *C. retusa* megalopae, stocking density should not be too high, and culture vessels used should have a large basal surface. Different from individual culture recommended for megalopae of some brachyuran species, *C. retusa* megalopae can be reared communally as long as stocking density is not too high and culture vessel basal area is large, which suggests a good potential of captive rearing for this species. In addition, providing 800 μm mesh sized nylon nets and conspecific adult exudates would also enhance megalopal survival. On the other hand, newly moulted juvenile crabs should be removed from the larval culture vessels as early as possible, since chemicals released from them could supress the settlement and metamorphosis of megalopae.

Chapter 6. Growth, development and sexual dimorphism of the decorator crab *Camposcia retusa* (Latreille, 1829) reared under individual culture system

C. retusa newly settled first crab stage juveniles were successfully cultured to market size (the tenth stage or C_{10}) with an individual rearing system. Intermoult duration increased sharply from 5.3 ± 1.2 days for C_1 to 47.5 ± 16.5 days for C_{10}, which followed an exponential model ($y = 4.910 \times e^{0.231x}$). The increments of carapace length and width similarly followed the exponential manner with progressing developmental stage. Based mainly on the morphology of pleopods (4 pairs pleopods in females while only 2 pairs in males), the sex of *C. retusa* can be distinguished from C_5 stage onward.

Based on the results of this chapter, it is estimated that the timeframe required for rearing newly settled *C. retusa* juveniles to marketable size is relatively short, i.e. about 7 months, suggesting high feasibility and potential for commercial production. The description of *C. retusa* juvenile from C_1 up to the C_{10} stage for the first time and identification of features of sexual dimorphism for the species should also provide useful keys for both juvenile stage and sex identification not only for aquaculture practice, but also for field studies on its biology and ecology.

Synthesis of results and outcomes

Through the research presented in this thesis, an effective larval culture protocol for *C. retusa* has been established, which can be summarised as follows: newly hatched larvae are to be fed solely on *Artemia* nauplii at a density of 10 ind./ml throughout larval development. The salinity of culture water for zoeae should be set at 35–37, while it should be reduced to 30–33 when they become megalopae. During zoeal development, larvae can be reared at relatively high initial stocking density (~ 40 larvae per litre), however, once they moult to megalopae, the stocking density should be reduced to 5–10 megalopae per litre; meanwhile, culture vessels used should have a large basal area. Where feasible, conspecific adult odour

and 800 μm mesh sized nylon nets should be provided for *C. retusa* megalopae to improve survival and accelerate development. Following such a protocol, a good larval survival to first crab stage (up to > 70%) could be expected. Such a result in combination with a relatively short 7-month growout time from first stage crabs to market size, suggest that captive breeding of *C. retusa* for the marine aquarium trade holds a high commercial potential.

Significance of the research and future research direction

This thesis has made a significant contribution to a small but growing body of literature related to captive breeding techniques for marine ornamental crustaceans. The outcomes of this thesis hold value and pave the way to commercial production of *C. retusa* for the marine aquarium trade. Hence, the work of this thesis should benefit the development of marine ornamental aquaculture (MOA) industry, particularly in Australia. While the current MOA industry in Australia is largely limited to small scale initiatives led by hobbyists, legislation prevents the importation of crustaceans into the country for the ornamental trade, which make prices of marine ornamental crustaceans being significantly higher in the country as compared to those recorded in international markets, which should help stimulate the development of this industry. In addition, owing to its relatively easy larval rearing, *C. retusa* may also be used in the broader contexts of marine biology and ecology research, for instance, being used as a 'new' model species for decorating behaviour studies, supplementing existing model species, such as *Tiarinia cornigera* and *Oregonia* spp (Berke & Woodin 2008; Berke & Woodin 2009; Brooker *et al.* 2017; Hein & Jacobs 2016; Hultgren & Stachowicz 2011).

This thesis has also contributed to general brachyuran larval biology. For example, Chapter 2 not only described the morphology of complete larval development and the first crab stage of *C. retusa*, but also provided larval morphological comparation within the family. The description of larvae morphology is believed to be a basic but important contribution to the study of crustacean larval biology, showing the primary ontogeny of

species-specific morphological traits (Anger 2006). The comparative studies of larval morphological features also aid the identification of phylogenetic relationships. In fact, the modern taxonomy system for the Decapoda is based predominantly on reproductive and larval traits (Bauer 2004). The zoeae, in particular, have been found useful for phylogenetic analyses at various taxonomic levels within the Brachyura, mostly within families (Clark 2000; Ng & Clark 2000; Pohle & Marques 2000; Rice 1983; Rice 1980). Similarly, the morphology of the megalopa stage may indicate phylogenetic relationships within or between brachyuran families (Rice 1988). On the other hand, the knowledge of larval development and morphology of a species is also useful for identifying the species and larval stage in field studies, thus aids the studies of larval dispersal, recruitment and behaviour.

Establishing larval feeding regime is a critical step for captive breeding of target crab species (Calado *et al.* 2017; Daly *et al.* 2009; Ruscoe *et al.* 2004). This thesis investigated the suitability and optimal feeding density of rotifer and *Artemia*, two widely used traditional hatchery live prey (Støttrup & McEvoy 2003), for feeding *C. retusa* larvae (Chapters 3). The results suggest that feeding *C. retusa* larvae with newly hatched *Artemia* nauplii alone can obtain a decent survival, showing low complexity and cost, but high potential for commercial hatchery culture of *C. retusa*.

Understanding the environmental requirements is another important aspect for crab larval culture. Knowledge on environmental requirements supporting brachyuran larval development is not only essential for the optimisation of commercial hatchery production, but also contributes to the understanding of general ecology of the species (Castejón *et al.* 2019; Hamasaki *et al.* 2011; Zmora *et al.* 2005). For example, this thesis investigated the effects of salinity on larval survival and revealed that both zoeal and megalopal of *C. retusa* are stenohaline, particularly that total mortality of newly hatched zoeal larvae can occur within 3 days when these are reared at salinity ≤ 32; this finding suggests that spawning ground and early larvae of the species are mostly likely to be offshore (Chapter 4). The thesis also examined other factors, such as stocking density and basal area of culture vessel, conspecific odours, and the present of substrates, as these all influenced *C. retusa* megalopal survival, development and metamorphosis (Chapter 5). The results of this thesis showed that by

lowering the stocking density and providing substrates that facilitate megalopae to hold on to can improve their survival, suggesting cannibalism due to high interaction rate between megalopae could be a reason of their mortality. However, unlike other highly aggressive species (e.g. *Callinectes sapidus*, *Cancer magister, Carcinus maenas*, etc) that can only be cultured at extremely low density (< 0.5 ind./L) or even individually (Dawirs 1982; Fernandez *et al.* 1994; Forward *et al.* 1996; Zmora *et al.* 2005), *C. retusa* megalopae can be reared at relatively high density (10 ind./L) with decent survival (>50%). This result, on the other hand, suggested that *C. retusa* megalopae probably are not as cannibalistic as those of the species mentioned above. Therefore, it would be worth to confirm whether missing bodies of megalopae in stocking density experiment were due to cannibalism since this can help to understand the severity of cannibalism of *C. retusa* megalopae and further optimise the larval culture protocol. In addition, another interesting outcome of this thesis for future research is that megalopae showed delayed metamorphosis when juvenile odour was presented. There were relatively few studies focused on the effects and dosage of conspecific cues on the settlement of megalopae, especially cues released by young juveniles (Simith *et al.* 2013a), which would form an interesting area for future research. Moreover, effects of other types of inducers that may enhanced settlement and metamorphosis of *C. retusa* megalopae, such as biofilms, vegetation, substrates with different texture and structures, as well as interactions between varying cues, are all warrant further research.

Detailed description of juvenile development and the appearance of sexual dimorphism for species from superfamily Majoidea is scarce. The current thesis also made an important contribution to this area, by providing necessary keys to assist in the identification of juvenile stages and sexes in both aquaculture practice and field study. The mathematical models for growth and development of *C. retusa* juveniles up to market size (C_{10} stage) provided crucial information that help evaluate the potential of captive growout of this species. However, since this is the first attempt to rear *C. retusa* juvenile in captive conditions, there is certainly room for future research to advance the culture of juvenile crabs, which could include optimising the culture environment, feeds and feeding, as well as evaluating the potential and techniques for communal culture.

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