Fisheries biology of *Metapenaeopsis* species (Decapoda, Penaeidae) in Kagoshima Bay, Japan

（鹿児島湾におけるアカエビ属エビ類（十脚目・クルマエビ科）の資源生物学的研究）

Md. Mosaddequr Rahman

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Md. Mosaddequr Rahman

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ABSTRACT

Metapenaeopsis Bouvier, 1905, with seventy-one species and seven subspecies, is the most speciose genus of Penaeidae Rafinesque, 1815. The genus has a worldwide distribution and its species are important ecologically as well as commercially. In Kagoshima Bay, southern Japan, five species and one subspecies of *Metapenaeopsis* are found: *M. acclivis* (Rathbun, 1902), *M. palmensis* (Haswell, 1879), *M. mogiensis mogiensis* (Rathbun, 1902), *M. kyushuensis* (Yokoya, 1933), *M. provocatoria owstoni* Shinomiya & Sakai, 2000, and *M. sibogae* (De Man, 1907). Among these, *M. sibogae*, *M. kyushuensis* and *M. provocatoria owstoni* are dominant in the benthic community of the bay and are commercially important. *M. sibogae* is the latest member of this genus being recorded from Japanese waters in 2004 in Kagoshima Bay. There is, however, no study available on any aspect of population biology of these three species. The present study therefore aims to provide the first information on their reproduction, growth, distribution patterns and population dynamics in Kagoshima Bay.

Monthly sampling was conducted at 8 stations established in the bay onboard the training vessel *Nansei Maru* (175 t) of the Faculty of Fisheries, Kagoshima University, using a simple trawl net during 2014-2017. The net was towed for a preset tow duration of 10 min at a speed of 2 knots. At each haul, target species were identified based on the shape of petasma in males or thelycum in females. It was not possible to distinguish between the males of *M. kyushuensis* and *M. provocatoria owstoni* due to the lack of identification key. Additional laboratory samples collected during 2004-2013 were also used. The effective tow duration was estimated and the catch per unit effort (CPUE) was standardized to the preset tow duration following Fulanda & Ohtomi (2011). Individual carapace length (CL), body length (BL) and body weight (BW) were measured accordingly. Ovaries were macroscopically classified as one of three initial maturity stages based on the ratio of ovary width to body width taken at the middle of the first abdominal segment: Stage I, ovary width < 1/5 body width; Stage II, 1/5

body width ≤ ovary width < 1/3 body width; and Stage III, ovary width ≥ 1/3 body width. The whole ovary was then removed and weighed and the gonadosomatic index (GSI) was calculated. Ovarian maturity status was then confirmed by histological observation. The growth patterns and longevity were estimated using length-frequency method. Histograms of CL by sex were constructed for each month using 1 mm class intervals. Hasselblad's maximum-likelihood method was applied to the CL histograms to estimate the parameters for the normal distributions in each histogram. Each component normal distribution represents an age group in the population. Age of individuals in months were assigned to the mean CLs belonging to each of the cohorts by arbitrarily assigning the approximate midpoint of main spawning season as the date the shrimp hatched. The von Bertalanffy growth function (VBGF) and the Pauly and Gaschutz growth function (PGGF) were fitted to the mean length-at-age data to identify any seasonal oscillation in growth rate and to suggest the best-fit model for describing growth. The relative growth of BL to CL was estimated hypothesizing that the morphological sexual maturity can be estimated based on abrupt shifts in allometry. The relationships of BL and BW with CL were determined by the least squares method. The occurrence of parasites were/ checked by observing the branchial chamber of the shrimp. Parasites were identified in collaboration with Ehime Prefecture Fisheries Research Center and Kochi University.

All the species exhibited asynchronous ovaries, containing oocytes at various developmental stages, signifying multiple spawning in a reproductive season. The stages of ovarian maturity were divided into three categories based on the developmental stage of the most advanced oocytes in the ovary: immature, maturing, and mature. Cortical crypts in the periphery of oocyte cytoplasm, widely recognized as a sign of oocyte prematuration in penaeid shrimps, were not identifiable in the studied shrimps. Females containing mature oocytes, i.e., those in which germinal vesicle breakdown had occurred, were defined as mature individuals. The performances of both GSI and macroscopic staging in ovarian maturity classification were histologically evaluated. The GSI exhibited a positive but gradual relationship with ovarian maturation progression. Macroscopic staging, in contrast, showed a sharper relationship with histological stages of ovarian maturity exposing its potential as a maturity index for the studied species, which could also be suitable for other small penaeid shrimps in absence of histological data. Females with Stage III ovaries were considered to be mature. The size at sexual maturity was estimated to be 14.3 mm, 13.7 mm and 12.8 mm in CL for *M. sibogae*, *M. kyushuensis* and *M. provocatoria owstoni*, respectively, and individuals of these sizes belong to the 1-year-old age class. *M. sibogae* spawned throughout the year but peaked September-October. *M. kyushuensis* spawned from April to January with a peak in June to September. The spawning season of *M. provocatoria owstoni* lasted from April to December with May-June being the peak.

Both males and females of *M. sibogae* were recruited during winter (January) with a modal size of around 9 mm CL. Almost no seasonality in growth rate were detected, therefore, the VBGF was adopted as the best fitting model to describe the growth of both sexes. The estimated equations were $L_t = 16.79\ [1 - \exp\{- 0.181\ (t + 0.867)\}]$ and $L_t = 19.56\ [1 - \exp\{- 0.139\ (t + 1.928)\}]$ for males and females, respectively. Growth differed significantly between the sexes. Longevity was estimated to be about 27 months for males and 28 months for females. Females of *M. kyushuensis* were first recruited in winter (December/January) with a modal size of around 10 mm CL. Seasonal oscillation in growth rate was detected in the females of *M. kyushuensis*, therefore, the PGGF was adopted as the best fitting model. The estimated equation was $L_t = 24.30\ [1 - \exp\{- 0.694\ (t/12 + 0.336) - (0.218/2\pi)\ \sin\ (2\pi\ (t/12 - 0.940)\}]$. The growth rate was the highest in November-December and the lowest in May-June. The longevity was estimated to be about 25 months. Females of *M. provocatoria owstoni* were first recruited in autumn (November) with a modal size of around 10 mm CL. Seasonal oscillation in growth rate was detected in the females of *M. provocatoria owstoni*, therefore, the PGGF was adopted as the best fitting model. The estimated growth equation was $L_t = 28.77 [1 - \exp \{- 0.521 (t/12 + 0.297) - (0.454/2\pi) \sin (2\pi (t/12 - 0.891)\}]$. The growth rate was the highest in October-November and the lowest in April-May. The longevity was estimated to be about 27 months.

Analysis of the sum of squared residuals indicated that dimorphic growth existed in all the studied species and that two separate linear BL/CL models fit the data better than a single linear model showing clear transition points, which could be associated with sexual maturity. The relationship between BW and CL showed negative allometric growth in all the studied shrimps.

All the studied species had wide spatial and bathymetric distributions (water depth, ~80 m to ~230 m) in the bay. They were mostly distributed in the central bay, with *M. sibogae* had higher CPUE in comparatively deeper areas (~180 m), whereas *M. kyushuensis* and *M. provocatoria owstoni* had higher CPUEs in the comparatively shallower areas (~130 m). *M. sibogae* was the most dominant among the studied species followed by *M. kyushuensis*. There were no significant relationships between bottom-water temperature or salinity and the distribution patterns of the studied *Metapenaeopsis* shrimps, but the sediment characteristics and water depth might appear to influence the distribution patterns. Similar distribution patterns with progressing age were observed for all the species with 0+ age group individuals were distributed throughout their distribution areas. However, an increased tendency of inhabiting the deeper waters being observed for individuals belong to the ≥1+ age groups.

Bopyrid parasites *Minicopenaeon intermedium* Bourdon, 1981, *M. liuruiyui* An, Boyko & Li 2013, and *Parapenaeon tertium* Nierstrasz and Brender à Brandi, 1932 were identified to infest *M. sibogae*, *M. kyushuensis* and *M. provocatoria owstoni*, respectively. The infestation rate varied significantly among species with the highest rate recorded for the females of *M. provocatoria owstoni* (8.91%). The infestation inhibited the reproductive ability of the females as all the parasitized females were found to be immature. The relative growth rate of BW/CL relationship of parasitized females were significantly lower than that of their non-parasitized counterparts.

M. sibogae, M. kyushuensis and M. provocatoria owstoni are commercially important fisheries resources in Kagoshima Bay. The present study provided the first information on reproduction, growth, distribution patterns and population dynamics of all these species in addition to reports on parasite infestation and impact assessment of parasite infestation on host, which will be helpful for their sustainable management.

CHAPTER 1: GENERAL INTRODUCTION

Shrimps belonging to the suborder Dendrobranchiata are among the most important marine fishery resources in the world, which includes several commercially important species constituting an important food resource for humans (Costa et al., 2007). Among this suborder, shrimps belonging to the family Penaeidae Rafinesque, 1815 are a valuable fishery resource (Dall *et al.*, 1990), comprising 42.2% of the total worldwide shrimp catch between 1970 and 2000 (FAO, 2009). There are 48 recognized genera in the family Penaeidae (De Grave *et al.*, 2009), of which *Metapenaeopsis* Bouvier, 1905, with 71 species and 7 subspecies, is the most speciose genus (De Grave & Fransen, 2011). These species are widely distributed and found in the waters of Australia (Racek & Dall, 1965), New Caledonia (Crosnier, 2007), Papua New Guinea (Hall, 1962), Solomon Islands (Racek & Dall, 1965), Borneo, Brunei, Indonesia, Malaysia, Sarawak (Kubo, 1949; Racek & Dall, 1965; Champion, 1973; Johnson, 1976; Tseng & Cheng, 1980), Singapore (Hall, 1962; Johnson, 1976), Philippines, Thailand, Vietnam (Johnson, 1976; Tseng & Cheng, 1980), China, Hong Kong (Tseng & Cheng, 1980), Taiwan (Lee *et al.*, 1999), Japan (Kubo, 1949), Korea (Kim, 2012), India (Suseelan *et al.*, 1982), Maldives, Sri Lanka (Pérez Farfante & Kensley, 1997), the Arabian-Persian Gulf and Red Sea (Miquel, 1984), southeastern Africa (Racek & Dall, 1965; Champion, 1973), North and South America (Bauer, 1985), the Gulf of Mexico, and the Caribbean (Tseng & Cheng, 1980). Japanese waters are inhabited by twelve species and two subspecies of *Metapenaeopsis* (Sakaji *et al.*, 2000; Shinomiya & Sakai, 2000; Ohtomi & Nagata, 2004): *M. acclivis* (Rathbun, 1902), *M. aegyptia* Galil & Golani, 1990, *M. barbata* (De Haan, 1844 in De Haan, 1833–1850), *M. dalei* (Rathbun, 1902), *M. dura* Kubo, 1949, *M. gallensis* (Pearson, 1905), *M. palmensis* (Haswell, 1879), *M. sibogae* (De Man, 1907), *M. sinica* Liu and Zhong, 1988, *M. toloensis* Hall, 1962, *M. lamellata* (De Haan, 1844 in De Haan, 1833–1850), *M. kyushuensis* (Yokoya, 1933), *M. mogiensis mogiensis* (Rathbun, 1902), and *M. provocatoria owstoni* Shinomiya & Sakai, 2000.

Species of the genus *Metapenaeopsis* play an important role in marine benthic food webs and serve as prey species to various benthic fishes (Linke *et al.*, 2001; Raymundo-Huizar *et al.*, 2005; Chen *et al.*, 2014). Some species of *Metapenaeopsis*, such as *M. barbata*, *M. dalei*, *M. palmensis*, and *M. rosea* Racek & Dall, 1965, also have commercial importance in many areas of the world, including Australia, India, Japan, Korea, Taiwan, and Thailand (Longhurst, 1970; Hayashi & Sakamoto, 1978; Wu, 1985; Watson & Keating, 1989; Sakaji *et al.*, 2000; Sakaji, 2003; De Young, 2006). Despite their wide distribution and ecological and commercial significance, studies on the population biology of species of *Metapenaeopsis* are scarce and primarily concentrate on a few commercially exploited species. Existing studies have investigated the ecology of *M. dalei* in Sendai Bay (Kosaka, 1977); taxonomy and biology of *M. palmensis* in Kii Strait, central Japan (Hayashi & Sakamoto, 1978); aspects of the population biology of *M. rosea* in Torres Strait, Australia (Watson & Keating, 1989); growth and maturation of *M. barbata* in the Aki-nada area, the Seto Inland Sea, Japan (Sakaji *et al.*, 1992); oocyte development; maturation, and spawning of *M. dalei* in Tosa Bay, Japan (Sakaji *et al.*, 2000; Sakaji, 2001a); oocyte development of *M. aegyptia*, *M. barbata*, and *M. sinica* from the Tosa Bay, Japan (Sakaji, 2001b); growth and mortality of *M. barbata* off the northeastern coast of Taiwan (Tzeng *et al.*, 2005); growth and reproduction of *M. dalei* in the western sea of Korea (Choi *et al.*, 2005); and the life history of *M. palmensis* off southwestern Taiwan (Chen *et al.*, 2014).

Kagoshima Bay, southern Japan (31°25' N, 130°38' E), is a semi-enclosed deep-water bay with a maximum water depth of more than 230 m (Ohtomi, 2001). It is comprised of two

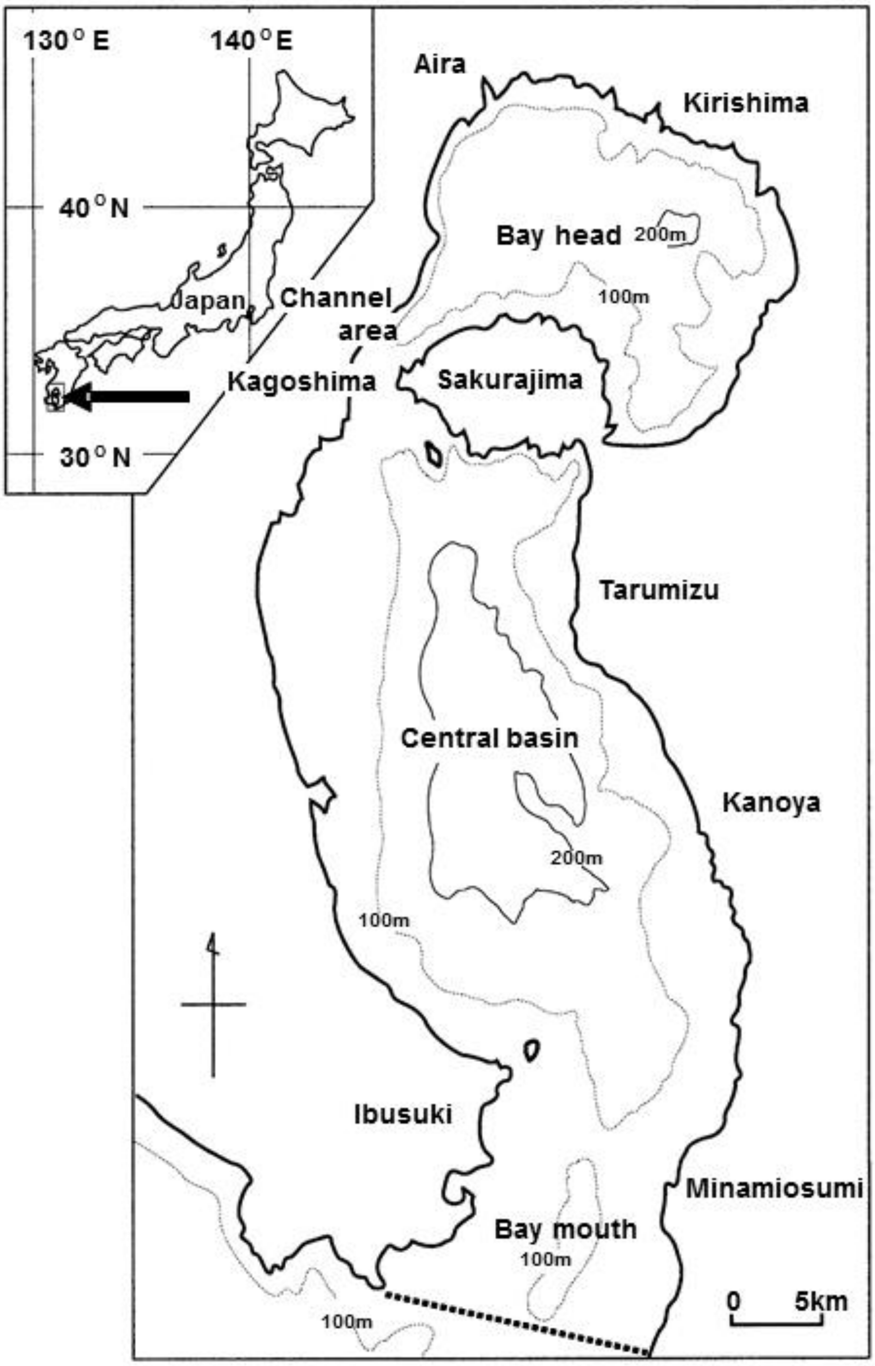


Figure 1.1. Map of Kagoshima Bay, southern Japan.

basins; a central basin and a bay head connected by a shallow channel area (Ohtomi *et al.*, 2018). The bay measures 80 km long and 25 km wide and is sandwiched between the Hokusatsu area of Kyushu Island, and the sinuate Satsuma and Osumi peninsulas to the west and east, respectively. The Satsuma Peninsula borders the East China Sea to the west while the eastern coast of the Osumi Peninsula is washed by the Pacific Ocean. The bay is uniquely characterized by the steep slopes on both sides and the bottom topography impacted by volcanic formation (Matumoto, 1943). Kagoshima Bay is influenced by the warm Kuroshio Current from the south (Schmeits & Dijkstra, 2001). Therefore, the bay is relatively warm and the bottom-water temperature below 100 m depth is relatively stable (Matumoto, 1943; Kohno *et al.*, 2004). Water circulation and surface currents within the bay deduced from surface temperature, salinity and transparency data indicate a counter-clockwise current system in the central basin (Sakurai & Maeda, 1980). Despite its unique structure, bottom topography, and influence of volcanic eruption from one of the most active volcanoes of the world, Sakurajima, Kagoshima Bay is rich in benthic biodiversity, inhabited by 124 species from 33 different families of decapod crustacean (Ohtomi *et al.*, 2008). The bay is home to an estimated 7800 fishing vessels, with 139 fishing ports in Kagoshima Prefecture (Fulanda & Ohtomi, 2008). A large number of fish and decapod crustacean species including *S. melantho*, *P. semilaevis* and *T. curvirostris* occur in the bay and are target species of the small-scale bottom seine fishery (Ohtomi & Nagata, 2004).

There are 4 species and 2 subspecies of *Metapenaeopsis* were found: *M. acclivis, M. palmensis, M. mogiensis mogiensis*, *M. kyushuensis*, *M. provocatoria owstoni*, *M. sibogae* (Rahman & Ohtomi, unpublished). Among these, *M. sibogae* (De Man, 1907) is the latest to be recorded from Kagoshima Bay, the first time it was found in Japanese waters (Ohtomi & Nagata, 2004). The species is also reported to inhabit deep water (from around 250 m to more than 400 m) of Indonesia, New Caledonia, and the Philippines (Crosnier, 1987, 2007; De Grave

M. acclivis

M. mogiensis mogiensis

M. palmensis

M. provocatoria owstoni

M. kyushuensis

M. sibogae

Figure 1.2. *Metapenaeopsis* species recorded in Kagoshima Bay, southern Japan. The specimens showed here are around 75 mm in body length.

& Fransen, 2011). Since the time it was first reported, *M. sibogae* has become one of the five most dominant benthic species of Kagoshima Bay in terms of number of individuals caught per haul, surpassing the two native species, *M. kyushuensis* and *M. provocatoria owstoni* (Rahman & Ohtomi, unpublished). *M. sibogae* is morphologically quite similar to *M. kyushuensis* and *M. provocatoria owstoni* and are commercially important as they are marketed as *Metapenaeiospis* spp. complex. The adaptation and abundance of *M. sibogae* in such a unique environment is astounding. It is extremely interesting to know whether there is any association in the spatio-temporal distribution and abundance of *M. sibogae* with that of the other two *Metapenaeopsis* species and with that of other major benthic species in the bay. Furthermore, my preliminary research has detected the occurrence of parasites in all the three *Metapenaeopsis* shrimps in the bay. It is important to identify the parasite species and to know the variations in prevalence and the impact of parasite on the hosts' reproduction and growth, for the formulation of appropriate management and conservation measures.

A number of studies has been conducted on the population biology of some major crustacean species in Kagoshima Bay including the reproduction and growth of *Plesionika semilaevis* Spence Bate, 1888 (Ohtomi, 1997); reproduction and growth of *Solenocera melantho* De Man, 1907 (Ohtomi & Irieda, 1997; Ohtomi *et al.*, 1998) and *Trachysalambria curvirostris* (Stimpson, 1860) (Hossain & Ohtomi, 2008, 2010), reproduction, growth, and relative growth and sexual maturity of *Plesionika izumiae* Omori, 1971 (Ahmed & Ohtomi, 2011, 2012, 2014), reproduction and growth of *Parapenaeus fissuroides* Crosnier, 1986 (Farhana & Ohtomi, 2016, 2017), and distribution patterns and population dynamics of *P. izumiae* (Ohtomi *et al.*, 2018). There is, however, no study available on the population biology of *M. sibogae*, *M. kyushuensis* and *M. provocatoria owstoni* from any of their distribution areas.

Detail information on the population biology of a species is important to know their gonadal development, size and age at sexual maturity, spawning season, recruitment patterns, nursery and spawning grounds, mortality, possible migratory behavior, and to explain the seasonal distribution and abundance patterns, which are indispensable for supporting optimal exploitation and sustainable management regimes (Ohtomi *et al.*, 2018). The population biology of shrimps in a unique bay environment, like Kagoshima Bay, is particularly interesting ecologically and important for sustainable management and conservation protocols. The present study, therefore, aimed at providing the first information on the reproductive biology, growth patterns, longevity, relative growth, distribution patterns, population dynamics of *M. sibogae*, *M. kyushuensis* and *M. provocatoria owstoni* species in Kagoshima Bay, southern Japan. In addition, the parasites infesting the three *Metapenaeopsis* species were identified, their variations in prevalence and effect on host's reproduction and growth were evaluated.

CHAPTER 2: REPORDUCTIVE BIOLOGY OF *METAPENAEOPSIS* SPECIES IN KAGOSHIMA BAY, SOUTHERN JAPAN

2.1. BACKGROUND OF THE STUDY

Understanding the reproductive biology of a species is imperative for fisheries management, as reproductive biology largely determines the productivity of a stock and therefore its resilience to exploitation (Morgan, 2008). The reproductive strategies of a species also play a major role in its biogeography and its population dynamics (Sastry, 1983; Ramirez Llodra, 2002). Information on the reproductive biology of *Metapenaeopsis* species, including ovarian maturation, size and age at sexual maturity, and spawning season is, therefore, necessary as their exploitation increases if management of the fishery is to succeed.

Lack of cortical crypts in the periphery of oocyte cytoplasm during maturation has been reported in small penaeid shrimps of the genus *Metapenaeopsis* (Sakaji *et al.*, 2000; Sakaji, 2001b; Chen *et al.*, 2014). Differences in spawning seasonality have also been described for some species of *Metapenaeopsis* by several authors who pointed out different factors, including water depth, water temperature, and latitudinal variations of the sampling areas, among others, are responsible for this phenomenon (e.g., Sakaji *et al.*, 1992; Choi *et al.*, 2005; Chen *et al.*, 2014). It is important to know whether the absence of cortical crypts in oocyte development and seasonality in spawning are general phenomena in *Metapenaeopsis*. The usefulness of gonadosomatic index (GSI) as a maturity index in penaeid shrimps is often scrutinized due to its dependence on body size (Courtney *et al.*, 1995) and its gradual relationship with the progression of ovarian maturation (Ohtomi *et al.*, 2003). The suitability of GSI as an index of ovarian maturation in *Metapenaeopsis* species should be investigated, and in case GSI to be a poor index of ovarian maturation, effectiveness of a new index, as a reliable, faster and cost-effective method of ovarian maturity classification, needs to be tested.

Studies on the reproductive biology of species of *Metapenaeopsis* are scarce and primarily concentrate on a few commercially exploited species. Existing studies have investigated aspects of the population biology of *M. rosea* in Torres Strait, Australia (Watson & Keating, 1989); growth and maturation of *M. barbata* in the Aki-nada area, the Seto Inland Sea, Japan (Sakaji *et al.*, 1992); oocyte development; maturation, and spawning of *M. dalei* in Tosa Bay, Japan (Sakaji *et al.*, 2000; Sakaji, 2001a); oocyte development of *M. aegyptia*, *M. barbata*, and *M. sinica* from the Tosa Bay, Japan (Sakaji, 2001b); growth and reproduction of *M. dalei* on the western coast of Korea (Choi *et al.*, 2005); and the life history of *M. palmensis* off southwestern Taiwan (Chen *et al.*, 2014). There is, however, no study available on the reproductive biology of *M. sibogae*, *M. kyushuensis* and *M. provocatoria owstoni* in the literature presenting a stumbling block for formulating and implementing effective management and conservation measures of these species.

2.2: REPRODUCTIVE BIOLOGY OF *METAPENAEOPSIS SIBOGAE*

2.2.1. INTRODUCTION

M. sibogae was reported from Kagoshima Bay in 2004, the first time it was found in Japanese waters (Ohtomi & Nagata, 2004). The species is also reported to inhabit deep waters (from around 250 m to more than 400 m) of Indonesia, New Caledonia, and the Philippines (Crosnier, 1987, 2007; De Grave & Fransen, 2011). *M. sibogae* has become one of the five most dominant benthic species of Kagoshima Bay in terms of number of individuals caught per haul and has become commercially important. Studies on the reproductive biology of *M. sibogae* from Japan and elsewhere are lacking. The present study, therefore, aims to delineate the ovarian maturity, size at sexual maturity and spawning season of *M. sibogae* for the first time for formulating and implementing effective management and conservation measures.

2.2.2. MATERIALS AND METHODS

2.2.2.1. *Study area*

Kagoshima Bay, southern Japan (31°25' N, 130°38' E), is a semi-enclosed deep-water bay with a maximum water depth of more than 230 m (Ohtomi, 2001) (Figure 2.2.1). The bay consists of two basins connected by a shallow channel and an opening to the Pacific Ocean in the south, and it is influenced by the warm Kuroshio Current (Mizuno & White, 1983). A large number of decapod crustaceans, including some commercially important species, are fished by small-scale bottom seiners in the bay (Ohtomi, 2001).

2.2.2.2. *Sampling*

Monthly samples of *M. sibogae* were obtained from experimental trawl surveys conducted in the central area of the bay at depths of 125–183 m (Figure 1) with the training vessel *Nansei-Maru* (175 t) of the Faculty of Fisheries, Kagoshima University from January

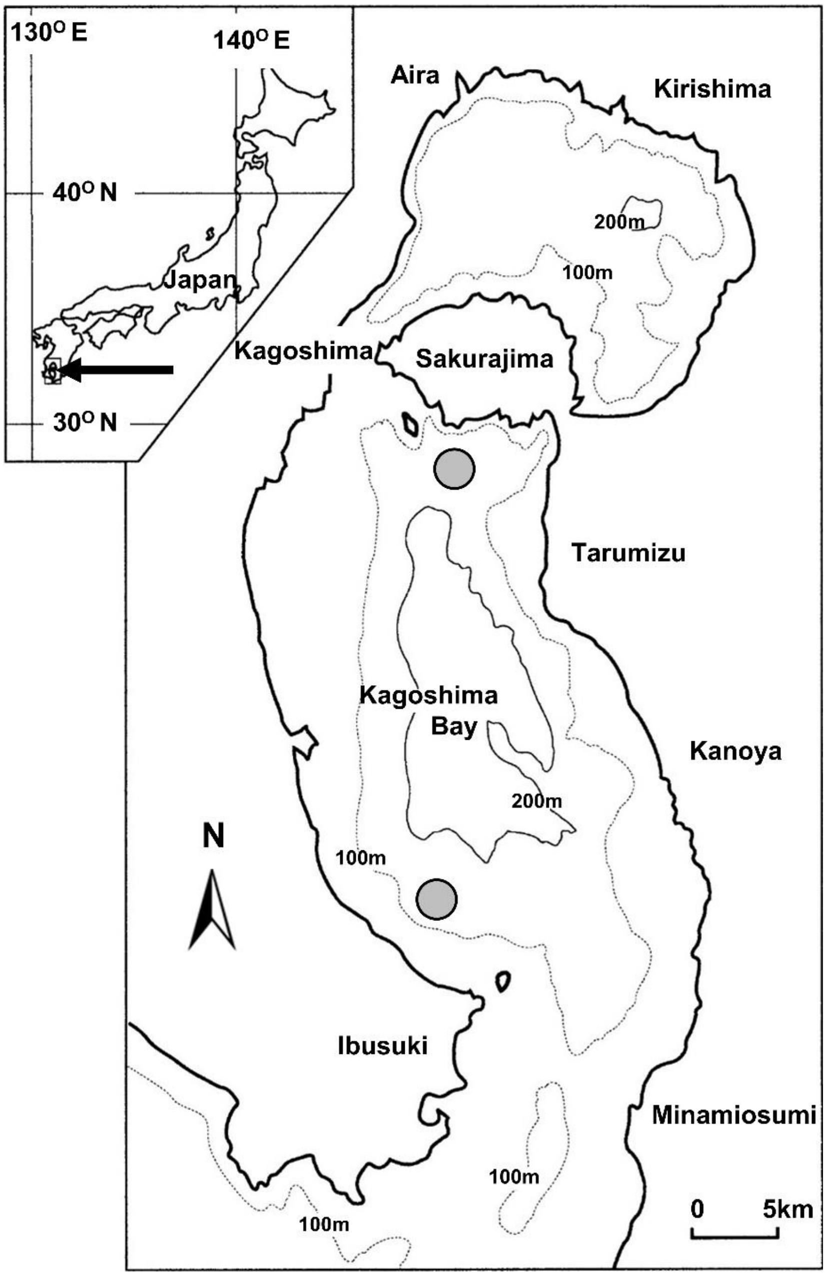


Figure 2.2.1. Kagoshima Bay, southern Japan, with indication of the collection of samples of *Metapenaeopsis sibogae* in central part of the bay (shaded areas).

2014 to December 2015. A simple trawl net (LC-VI; Nichimo Corporation, Tokyo), 23.5 m long, 37.9 mm mesh size in the body, 20.2 mm in the cod-end, carrying canvas kites on the tip of the wings (Ohtomi *et al.*, 2004) was used for sampling.

Net depth and water temperature were recorded at 1-min intervals using a compact temperature-depth (compact-TD) logger (ATD-HR; JFE Advantech, Nishinomiya, Japan) attached to the head rope of the net. The bottom depth was recorded at 1-min intervals with a split-beam quantitative echo sounder (KFC-3000; Kaijo Corp., Hamura, Japan). The net was towed for a pre-set tow duration of 10 min at a velocity of 2 knots. Bottom-water temperatures recorded during effective tow durations (see below) were used to calculate mean temperature for each haul and month. *M. sibogae* was sorted out of each haul, counted, and instantly preserved in ice onboard and then in formalin upon arrival at the laboratory. We could not collect samples in September 2014 due to a typhoon so data for samples collected in September 2013 were used in place of the September 2014 data in the analyses. Histological data of ovaries of 88 females collected during 2010 were also used.

2.2.2.3. *Measurements*

All specimens were classified by sex according to the presence of a petasma in males or a thelycum in females, and only females (*N* = 4,245) were used. For each female, carapace length (CL) was measured to the nearest 0.01 mm from the posterior margin of the eyestalk to the dorsal midline of the posterior margin of the carapace with slide calipers (CD-15PS; Mitutoyo Corp., Kawasaki, Japan), and body weight was measured to the nearest 0.01 g by an electronic balance (EB-430DW; Shimadzu Corp., Kyoto, Japan). The exoskeleton and muscles above the ovary of each female were removed, and the ovary and body widths were measured to the nearest 0.01 mm at the middle of the first abdominal segment (Figure 2.2.2). Ovaries were classified as one of three initial sexual maturity stages based on the ratio of ovary width

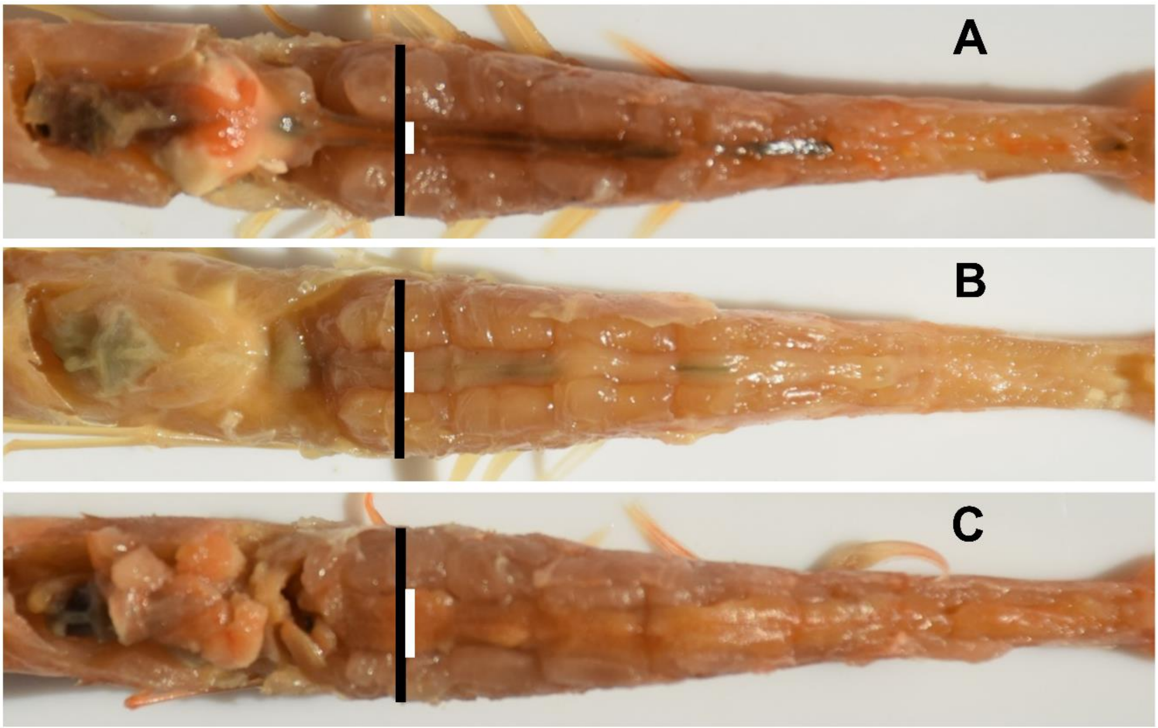


Figure 2.2.2. Macroscopic maturity stages of the ovaries of *Metapenaeopsis sibogae*. **A**, Stage I (ovary width < 1/5 body width); **B**, Stage II (1/5 body width ≤ ovary width < 1/3 body width); **C**, Stage III (ovary width ≥ 1/3 body width). Black line indicates body width; white line indicates ovary width.

to body width: Stage I, ovary width < 1/5 body width (Figure 2.2.2A); Stage II, 1/5 body width ≤ ovary width < 1/3 body width (Figure 2.2.2B); and Stage III, ovary width ≥ 1/3 body width (Figure 2.2.2C). The maturity status of these stages was assessed by histological observation (see below). The whole gonad was then removed and weighed to the nearest 0.001 g using the electronic balance. The gonadosomatic index was calculated as:

$$\text{GSI (\%)} = 100 \times [\text{ovary weight} / \text{body weight}]$$

2.2.2.4. *Histological analysis and measurement of oocytes*

A small portion of each ovary from the 130 females collected from January to December 2010 and October 2014 to September 2015 (CL, 6.73 to 20.75 mm; GSI, 0.06 to 9.80%) was dehydrated in an ethanol series, infiltrated with paraffin, and sliced into 6 µm sections. The sections were stained with Mayer's hematoxylin and eosin, cover-slipped with a mounting medium, and examined under a microscope (DMLB; Leica, Wetzlar, Germany). As a preliminary examination, sections from three parts of the ovary (the posterior region of the cephalothoracic lobe and the anterior and middle regions of the abdominal lobe) from three females (CL, 16.59 to 20.12 mm; GSI, 8.84 to 9.80%) were examined to differentiate the maturity stages in relation to the position in the ovary, but no difference was found in maturity stage with respect to ovary position. Tissue samples from the anterior regions of the abdominal lobes were thus used for histological analysis for the rest of the ovaries to determine the oocyte developmental stages. The terminology and criteria for describing oocyte development and ovarian maturation followed Yano (1988), Ohtomi & Yamamoto (1997), Ohtomi *et al.* (1998), Sakaji *et al.* (2000), and Chen *et al.* (2014) with few modifications.

Fifteen of the sectioned ovaries of the highest quality were selected for measuring oocyte diameters. Only oocytes sectioned through the nucleus (N = 1,391) were measured under a microscope. Due to histological processing and natural variation, most of the oocytes were not perfectly spherical in shape. Average values of the maximum and minimum oocyte

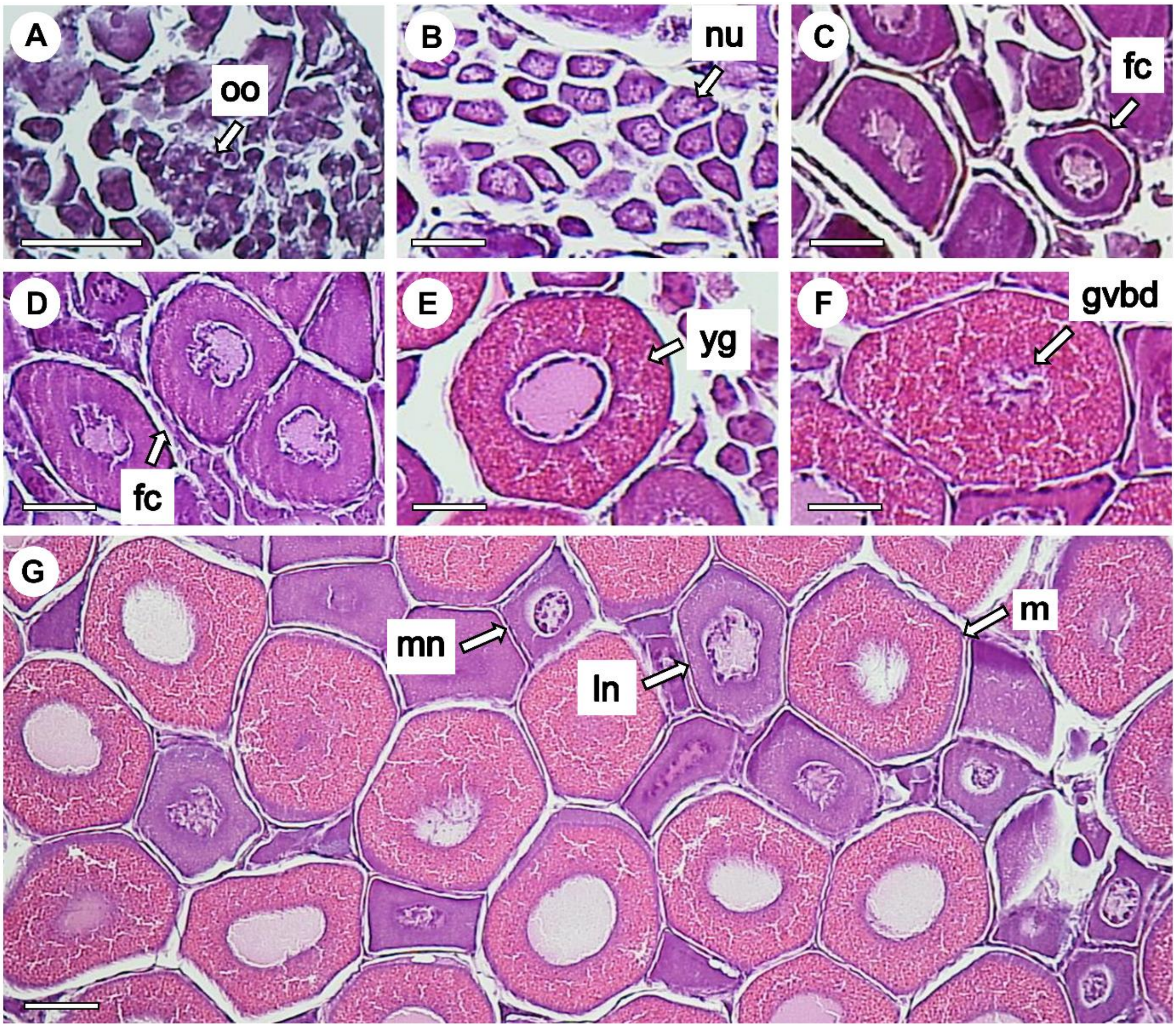


Figure 2.2.3. Histological sections of oocyte development and mature gonad of *Metapenaeopsis sibogae*. **A**, oogonium stage; **B**, early nucleolus stage; **C**, middle nucleolus stage; **D**, late nucleolus stage; **E**, yolk granule stage; **F**, mature stage; **G**, asynchronous oocyte development in the ovary; fc, follicle cells; gbvd, germinal vesicle break down; ln, late nucleolus-stage oocyte; m, mature-stage oocyte; mn, middle nucleolus-stage oocyte; nu, nucleoli; oo, oogonium; yg, yolk granules. Scale bar = 50 µm.

diameters were thus used to reduce the variance and avoid artificially increasing the overlap between oocytes of different developmental stages (see West, 1990; Farrell *et al.*, 2012).

2.2.2.5. ***Classification of developmental stages of oocytes***

Oocytes were assigned to one of six developmental stages based on histological observation: oogonium, early nucleolus, middle nucleolus, late nucleolus, yolk granule, and mature (Table 2.2.1). Oogonium-stage oocytes were 3–10 µm in diameter, spherical, and strongly basophilic (Figure 2.2.3A); early nucleolus-stage oocytes were 18–58 µm in diameter, basophilic, and contained thin cytoplasm (Figure 2.2.3B); middle nucleolus-stage oocytes were 43–113 µm in diameter with strongly basophilic chromatin in the nucleus and follicle cells visible around the oocytes (Figure 2.2.3C); late nucleolus-stage oocytes were 98–150 µm in diameter with weak hematoxylin staining in the cytoplasm and decreased thickness of the follicle layers (Figure 2.2.3D); yolk granule-stage oocytes were 133–183 µm in diameter with eosin-stained acidophilic yolk granules in the cytoplasm (Figure 2.2.3E); and mature-stage oocytes were 160–238 µm in diameter and comprised of acidophilic yolk granules concentrated in and filling the cytoplasm of the oocyte, and germinal vesicle breakdown (GVBD) occurred throughout the cytoplasm of this stage (Figure 2.2.3F).

2.2.2.6. ***Assessment of stages of ovarian maturity***

M. sibogae had asynchronous ovaries, containing oocytes at various developmental stages (Figure 2.2.3G). The stages of ovarian maturity were thus divided into three categories based on the developmental stage of the most advanced oocytes in the ovary: immature, maturing, and mature (Table 2.2.1). Ovaries with oogonium-stage oocytes and/or oocytes in the early-, middle-, or late nucleolus-stages (previtellogenic oocytes) were classified as immature. Maturing ovaries contained oocytes in the yolk granule-stage (vitellogenic oocytes) in addition to previtellogenic oocytes (except oogonium-stage oocytes). Mature ovaries contained mature-stage oocytes as well as previtellogenic (except oogonium-stage oocytes) and vitellogenic oocytes. Yano (1988) reported that germinal vesicle breakdown (GVBD) can last 5–11 h in the oocytes in the late phases of the prematuration- and maturation-stages in *Marsupenaeus japonicus* (Spence Bate, 1888). Females of *M. sibogae* were, therefore, determined to be mature if their oocytes had evidence of GVBD that would lead them to spawn within a few hours.

2.2.2.7. ***Size and age at sexual maturity***

Size at sexual maturity (CL_{50}) was defined as the CL at which 50% of the females become mature (King, 2007). The percentage of mature females (PMF) was plotted against CL, and a logistic function was fit to the data to determine the CL_{50} following King (2007):

$$PMF = 100 / [1 + \exp \{- f (CL_M - CL_{50})\}],$$

where f is the growth coefficient (the explanatory variable) and CL_M is the median value of each CL class. During the study period, PMF was less than 100% even in the largest CL class, which is common as not all mature individuals in a population are in a reproductive state at the same time (King, 2007). The data were therefore adjusted according to the method of King (2007) to avoid an unreasonably high estimate of CL_{50}. Minimum CL at sexual maturity was recorded from the histological analysis of ovaries. Age at minimum CL at maturity and at CL_{50} were calculated by transforming these CLs to their respective age classes following the CL frequency distribution and the estimated growth equation of *M. sibogae* (Rahman & Ohtomi, 2018a).

2.2.2.8. ***Spawning season***

The spawning season was estimated based on the monthly catch per unit effort (CPUE) of mature females. The monthly CPUE of mature females was standardized to a 10 min pre-set tow duration according to Fulanda & Ohtomi (2011) as follows:

$$CPUE = \text{number of mature females collected per haul} \times [10 / \text{effective tow duration (min)}]$$

The effective tow duration was determined from the initial and final times of net contact with the ocean bottom for each tow as determined from the logger-echo sounder depth plots (Fulanda & Ohtomi, 2011). The mouth opening height of the trawl net used was 2.5 ± 0.3 m (mean ± SD) (Fuwa *et al.*, 2010). The trawl net contact with the bottom was therefore confirmed as long as ≤ 2.8 m difference between the echo sounder and the compact TD-logger readings was maintained (Fulanda & Ohtomi, 2011). Females with a CL less than the CL_{50} were excluded from this analysis.

2.2.2.9. ***Statistical analyses***

The relationship between CL and GSI was analyzed by simple regression analysis using Microsoft Excel 2013. The size at sexual maturity was estimated by nonlinear regression analysis and fitting a logistic function based on the least-squares method using DeltaGraph 7. All statistical analyses were considered significant at 95% confidence interval ($P < 0.05$).

Table 2.2.1. Histological characteristics and diameter of oocytes in ovaries at different stages of maturity in female *Metapenaeopsis sibogae*. [a]Developmental stage of the most advanced oocytes in the ovary. Mean ± SD of oocyte diameters is given in parentheses; *N*, number of oocyte measured.

Ovarian maturity stage	Oocyte stage[a]	Histological characteristics	Oocyte diameter (µm)	*N*
Immature	Oogonium	Previtellogenesis	3-10 (5.2 ± 1.6)	54
	Early nucleolus		18-58 (30.7 ± 8.4)	339
	Middle nucleolus		43-113 (73.7 ± 14.3)	284
	Late nucleolus		98-150 (128.4 ± 11.9)	299
Maturing	Yolk granule	Vitellogenesis	133-183 (156.5 ± 8.0)	213
Mature	Mature	Germinal vesicle breakdown	160-238 (190.6 ± 15.6)	202

2.2.3. RESULTS

2.2.3.1. *Change in ovarian maturation with progression of gonadosomatic index*

There was no significant correlation between CL and gonadosomatic index (GSI) ($r = 0.061$, $P = 0.093$) for females determined as sexually mature using histological methods. The percent occurrence of females in each histologically determined stage of ovarian maturity gradually moved from most ovaries being immature to a majority being mature as GSI increased (Figure 2.2.4). Females with a GSI < 2% were mostly immature, whereas most females with a GSI of 2 to < 4% were maturing. The mature females first appeared in the GSI 2–3% class. More than 50% of the females with a GSI ≥ 4% were mature, whereas all the females with GSI ≥ 6% were mature.

2.2.3.2. *Histological verification of ovarian stages*

The maturity status of the different ovarian stages that were determined macroscopically was verified by histological analysis (Figure 2.2.5). Nearly 85% of Stage I ovaries were immature. Stage II ovaries included all the three maturity stages; however, the dominant stage was maturing (nearly 66%). Of the Stage III ovaries, nearly 80% were mature, and the remaining were maturing. The percentage of mature females steeply increased when classified macroscopically. The macroscopic determination of ovarian maturity based on the ovary and body widths can therefore be used as a simple and reliable index for estimating sexual maturity in *M. sibogae*. Females of *M. sibogae* with Stage III ovaries were considered to be mature.

2.2.3.3. *Size and age at sexual maturity*

The size at sexual maturity was calculated from the 2,995 females collected from April to December when the CPUE of mature females was relatively high (monthly CPUE of mature females ≥ 10; see below). The carapace length of mature females with Stage III ovaries ranged

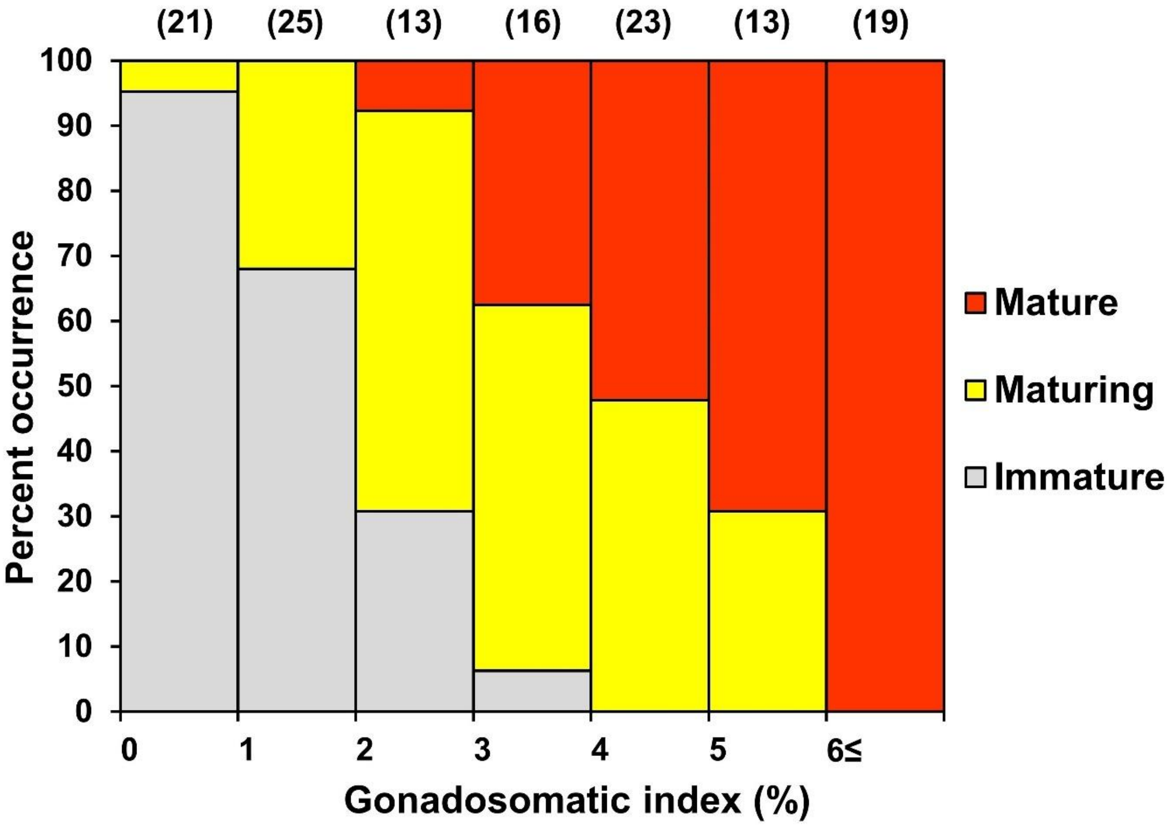


Figure 2.2.4. Percent occurrence of female *Metapenaeopsis sibogae* in histologically determined stages of ovarian maturity (immature, maturing, and mature) against the associated gonadosomatic index class. The number of females examined is given in parentheses.

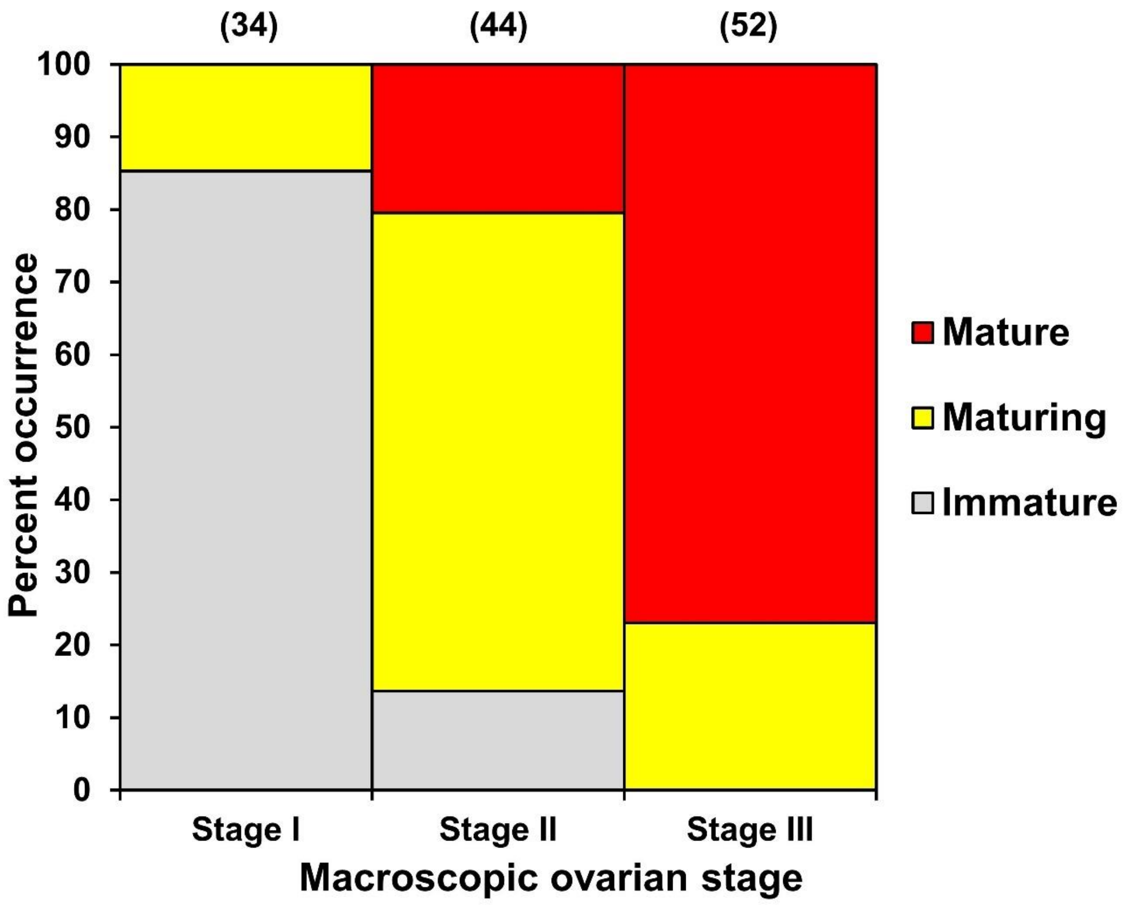


Figure 2.2.5. Percent occurrence of female *Metapenaeopsis sibogae* in histologically determined stages of ovarian maturity (immature, maturing, and mature) against the associated macroscopic ovarian stage. The number of females examined is given in parentheses.

from 12.5 mm to 21.5 mm. The relationship between CL and the percentage of mature females was expressed by the logistic function:

$$\mathrm{PMF} = 100 / [1 + \exp \{- 0.462 (\mathrm{CL_M} - 14.3)\}] \quad (N = 13, R^2 = 0.931, P < 0.001)$$

Size at sexual maturity was estimated to be 14.3 mm (Figure 2.2.6). The minimum CL of the histologically determined mature females was 12.5 mm. Both minimum CL at maturity and $\mathrm{CL_{50}}$ belonged to around 1-year-old age class.

2.2.3.4. *Spawning season*

Mature females occurred throughout the year though the mean CPUE of mature females from January to March was comparatively lower (monthly CPUE of mature females < 10) than that from April to December with a peak occurring in September to October (Figure 2.2.7). It was therefore concluded that *M. sibogae* spawns throughout the year in Kagoshima Bay with September-October being the main spawning season.

2.2.3.5. *Bottom-water temperature*

The monthly bottom-water temperature in the sampling area ranged from 15.4 °C in September to 16.3 °C in January. The monthly bottom-water temperature tended to be relatively stable and never dropped below 15 °C throughout the study period (Figure 2.2.8).

2.2.4. DISCUSSION

The precise determination of maturity is indispensable for the assessment and management of exploited stocks (Vitale *et al.*, 2006). The ovarian maturity status of *M. sibogae* was confirmed by the use of histological observation, which is known to be one of the most reliable methods for determining mature females of fishes and shrimps (West, 1990; Ohtomi *et al.*, 2003; Carbonell *et al.*, 2006). Histological evaluations allow for the precise and thorough examination of oocyte development, which results in less ambiguity when classifying ovarian maturity status (Williams, 2007).

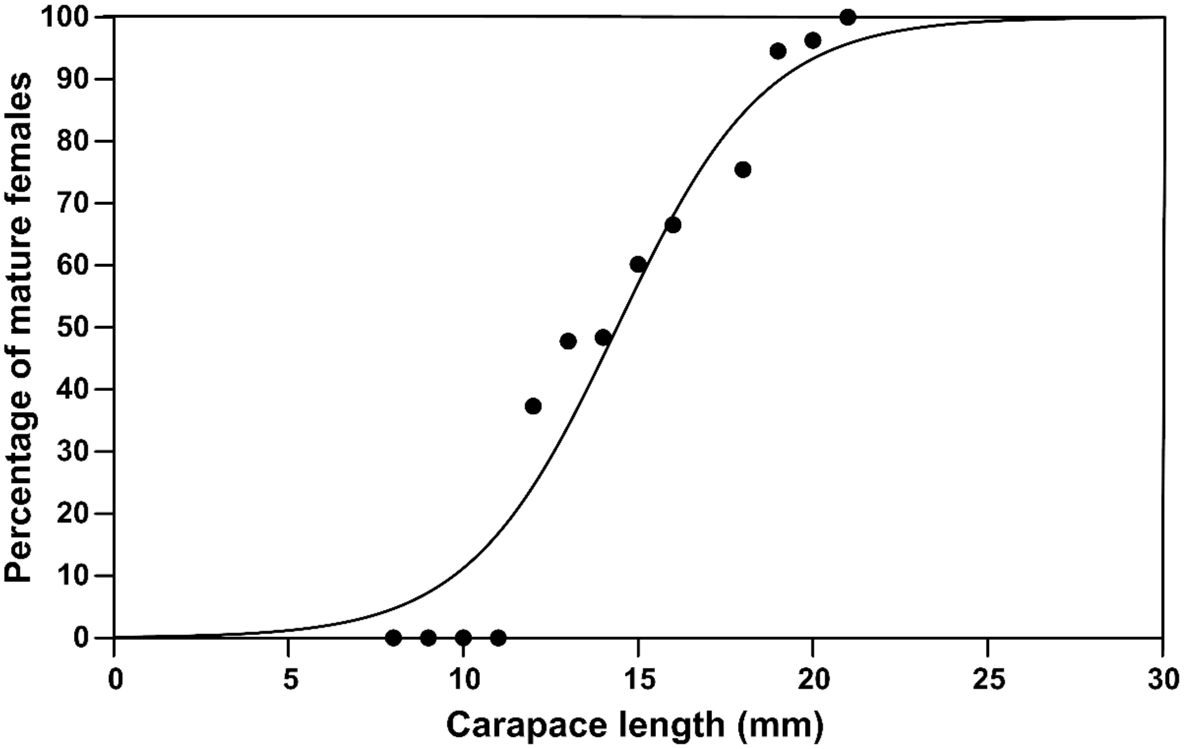


Figure 2.2.6. Adjusted percentage of mature females of *Metapenaeopsis sibogae versus* carapace length showing the logistic curve fitted to the data.

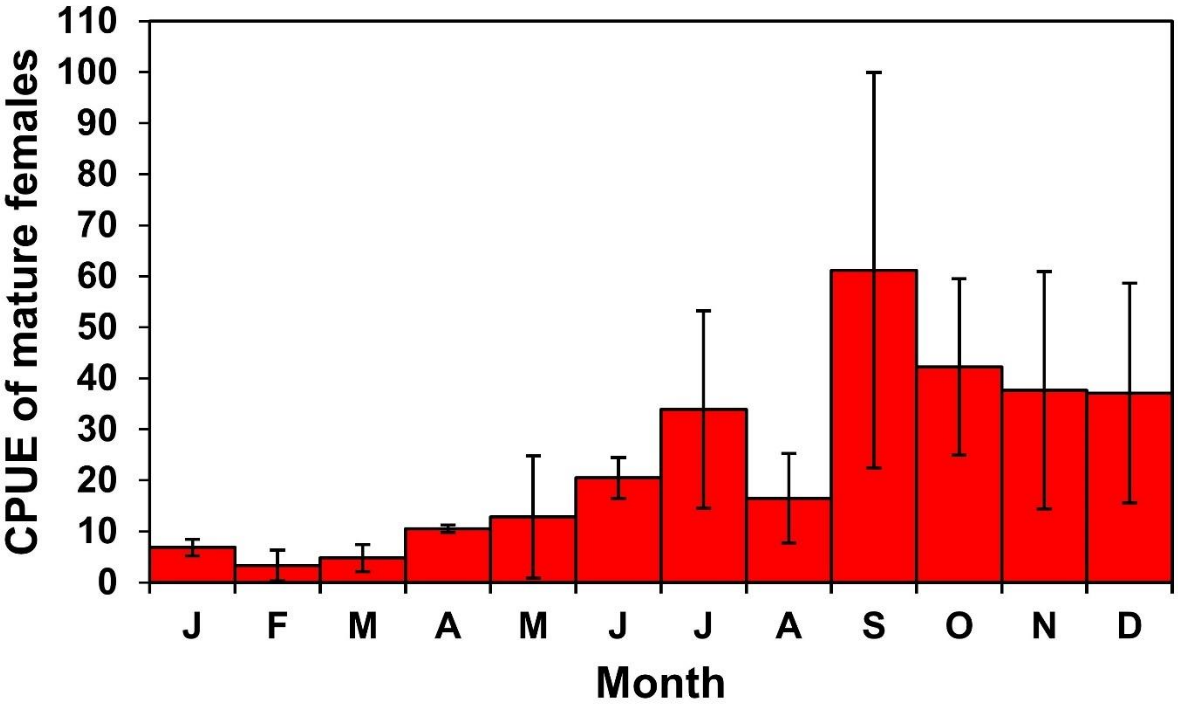


Figure 2.2.7. Monthly changes in the mean catch per unit effort (CPUE) of mature female *Metapenaeopsis sibogae* in Kagoshima Bay, southern Japan, from January 2014 to December 2015 and September 2013. Error bars show standard deviations.

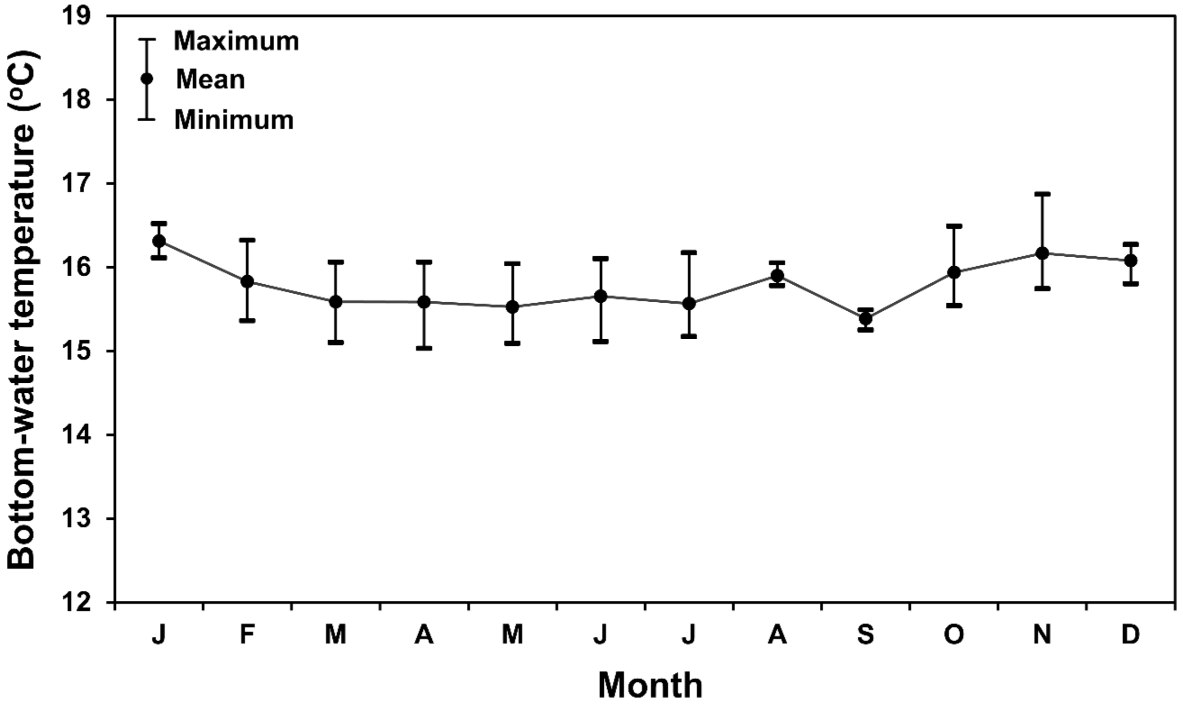


Figure 2.2.8. Monthly changes in bottom-water temperature at the sampling areas in Kagoshima Bay, southern Japan, from January 2014 to December 2015 and September 2013.

M. sibogae had asynchronous oocyte development in the ovary, which is a common phenomenon in penaeid shrimps including in other species of *Metapenaeopsis* such as *M. aegyptia*, *M. barbata*, *M. dalei*, *M. sinica*, and *M. palmensis* (Yano, 1988; Ohtomi & Yamamoto, 1997; Sakaji *et al.*, 2000; Sakaji, 2001a, 2001b; Yamada *et al.*, 2007; Chen *et al.*, 2014; Farhana & Ohtomi, 2016). This type of oocyte development is indicative of multiple spawning in a reproductive season (Bauer, 1989; Yamada *et al.*, 2007; Chen *et al.*, 2014). The ovarian maturity stage (immature, maturing, and mature) was assigned based on the most advanced oocytes present in the ovary. Females with spent ovaries, however, could not be detected during the present study because of the difficulty in distinguishing spent-stage ovaries from the developing-stage (Penn, 1980; Courtney & Dredge, 1988; Ohtomi *et al.*, 1998).

The appearance of cortical crypts and/or rods in the peripheral region of the oocyte cytoplasm based on histological observation is recognized as a sign of prematuration, and that the individual is capable of spawning within a few days, in many species of penaeoid shrimps (e.g., Bell & Lightner, 1988; Yano, 1988; Ohtomi & Yamamoto, 1997; Ohtomi *et al.*, 1998, 2003; Farhana & Ohtomi, 2016). It was impossible in the present study to distinguish the yolk's granule-stage from the prematuration-stage because of the absence of cortical crypts and/or rods. Previous studies of species of *Metapenaeopsis*, including *M. aegyptia*, *M. barbata*, *M. sinica*, and *M. palmensis*, have also reported the absence of cortical crypts (Sakaji, 2001b; Chen *et al.*, 2014), and no cortical crypts were found in *M. dalei* even when using samples collected at night (Sakaji *et al.*, 2000). The absence of cortical crypts and/or rods in oocyte development may therefore be a characteristic of *Metapenaeopsis* as compared to other penaeid shrimps (Sakaji *et al.*, 2000; Sakaji, 2001b; Chen *et al.*, 2014). In place of cortical crypts and/or rods, (e.g., Clark *et al.*, 1980; Yano, 1988; Ohtomi & Yamamoto, 1997; Farhana & Ohtomi, 2016), species of *Metapenaeopsis* have the characteristic germinal vesicle breakdown (GVBD) indicating maturity (Sakaji *et al.*, 2000; Sakaji, 2001b; Chen *et al.*, 2014).

Macroscopic analyses and biological indices are the quickest, relatively most inexpensive, and most widely applied methods used to assign maturity stages (West, 1990; Farrell *et al.*, 2012; Flores *et al.*, 2015). Both methods, however, could involve high amounts of error due to the difficulties in distinguishing different maturity stages. Any macroscopic determination of maturity or biological index should, therefore, be validated by histology to ensure its accuracy (West, 1990). The gonadosomatic index (GSI) has been used extensively in reproductive studies of shrimps (e.g., Gab-Alla *et al.*, 1990; Ohtomi & Yamamoto, 1997; Ohtomi & Matsuoka, 1998; Ohtomi *et al.*, 1998; Hossain & Ohtomi, 2008; Farhana & Ohtomi, 2016). Courtney *et al.* (1995), in contrary, proposed that GSI is a poor indicator of ovarian maturity in *Melicertus plebejus* (Hess, 1865) due to its dependence on body size. The relationship between CL and GSI in mature females of *M. sibogae* was not significant; however, the relationship between the two variables was gradual, and similar relationships have been reported for other penaeid shrimps such as *Marsupenaeus japonicus* (Ohtomi *et al.*, 2003) and *M. palmensis* (Chen *et al.*, 2014). Due to the lack of a sharp relationship between GSI and ovarian maturity stages, GSI was not used as an index for estimating the sexual maturity of female *M. sibogae*.

The limitations of GSI as an index of maturity call for alternative approaches to be explored for the purposes of simple yet reliable maturity staging. Several authors have used oocyte diameter and the color and shape of the ovary for maturity staging in penaeids (e.g., Gab-Alla *et al.*, 1990; Choi *et al.*, 2005; López-Martínez *et al.*, 2005; Hossain & Ohtomi, 2008; Silva *et al.*, 2016). These attempts were not flawless as morphological differences between fresh and frozen gonads have been documented (McPherson *et al.*, 2011), whereas measuring oocyte diameter is a laborious and time-consuming procedure, and in many cases, these indices were not verified through histology (e.g., Choi *et al.*, 2005; López-Martínez *et al.*, 2005; Hossain & Ohtomi, 2008). In search of a faster, cost-effective, and reliable method of assessing ovarian maturity, the ratio of ovary width to body width was investigated, and it yielded promising results. Histological observation revealed a sharp relationship between macroscopically and histologically determined ovarian stages with nearly 80% of the Stage III ovaries being confirmed as mature. The ratio of ovary width to body width can, therefore, be used as a simple and reliable index for estimating ovarian maturity in *M. sibogae*. Females with Stage III ovaries were identified as mature, and this index may also be useful for classifying ovarian maturity in other small penaeid shrimps in absence of data based on a histological classification.

To successfully manage an exploited fishery, it is imperative to know the size at sexual maturity of the target species, and this value should be regularly re-evaluated, particularly early in the process (Watters & Hobday, 1998). Minimum CL at maturity and CL_{50} for female *M. sibogae* were estimated to be 12.5 and 14.3 mm, respectively. Transforming these values to ages indicated that individuals at these sizes approximately belonged to a 1-year-old age class. This information is helpful for setting the cod-end mesh size to restrict catching the first mature shrimp and thus leaving the smallest mature shrimp to spawn (Chen *et al.*, 2014).

Results of the present study suggest a year-round spawning in *M. sibogae*, with September-October being the peak season. Year-round spawning is a rare phenomenon in Japanese penaeids as most for which maturation data are available, mature individuals appear during spring to autumn (e.g., Hudinaga, 1942; Yasuda, 1949; Kosaka, 1977; Sakaji *et al.*, 1992; Ohtomi *et al.*, 1998, 2003; Hossain & Ohtomi, 2008; Farhana & Ohtomi, 2016). Sakaji (2001a), however, reported year-round spawning in *M. dalei* from Tosa Bay, and several studies have indicated that an extended reproductive period may be a typical feature of deep-water species (George & Menzies, 1967; Harrison, 1988; Gage & Tyler, 1991). Both Kagoshima Bay and Tosa Bay (maximum water depth of over 400 m; Sakaji *et al.*, 2006) are deep-water bays. Seasonality in the reproduction of penaeids is also affected by water temperature, availability of food, and planktonic larvae, among other factors (Thorson, 1950; Giese & Pearse, 1974; Sastry, 1983; Tyler, 1986; Bauer, 1989, 1992; Bishop & Shalla, 1994; Cha *et al.*, 2004a). During the study period, bottom-water temperature was relatively constant and never fell below 15 °C even during the winter months. The stable bottom-water temperature in Kagoshima Bay in winter is caused by the intrusion of warm Kurushio Current into the bay (Kohno *et al.*, 2004). Bottom-water temperature rarely falls below 15 °C in Tosa Bay (Sakaji, 2001a). Kobari *et al*. (2002) further reported that phytoplankton is predominant throughout the year in Kagoshima Bay. Deep-water habitats, relatively constant bottom-water temperatures, and the availability of larval food may, therefore, have an influence on the extended spawning season of *M. sibogae*.

The spawning season of other Japanese species of *Metapenaeopsis* such as *M. acclivis* and *M. barbata* from Kasaoka Bay in the Ariake Sea and Aki-nada in the Seto Inland Sea is from early summer to autumn (Yasuda, 1949; Ikematsu, 1963), whereas mature females of *M. dalei* were reported to occur in July and August in Suo-nada in the Seto Inland Sea, and Sendai Bay (Yatsuyanagi & Maekawa, 1957; Kosaka, 1977). The water temperature of these semi-enclosed, shallow water bodies (average water depth ~ 30–50 m), however, falls below 10 °C in winter (Onbe & Kakuda, 1962; Sakaji, 2001a; Tabata *et al.*, 2015) when no spawning was reported. Chen *et al.* (2014) reported the spawning season of *M. palmensis* as February to June in waters off Taiwan (water depth, 10–40 m) whereas Choi *et al.* (2005) reported the spawning season of *M. dalei* as July to August in the western sea of Korea (water depth ≤ 30 m). They noted bottom-water temperatures and latitudinal effects as acting as the drivers of spawning seasonality in these species. The bottom-water temperature in the spawning season of *M. palmensis* ranged from 22.6 to 29.1 °C (Chen *et al.*, 2014), whereas spawning starts when the bottom-water temperature rises to over 23 °C in *M. dalei* (Choi *et al.*, 2005). The monthly bottom-water temperature in Kagoshima Bay during the present study varied between 15.4 °C and 16.3 °C. The general latitudinal pattern observed in shrimp reproductive strategies is often referred to as ‘the classical paradigm’, indicating continuous reproduction at low latitudes with increased seasonality towards the poles (Costa & Fransozo, 2004; Costa *et al.*, 2010; van de Kerk *et al.*, 2016). The spawning season of *M. palmensis* could last throughout the year in tropical waters (9°30' S, 10°10' S) (Watson & Keating, 1989), whereas in southwestern Taiwan (22°15' N, 22°45' N) it lasts for close to half a year (February to June) (Chen *et al.*, 2014) but is relatively shorter (June-September) in higher latitude waters (35°20' N, 35°30' N) (Hayashi & Sakamoto, 1978). The spawning season of *M. dalei* along the western coast of Korea is much shorter than that in Japanese waters (Yatsuyanagi & Maekawa, 1957; Kosaka, 1977, Choi *et al.*, 2005). Even though the study site, Kagoshima Bay, is located in a higher latitude (31°25' N), *M. sibogae* spawned throughout the year, thus not following this ‘classical paradigm.’ *Metapenaeopsis sibogae* rather represents a tropical/subtropical species reproductive pattern of continuous spawning (Garcia, 1988) in Japanese waters, with a single peak. The discrepancies in the reproductive pattern of *M. sibogae* in contrast to the other species of *Metapenaeopsis* could therefore be attributable to variations in water depth, bottom-water temperature, and food availability as well as other environmental factors.

Elucidation of the spawning season of *M. sibogae* should help fishery managers in establishing fishery closures targeting large spawning aggregations to protect the spawning population to allow a sufficient proportion of the spawning stock to be able to spawn (Russell *et al.*, 2012; van Overzee & Rijnsdorp, 2015) and thereby enhancing the reproductive output of this commercially exploited shrimp. This information is indispensable for the formulation of fishing regulations for sustainable management and conservation of this emergent fishery. Further studies on the spawning frequency and fecundity, as well as a breeding stock assessment, are needed to gain a broader understanding of the reproductive potential of the population, which in turn will form the basis for adequate management regimes.

2.3: REPRODUCTIVE BIOLOGY OF *METAPENAEOPSIS KYUSHUENSIS*

2.3.1. INTRODUCTION

M. kyushuensis is reported from the coastal waters of Japan and is a dominant species in the benthic community of Kagoshima Bay. It locally known as “Shiroebi” in Japanese. There is, however, no study available on the reproductive biology of this species. The present study aims to describe the ovarian maturation, size and age at maturity and spawning season of *M. kyushuensis* for the first time using samples collected from Kagoshima Bay, southern Japan.

2.3.2. MATERIALS AND METHODS

2.3.2.1. *Sampling*

Monthly samples of *M. kyushuensis* were obtained from experimental trawl surveys conducted in the central area of Kagoshima Bay, southern Japan at depths of 125–183 m (Figure 2.3.1). The sampling was conducted onboard the training vessel *Nansei-Maru* (175 t) of the Faculty of Fisheries, Kagoshima University from January 2016 to December 2017 using a simple trawl net (LC-VI; Nichimo Corporation, Tokyo), 23.5 m long, 37.9 mm mesh size in the body, 20.2 mm in the cod-end, carrying canvas kites on the tip of the wings (Ohtomi *et al.*, 2004) was used for sampling. Net depth and water temperature were recorded at 1-min intervals using a compact temperature-depth (compact-TD) logger (ATD-HR; JFE Advantech, Nishinomiya, Japan) attached to the head rope of the net. The bottom depth was recorded at 1-min intervals with a split-beam quantitative echo sounder (KFC-3000; Kaijo Corp., Hamura, Japan). The net was towed for a pre-set tow duration of 10 min at a speed of 2 knots. Bottom-water temperatures recorded during effective tow durations (see below) were used to calculate mean temperature for each haul and month. At each haul, individuals of *M. kyushuensis* were sorted out, counted, and instantly preserved in ice on-board and then in formalin upon arrival

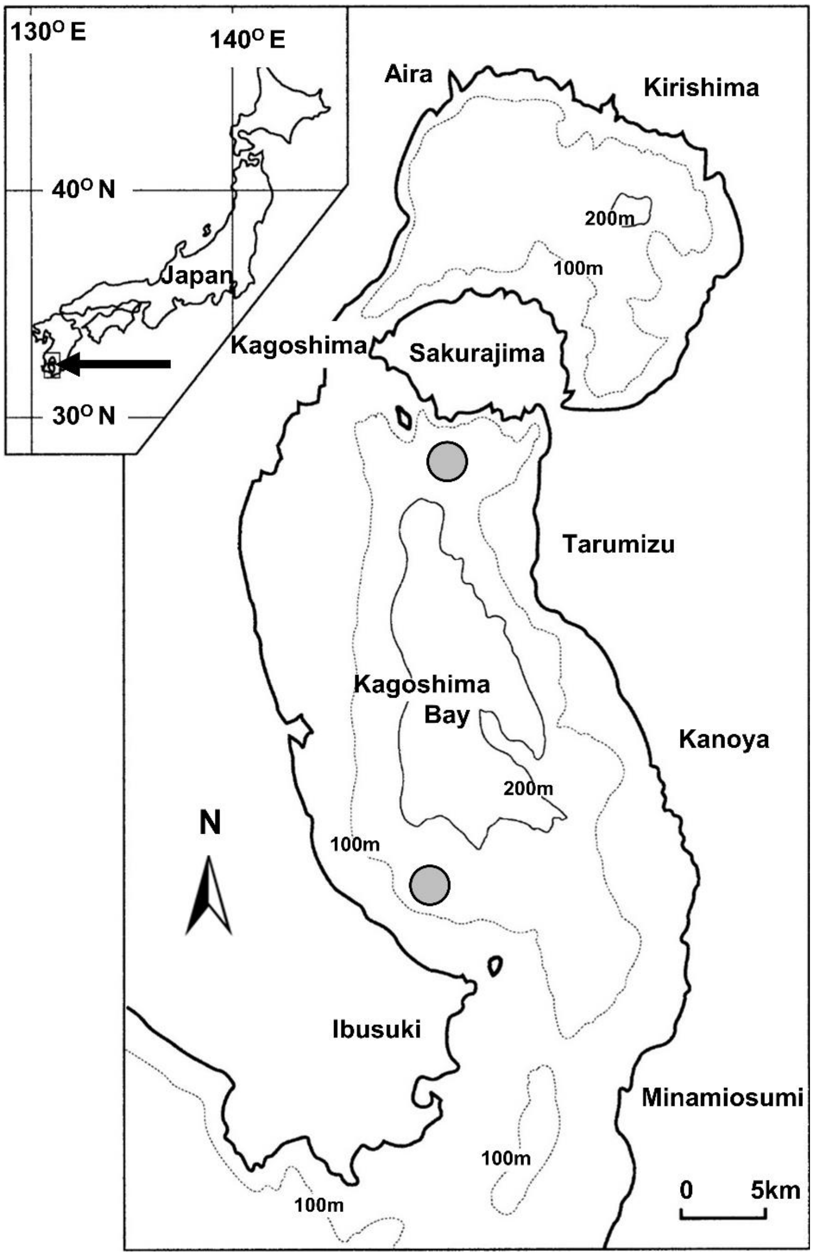


Figure 2.3.1. Kagoshima Bay, southern Japan, with indication of the collection of samples of *Metapenaeopsis kyushuensis* in central part of the bay (shaded areas).

at the laboratory.

2.3.2.2. *Measurements*

All specimens were classified by sex according to the presence of a petasma in males or a thelycum in females, and only females (N = 2006) were used. For each female, carapace length (CL) was measured to the nearest 0.01 mm from the posterior margin of the eyestalk to the dorsal midline of the posterior margin of the carapace with slide calipers (CD-15PS; Mitutoyo Corp., Kawasaki, Japan), and body weight was measured to the nearest 0.01 g by electronic balance (EB-430DW; Shimadzu Corp., Kyoto, Japan). The exoskeleton and muscles above the ovary of each female were removed, and the ovary and body widths were measured to the nearest 0.01 mm at the middle of the first abdominal segment (Figure 2.3.2). Ovaries were classified as one of three initial sexual maturity stages based on the ratio of ovary width to body width following Rahman & Ohtomi (2017): Stage I, ovary width < 1/5 body width (Figure 2.3.2A); Stage II, 1/5 body width ≤ ovary width < 1/3 body width (Figure 2.3.2B); and Stage III, ovary width ≥ 1/3 body width (Figure 2.3.2C). The performance of this macroscopic ovarian maturity staging was verified by histological observation (see below). The whole gonad was then removed and weighed to the nearest 0.001 g using the electronic balance. The gonadosomatic index was calculated as:

$$\text{GSI (\%)} = 100 \times [\text{ovary weight} / \text{body weight}]$$

2.3.2.3. *Histological analysis and measurement of oocytes*

A small portion of each ovary from the 92 females collected from January 2016 to December 2017 (CL, 8.4 to 18.4 mm; GSI, 0.11 to 8.70%) was dehydrated in an ethanol series, infiltrated with paraffin, and sliced into 6 µm sections. The sections were stained with Mayer's hematoxylin and eosin, cover-slipped with a mounting medium, and examined under a microscope (DMLB; Leica, Wetzlar, Germany). As a preliminary examination, sections from

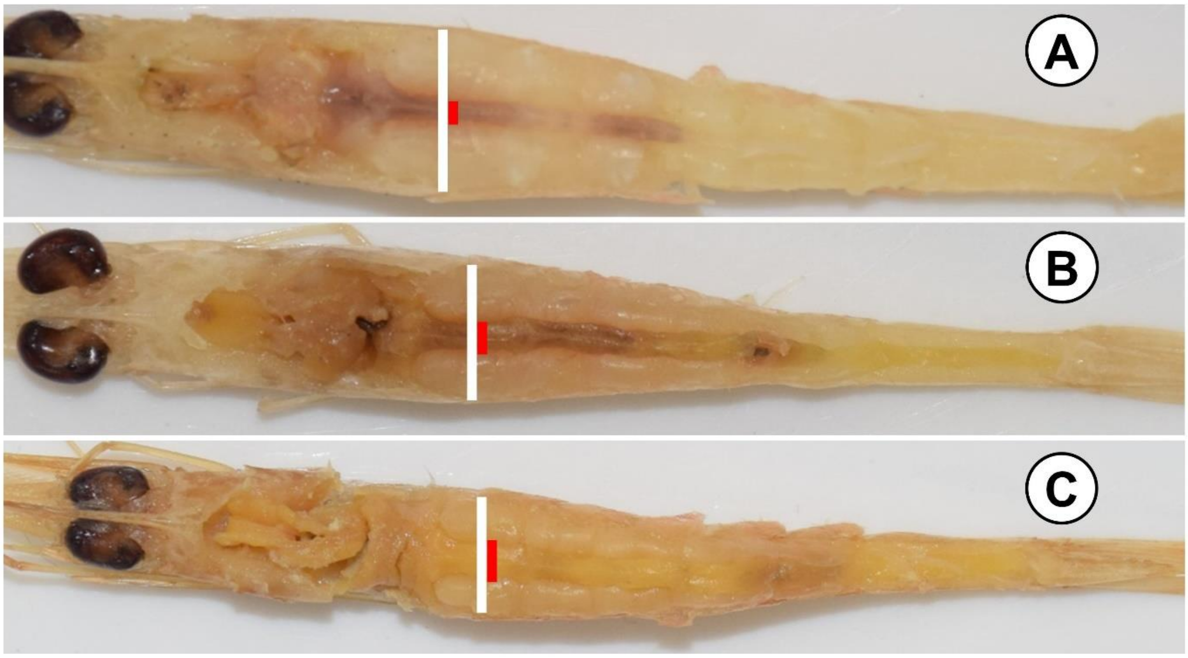


Figure 2.3.2. Macroscopic maturity stages of the ovaries of *Metapenaeopsis kyushuensis*. **A**, Stage I (ovary width < 1/5 body width); **B**, Stage II (1/5 body width ≤ ovary width < 1/3 body width); **C**, Stage III (ovary width ≥ 1/3 body width). Black line indicates body width; white line indicates ovary width.

three parts of the ovary (the posterior region of the cephalothoracic lobe and the anterior and middle regions of the abdominal lobe) from one female (CL, 17.7 mm; GSI, 7.65%) were examined to differentiate the maturity stages in relation to the position in the ovary, but no difference was found in maturity stage with respect to ovary position. Tissue samples from the anterior regions of the abdominal lobes were thus used for histological analysis for the rest of the ovaries to determine the oocyte developmental stages. The terminology and criteria for describing oocyte development and ovarian maturation followed Yano (1988), and Rahman & Ohtomi (2017) with few modifications.

Thirteen of the sectioned ovaries of the highest quality were selected for measuring oocyte diameters. Only oocytes sectioned through the nucleus (N = 983) were measured under a microscope. Due to histological processing and natural variation, most of the oocytes were not perfectly spherical in shape (Rahman & Ohtomi, 2017). Average values of the maximum and minimum oocyte diameters were thus used to reduce the variance and avoid artificially increasing the overlap between oocytes of different developmental stages (see West, 1990; Farrell *et al.*, 2012; Rahman & Ohtomi, 2017).

2.3.2.4. *Classification of developmental stages of oocytes*

Based on histological observation, oocytes were assigned to one of five developmental stages: early nucleolus, middle nucleolus, late nucleolus, yolk granule, and mature (Table 1). The criteria of these stages are as follows:

1. **Early nucleolus-stage:** oocytes were 20–53 µm in diameter, basophilic, and contained thin cytoplasm (Figure 2.3.3A);
2. **Middle nucleolus-stage:** oocytes were 40–108 µm in diameter with strongly basophilic chromatin in the nucleus and follicle cells visible around the oocytes (Figure 2.3.3B);
3. **Late nucleolus-stage:** oocytes were 79–134 µm in diameter with weak hematoxylin staining in the cytoplasm and decreased thickness of the follicle layers (Figure 2.3.3C);

4. **Yolk granule-stage:** oocytes were 118–223 µm in diameter with eosin-stained acidophilic yolk granules in the cytoplasm (Figure 2.3.3D); and
5. **Mature-stage:** oocytes were 148–231 µm in diameter and comprised of acidophilic yolk granules concentrated in and filling the cytoplasm of the oocyte, and germinal vesicle breakdown (GVBD) occurred throughout the cytoplasm of this stage (Figure 2.3.3E).

2.3.2.5. *Assessment of stages of ovarian maturity*

M. kyushuensis had asynchronous ovaries, containing oocytes at various developmental stages (Figure 2.3.3G). The stages of ovarian maturity were thus divided into three categories based on the developmental stage of the most advanced oocytes in the ovary: immature, maturing, and mature (Table 1).

1. **Immature:** Ovaries with oocytes in the early-, middle-, or late nucleolus-stages (previtellogenic oocytes) were classified as immature.
2. **Maturing:** Maturing ovaries contained oocytes in the yolk granule-stage (vitellogenic oocytes) in addition to previtellogenic oocytes.
3. **Mature:** Mature ovaries contained mature-stage oocytes as well as previtellogenic and vitellogenic oocytes.

Yano (1988) reported that germinal vesicle breakdown (GVBD) can last 5–11 h in the oocytes in the late phases of the prematuration- and maturation-stages in *M. japonicus*. Females of *M. kyushuensis* were, therefore, determined to be mature if their oocytes had evidence of GVBD that would lead them to spawn within a few hours.

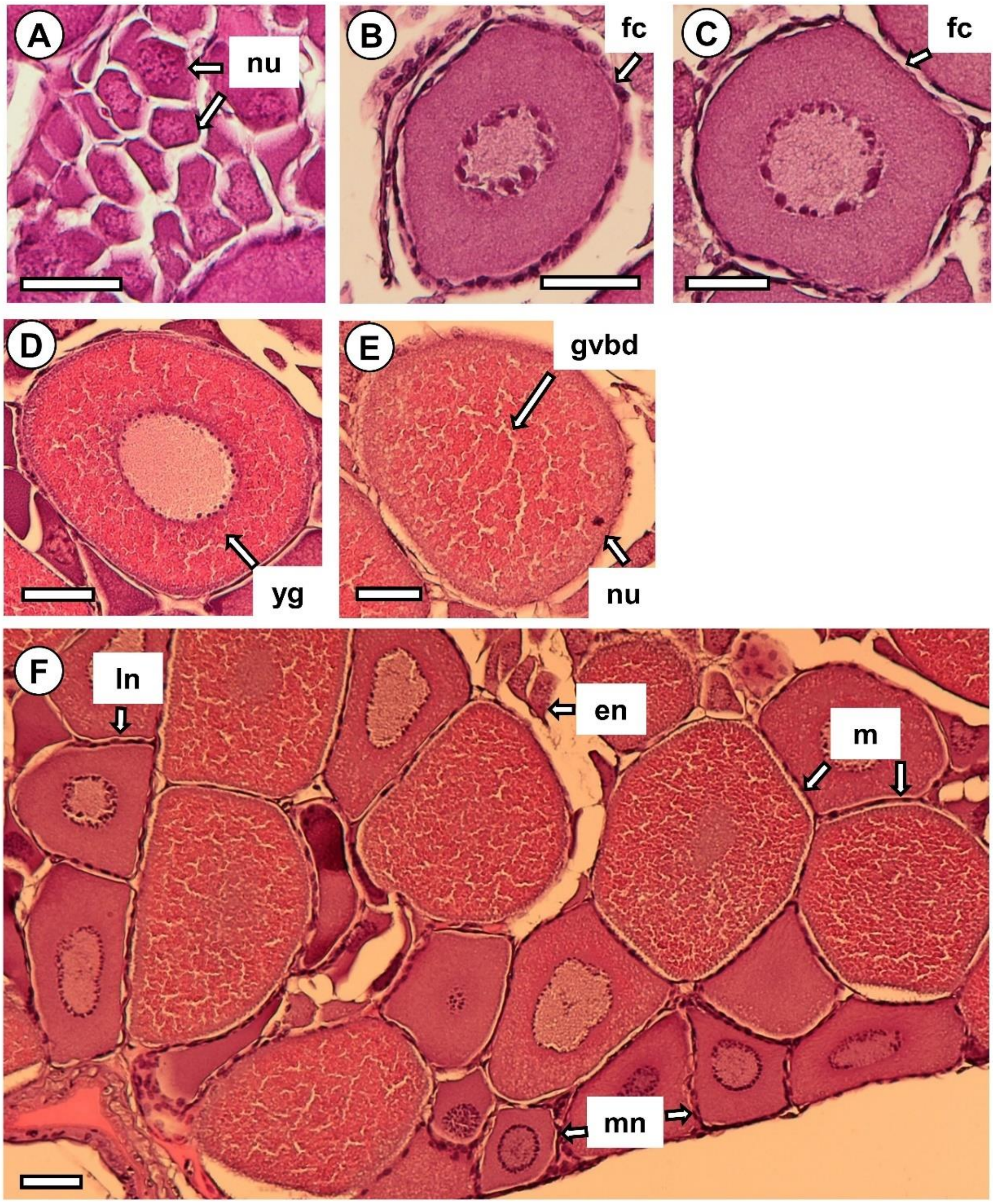


Figure 2.3.3. Histological sections of oocyte development and mature gonad of *Metapenaeopsis kyushuensis*. **A**, early nucleolus stage; **B**, middle nucleolus stage; **C**, late nucleolus stage; **D**, yolk granule stage; **E**, mature stage; **F**, asynchronous oocyte development in the ovary; fc, follicle cells; gbvd, germinal vesicle break down; en, early nucleolus-stage; ln, late nucleolus-stage oocyte; m, mature-stage oocyte; mn, middle nucleolus-stage oocyte; nu, nucleoli; yg, yolk granules. Scale bar = 50 µm.

Table 2.3.1. Histological characteristics and diameter of oocytes in ovaries at different stages of maturity in female *Metapenaeopsis kyushuensis*.

Ovarian maturity stage	Oocyte stage[a]	Histological characteristics	Oocyte diameter (µm)	*N*
Immature	Early nucleolus	Previtellogenesis	20-53 (28.2 ± 6.5)	339
	Middle nucleolus		40-108 (64.2 ± 17.1)	284
	Late nucleolus		79-134 (104.7 ± 12.8)	299
Maturing	Yolk granule	Vitellogenesis	118-223 (150.3 ± 11.6)	213
Mature	Mature	Germinal vesicle breakdown	148-231 (179.8 ± 18.5)	202

[a] Developmental stage of the most advanced oocytes in the ovary. Mean ± SD of oocyte diameters is given in parentheses; *N*, number of oocyte measured

2.3.2.6. *Size and age at sexual maturity*

Size at sexual maturity (CL_{50}) was defined as the CL at which 50% of the females become mature (King, 2007). The percentage of mature females (PMF) was plotted against CL, and a logistic function was fit to the data to determine the CL_{50} following King (2007):

$$PMF = 100 / [1 + \exp\{- f (CL_M - CL_{50})\}],$$

where f is the growth coefficient (the explanatory variable) and CL_M is the median value of each CL class. During the study period, PMF was less than 100% even in the largest CL class, which is common as not all mature individuals in a population are in a reproductive state at the same time (King, 2007; Rahman & Ohtomi, 2017). The data were therefore adjusted according to the method of King (2007) to avoid an unreasonably high estimate of CL_{50}. Minimum CL at sexual maturity was recorded from the histological analysis of ovaries. Age at minimum CL at maturity and at CL_{50} were calculated by transforming these CLs to their respective age classes following the CL frequency distribution and the estimated growth equation of *M. kyushuensis* (see Chapter 3, Section 3.3).

2.3.2.7. *Spawning season*

The spawning season was estimated based on the monthly catch per unit effort (CPUE) of mature females. The monthly CPUE of mature females was standardized to a 10 min pre-set tow duration according to Fulanda & Ohtomi (2011) as follows:

$$CPUE = \text{number of mature females collected per haul} \times [10 / \text{effective tow duration (min)}]$$

The effective tow duration was determined from the initial and final times of net contact with the ocean bottom for each tow as determined from the logger-echo sounder depth plots (Fulanda & Ohtomi, 2011). The mouth opening height of the trawl net used was 2.5 ± 0.3 m (mean ± SD) (Fuwa *et al.*, 2010). The trawl net contact with the bottom was therefore confirmed as long as ≤ 2.8 m difference between the echo sounder and the compact TD-logger readings was maintained (Fulanda & Ohtomi, 2011). Females with a CL less than the CL_{50} were excluded from this analysis.

2.3.2.8. *Statistical analyses*

The relationship between CL and GSI was analyzed by simple regression analysis using Microsoft Excel 2013. The size at sexual maturity was estimated by nonlinear regression analysis and fitting a logistic function based on the least-squares method using DeltaGraph 7. All statistical analyses were considered significant at 95% confidence interval ($P < 0.05$).

2.3.3. RESULTS

2.3.3.1. *Change in ovarian maturation with progression of gonadosomatic index*

There was no significant correlation between CL and gonadosomatic index (GSI) ($r = 0.033$, $P = 0.645$) for females determined as sexually mature using histological methods. The percent occurrence of females in each histologically determined stage of ovarian maturity gradually moved from most ovaries being immature to a majority being mature as GSI increased (Figure 2.3.4). Females with a GSI < 2% were mostly immature, whereas most females with a GSI of 2 to < 4% were maturing. The mature females first appeared in the GSI 2–3% class. More than 50% of the females with a GSI ≥ 5% were mature, whereas all the females with GSI ≥ 7% were mature.

2.3.3.2. *Histological verification of ovarian stages*

The maturity status of the different ovarian stages that were determined macroscopically was verified by histological analysis (Figure 2.3.5). Nearly 70% of Stage I ovaries were immature whereas ~23% were maturing and ~6% were mature. Stage II ovaries also included all the three maturity stages; however, the dominant stage was maturing (~56%). Of the Stage III ovaries, ~81% were mature, and the remaining were maturing. The percentage of mature females steeply increased when classified macroscopically. The macroscopic

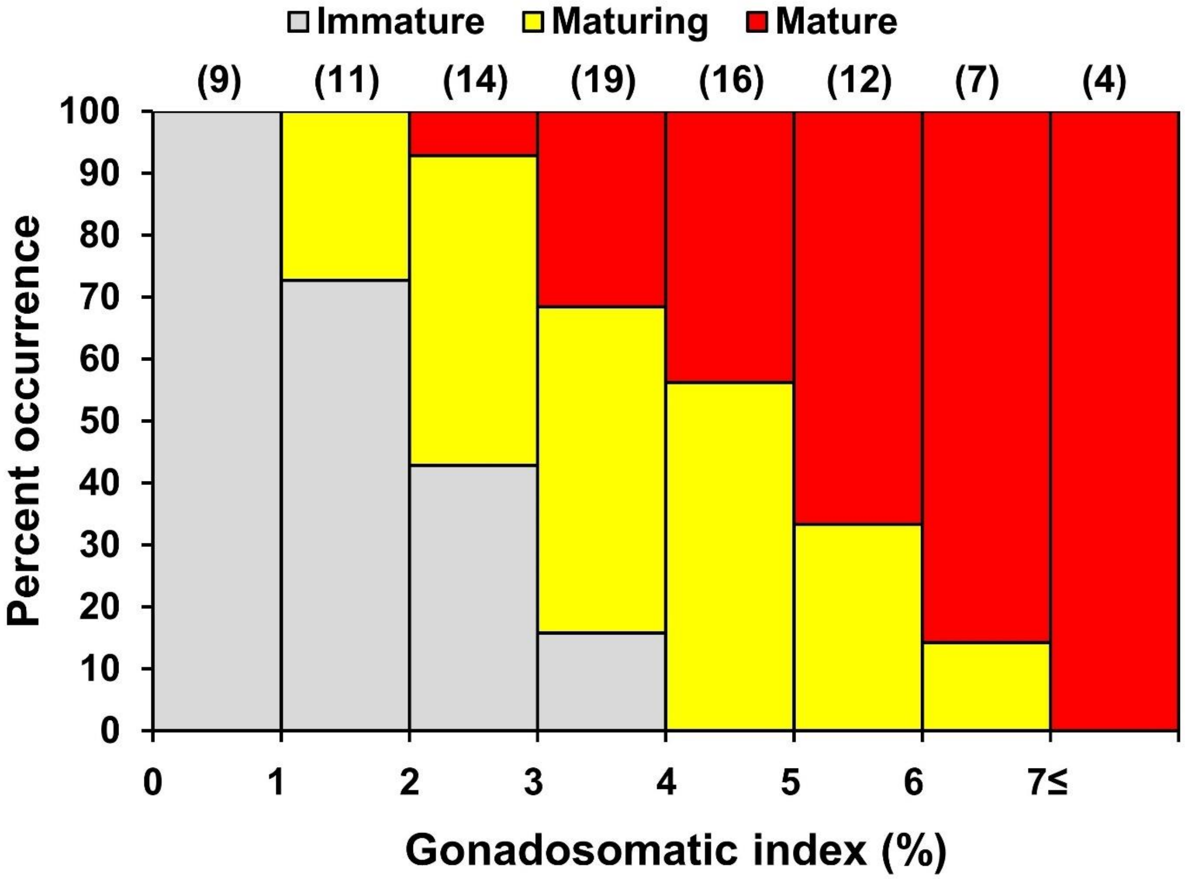


Figure 2.3.4. Percent occurrence of female *Metapenaeopsis kyushuensis* in histologically determined stages of ovarian maturity (immature, maturing, and mature) against the associated gonadosomatic index class. The number of females examined is given in parentheses.

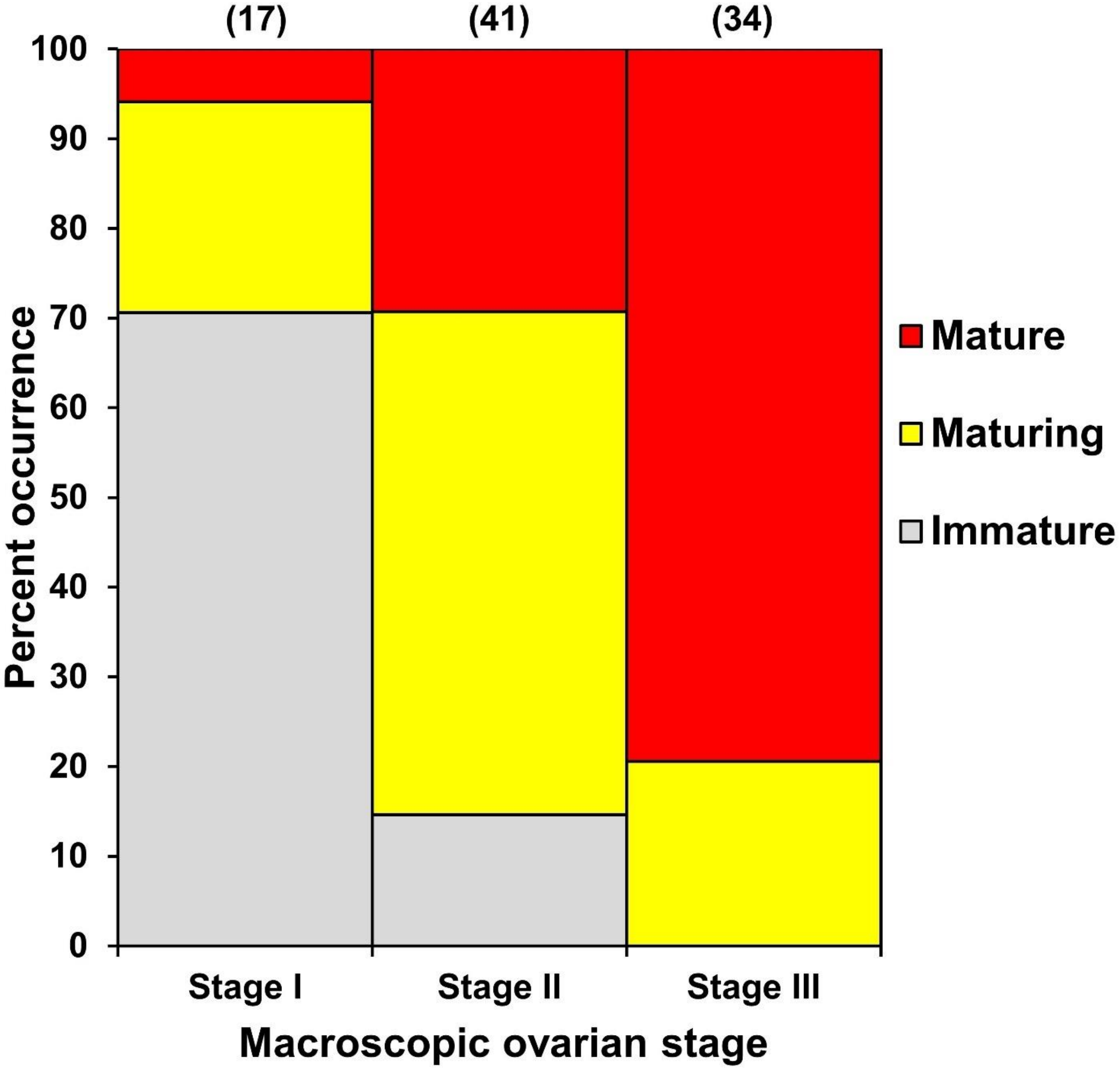


Figure 2.2.5. Percent occurrence of female *Metapenaeopsis kyushuensis* in histologically determined stages of ovarian maturity (immature, maturing, and mature) against the associated macroscopic ovarian stage. The number of females examined is given in parentheses.

determination of ovarian maturity based on the ovary and body widths can therefore be used as a simple and reliable index for estimating sexual maturity in *M. kyushuensis*. We considered females with Stage III ovaries to be mature.

2.3.3.3. *Size and age at sexual maturity*

The size at sexual maturity was calculated from 758 females collected from May to September when the CPUE of mature females was relatively high. The carapace length of mature females with Stage III ovaries ranged from 11.5 mm to 19.7 mm. The relationship between CL and the percentage of mature females was expressed by the logistic function:

$$\mathrm{PMF} = 100 / [1 + \exp \{-0.518 (\mathrm{CL_M} - 13.7)\}] \ (N = 12, R^2 = 0.930, P < 0.001)$$

Size at sexual maturity was estimated to be 13.7 mm (Figure 2.3.6). The minimum CL of the histologically determined mature females was 11.5 mm. Both minimum CL at maturity and CL_{50} belonged to around 1-year-old age class.

2.3.3.4. *Spawning season*

Mature females occurred from April to January though the mean CPUE of mature females from October to January and in April was comparatively lower (monthly CPUE of mature females < 5) than that from May to September with a peak occurring in June to September (Figure 2.3.7). We therefore concluded that *M. kyushuensis* spawns from April to January in Kagoshima Bay with June-September being the main spawning season.

2.3.3.5. *Bottom-water temperature*

The monthly bottom-water temperature in the sampling area ranged from 15.1 °C in April to 17.5 °C in January. The monthly bottom-water temperature tended to be relatively stable and never dropped below 15 °C throughout the study period (Figure 2.3.8).

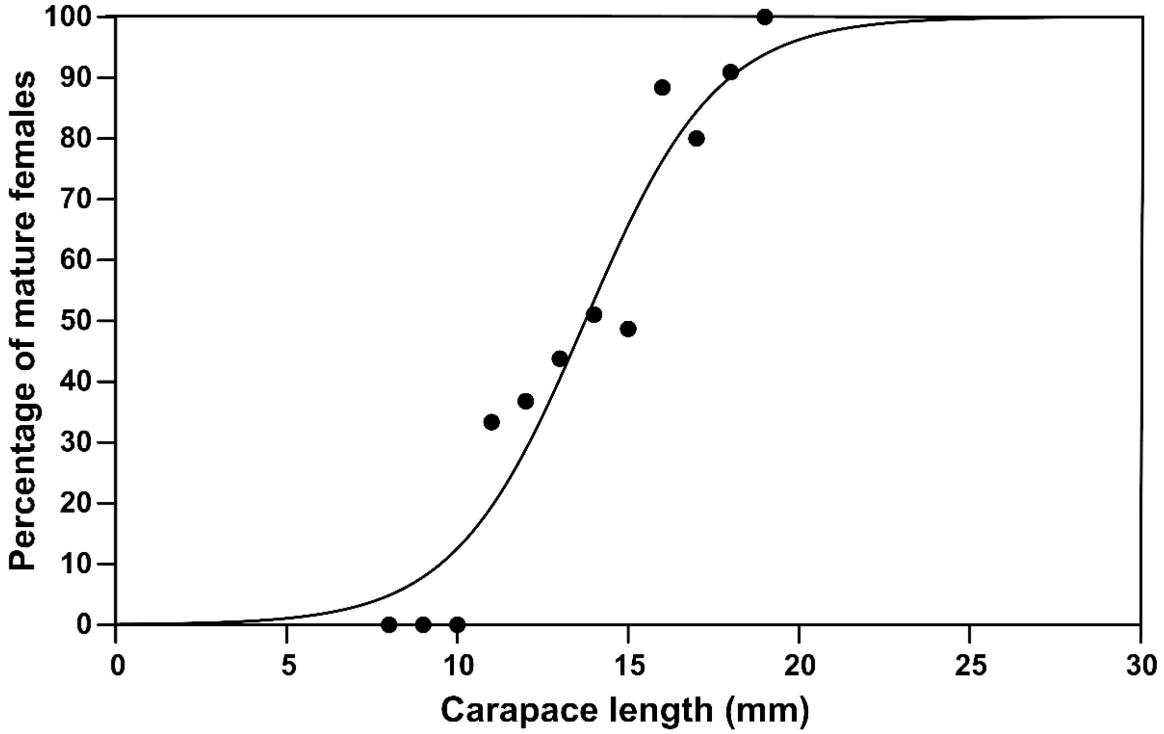


Figure 2.3.6. Adjusted percentage of mature females of *Metapenaeopsis kyushuensis* against carapace length showing the logistic curve fitted to the data.

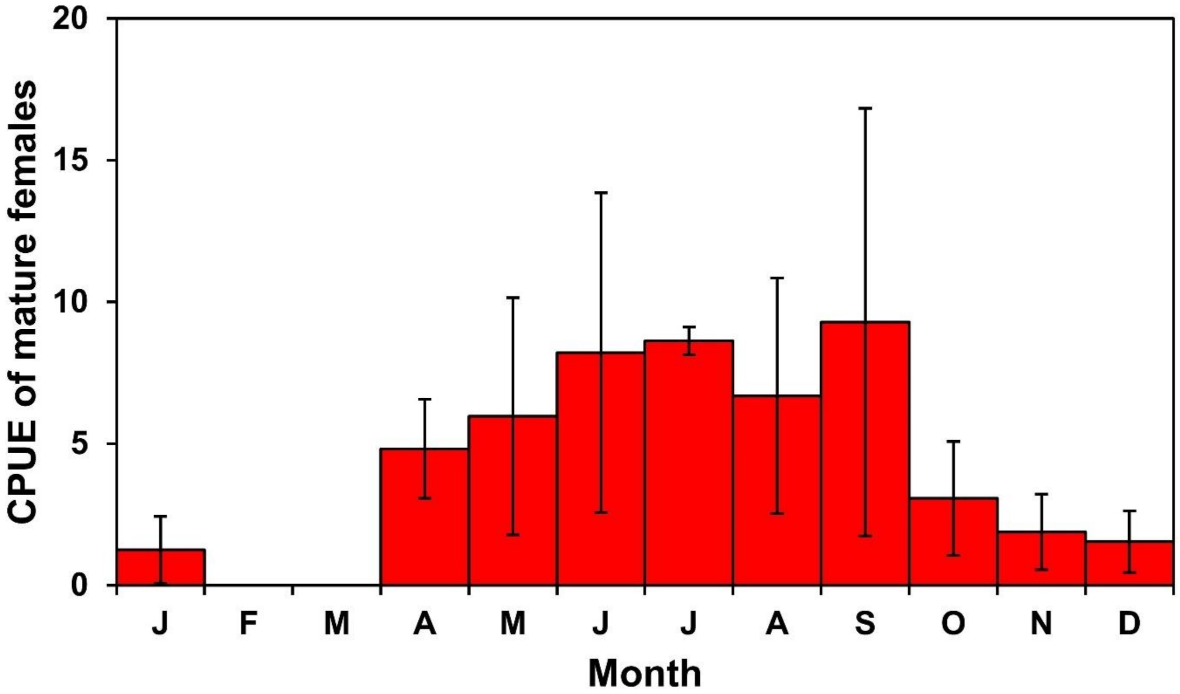


Figure 2.3.7. Monthly changes in the mean catch per unit effort (CPUE) of mature female *Metapenaeopsis kyushuensis* in Kagoshima Bay, southern Japan, from January 2016 to December 2017. Error bars show standard deviations.

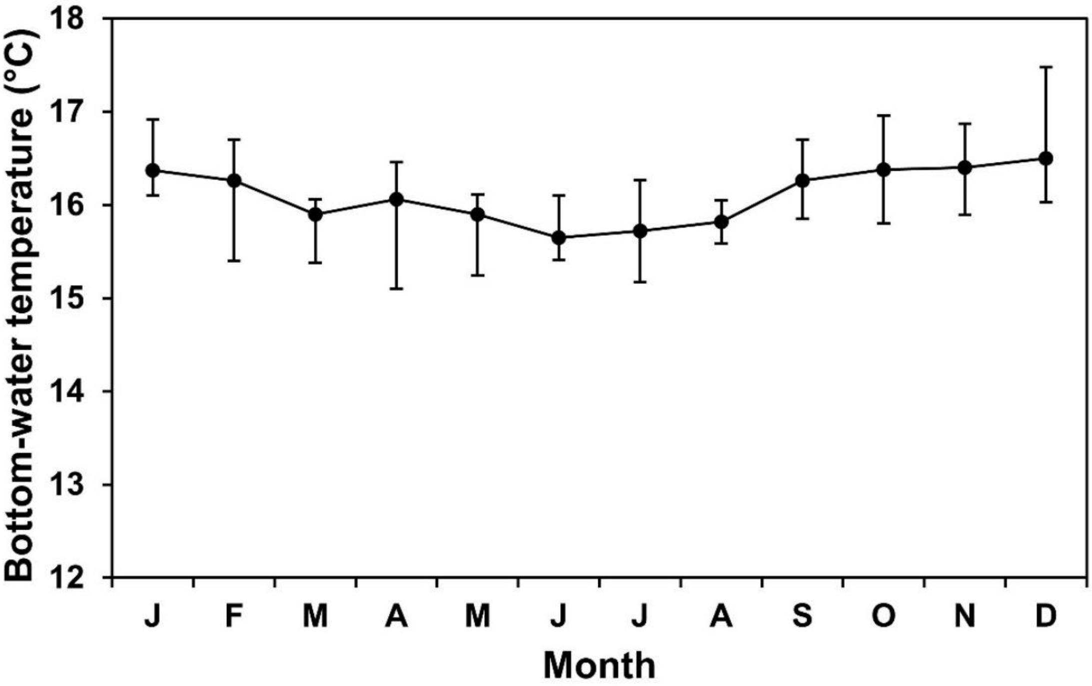


Figure 2.3.8. Monthly changes in bottom-water temperature at the sampling areas in Kagoshima Bay, southern Japan, from January 2016 to December 2017.

2.3.4. DISCUSSION

Histological observation revealed asynchronous oocyte development in the ovary, i.e. containing occytes of different developmental stages, of *M. kyushuensis*, which is a common phenomenon in penaeid shrimps including in other species of *Metapenaeopsis* such as *M. aegyptia*, *M. barbata*, *M. dalei*, *M. sinica*, *M. palmensis*, and *M. sibogae* (Yano, 1988; Ohtomi & Yamamoto, 1997; Sakaji *et al.*, 2000; Sakaji, 2001a, 2001b; Yamada *et al.*, 2007; Chen *et al.*, 2014; Farhana & Ohtomi, 2016; Rahman & Ohtomi, 2017). Asynchronous oocyte development is indicative of multiple spawning in a reproductive season (Bauer, 1989; Yamada *et al.*, 2007; Chen *et al.*, 2014; Rahman & Ohtomi, 2017). The ovarian maturity stage (immature, maturing, and mature) was, therefore, assigned based on the most advanced oocytes present in the ovary. Females with spent ovaries could not be detected during the present study because of the difficulty in distinguishing spent-stage ovaries from the developing-stage (Penn, 1980; Courtney & Dredge, 1988; Ohtomi *et al.*, 1998; Rahman & Ohtomi, 2018).

It was impossible to distinguish the yolk’s granule-stage from the prematuration-stage because of the absence of cortical crypts and/or rods. Previous studies of species of *Metapenaeopsis*, including *M. aegyptia*, *M. barbata*, *M. sinica*, *M. palmensis*, and *M. sibogae* have also reported the absence of cortical crypts (Sakaji, 2001b; Chen *et al.*, 2014; Rahman & Ohtomi, 2017), and no cortical crypts were found in *M. dalei* even when using samples collected at night (Sakaji *et al.*, 2000). The results of this study further ensure the absence of cortical crypts and/or rods in oocyte development of *Metapenaeopsis*. Germinal vesicle breakdown (GVBD) is considered to be the characteristic indication of maturity in species of *Metapenaeopsis* (Sakaji *et al.*, 2000; Sakaji, 2001b; Chen *et al.*, 2014), which was observed clearly in *M. kyushuensis*.

The relationship between CL and GSI in mature females of *M. kyushuensis* was not significant; however, the relationship between the two variables was gradual as was reported for *M. sibogae* (Rahman & Ohtomi, 2017). This type of relationship has been reported for other penaeid shrimps such as *M. japonicus* (Ohtomi *et al.*, 2003) and *M. palmensis* (Chen *et al.*, 2014). Due to the lack of a sharp relationship between GSI and ovarian maturity stages, it was not suitable to use GSI as an index for estimating the maturity status of female *M. kyushuensis*.

Even though GSI has been used extensively as an index for ovarian maturation of shrimps (e.g., Gab-Alla *et al.*, 1990; Ohtomi & Yamamoto, 1997; Ohtomi & Matsuoka, 1998; Ohtomi *et al.*, 1998; Hossain & Ohtomi, 2008; Farhana & Ohtomi, 2016), however, it was deemed not to be suitable for *M. kyushuensis*. The macroscopic ovarian staging based on the ratio of ovary width to body width was, therefore, evaluated for *M. kyushuensis* to be used as a quicker, simpler and inexpensive method of assigning maturity stages following Rahman & Ohtomi (2017). Histological observation revealed promising outcome. The relationship between macroscopically and histologically determined ovarian stages was steep with nearly 80% of the Stage III ovaries being confirmed as mature. The ratio of ovary width to body width can, therefore, be used as a simple and reliable index for estimating ovarian maturity in *M. kyushuensis*. In this study, females with Stage III ovaries were identified as mature. The positive outcome of the macroscopic staging in assigning maturity stages in *M. kyushuensis* showed that this index may also be useful for classifying ovarian maturity in other small penaeid shrimps in absence of data based on a histological classification.

The minimum CL at maturity and CL_{50} for female *M. kyushuensis* were estimated to be 11.5 and 13.7 mm, respectively. Transforming these values to ages indicated that individuals at these sizes approximately belonged to a 1-year-old age class. The CL_{50} of *M. sibogae* in Kagoshima Bay was also in the similar range and in the same age class. The size at sexual maturity is of special interest in fisheries management and is widely used as an indicator for minimum permissible capture size (Lucifora *et al.*, 1999). The information on minimum CL at maturity and CL_{50} is helpful for setting the cod-end mesh size to restrict catching the first mature shrimp and thus leaving the smallest mature shrimp to spawn (Chen *et al.*, 2014; Rahman & Ohtomi, 2017). Regular evaluation of these values is recommended for successful management of this exploited fishery.

The mean CPUE of mature females suggested an extended spawning season in *M. kyushuensis* lasting from April to January, with June to September being the main season. Several studies have indicated that an extended reproductive period may be a typical feature of deep-water species (George & Menzies, 1967; Harrison, 1988; Gage & Tyler, 1991). Rahman & Ohtomi (2017) reported year-round spawning season of *M. sibogae* in Kagoshima Bay. Sakaji (2001a) also reported year-round spawning in *M. dalei* from Tosa Bay. Both Kagoshima Bay and Tosa Bay (maximum water depth of over 400 m; Sakaji *et al.*, 2006) are deep-water bays, which justifies the hypothesis of extended spawning seasons of deep-water species. Seasonality in the reproduction of penaeids is also affected by water temperature, availability of food, and planktonic larvae, among other factors (Thorson, 1950; Giese & Pearse, 1974; Sastry, 1983; Tyler, 1986; Bauer, 1989, 1992; Bishop & Shalla, 1994; Cha *et al.*, 2004a). The bottom-water temperature in Kagoshima Bay was relatively constant and never fell below 15 °C even during the winter months as was also reported by Rahman & Ohtomi (2017). The stable bottom-water temperature in Kagoshima Bay in winter is caused by the intrusion of warm Kurushio Current into the bay (Kohno *et al.*, 2004). Sakaji (2001a) reported that the bottom-water temperature in Tosa Bay also rarely falls below 15 °C. Furthermore, phytoplankton was found to be predominant throughout the year in Kagoshima Bay (Kobari *et al.*, 2002). It is therefore most likely that the deep-water habitats, relatively constant bottom-water temperatures, and the availability of larval food may have an influence on the extended spawning season of *M. kyushuensis*.

Shorter spawning seasons are well documented for Japanese species of *Metapenaeopsis*, such as *M. acclivis* (Rathbun, 1902) and *M. barbata* from Kasaoka Bay in the Ariake Sea and Aki-nada in the Seto Inland Sea is from early summer to autumn (Yasuda, 1949; Ikematsu, 1963). Mature females of *M. dalei* were reported to occur in July and August in Suo-nada in the Seto Inland Sea, and Sendai Bay (Yatsuyanagi & Maekawa, 1957; Kosaka, 1977). The water temperature and depth might be the main factors acting behind the differences in spawning seasonality of these species with that of *M. kyushuensis*. The water temperature of these semi-enclosed, shallow water bodies (average water depth ~ 30–50 m) falls below 10 °C in winter (Onbe & Kakuda, 1962; Sakaji, 2001a; Tabata *et al.*, 2015) when no spawning was reported. The spawning season of *M. palmensis* extends from February to June in waters off Taiwan (water depth, 10–40 m) (Chen *et al.*, 2014) whereas it was from July to August for *M. dalei* in the western sea of Korea (water depth ≤ 30 m) (Choi *et al.*, 2005). The bottom-water temperatures and latitudinal effects were reported to be acting as the drivers of spawning seasonality in these species. The bottom-water temperature in the spawning season of *M. palmensis* ranged from 22.6 to 29.1 °C (Chen *et al.*, 2014), whereas spawning starts when the bottom-water temperature rises to over 23 °C in *M. dalei* (Choi *et al.*, 2005) in contrast to the relatively stable bottom-water temperature recorded in Kagoshima Bay.

The spawning season of *M. palmensis* lasted throughout the year in tropical waters (9°30' S, 10°10' S) (Watson & Keating, 1989), whereas in southwestern Taiwan (22°15' N, 22°45' N) it lasts for close to half a year (February to June) (Chen *et al.*, 2014) but is relatively shorter (June-September) in higher latitude waters (35°20' N, 35°30' N) (Hayashi & Sakamoto, 1978). The spawning season of *M. dalei* along the western coast of Korea is much shorter than that in Japanese waters (Yatsuyanagi & Maekawa, 1957; Kosaka, 1977, Choi *et al.*, 2005). Even though Kagoshima Bay is located in a higher latitude (31°25' N), an extended spawning season was noted for *M. kyushuensis* showing this species does not following the so called ‘classical paradigm’ as also reported for *M. sibogae* from the same habitat (Rahman & Ohtomi, 2017). The present study provides the first information on reproductive biology of *M. kyushuensis*, which will be helpful for the formulation of fishing regulations for the sustainable management and conservation of this emergent fishery. Further studies on the spawning frequency and fecundity, as well as a breeding stock assessment, are strongly recommended.

2.4: REPRODUCTIVE BIOLOGY OF *METAPENAEOPSIS PROVOCATORIA OWSTONI*

2.4.1. INTRODUCTION

M. provocatoria owstoni is a small penaeid shrimp distributed in the waters of Japan, Korea and Taiwan (Shinomiya & Sakai, 2000). It is one of the dominant *Metapenaeopsis* species in Kagoshima and is commercially important as it is marketed together with other species of the same genus (Rahman & Ohtomi, unpublished). There is no information available on any aspects of population biology of this species in literature, which represents an obstacle for formulating the proper management strategies for this important shrimp fishery. The present study therefore aimed to delineate for the first time its ovarian maturation, size at sexual maturity and spawning season using samples collected from Kagoshima Bay, southern Japan.

2.4.2. MATERIALS AND METHODS

2.4.2.1. *Sampling*

Monthly samples of *M. provocatoria owstoni* were collected from the experimental trawl surveys conducted in Kagoshima Bay, southern Japan (31°25' N, 130°38' E) at depths of ~80 to ~230 m (Figure 2.4.1). Sampling was conducted on-board the training vessel *Nansei-Maru* (175 t) of the Faculty of Fisheries, Kagoshima University from January 2013 to December 2017 using a simple trawl net (LC-VI; Nichimo Corporation, Tokyo), 23.5 m long, 37.9 mm mesh size in the body, 20.2 mm in the cod-end, carrying canvas kites on the tip of the wings (Ohtomi *et al.*, 2004). The net was towed for a preset tow duration of 10 min at a speed of 2 knots. *M. provocatoria owstoni* was sorted out of each haul, counted, and instantly preserved in ice on-board and then in formalin upon arrival at the laboratory.

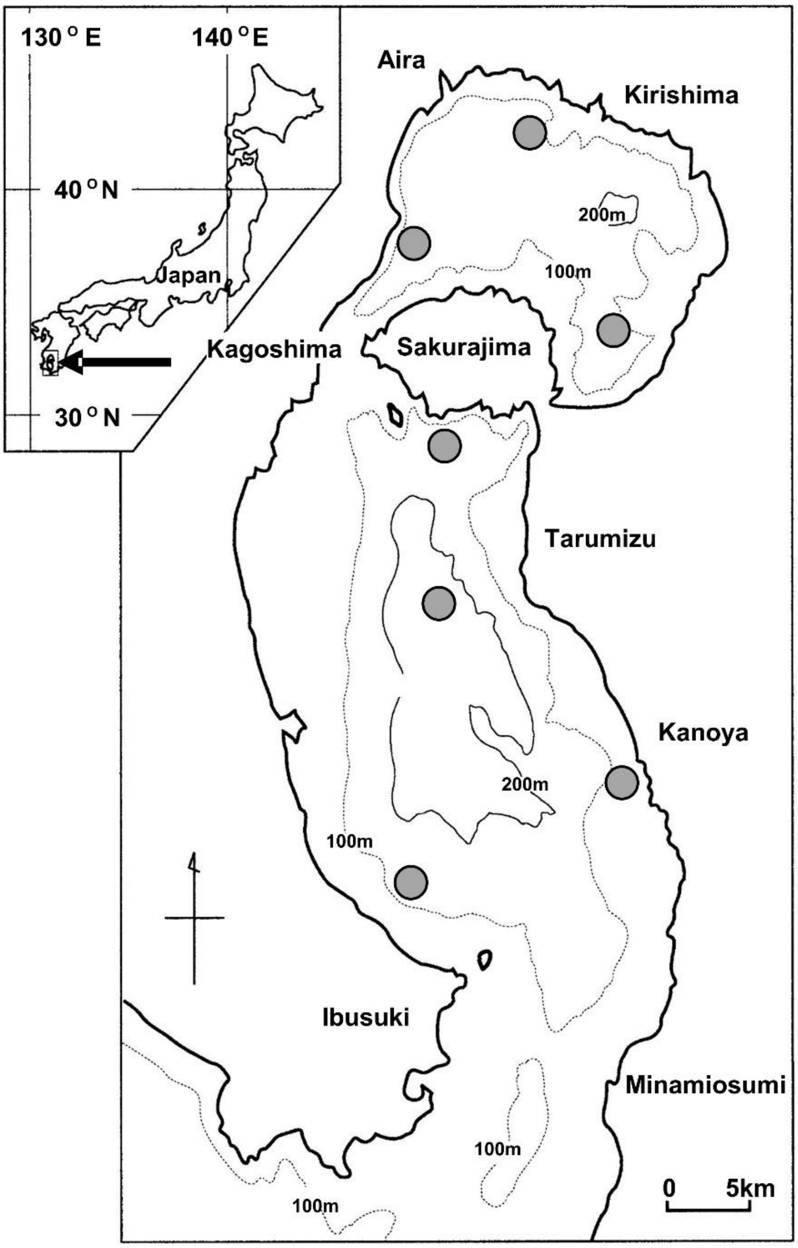


Figure 2.4.1. Kagoshima Bay, southern Japan, with indication of the collection of samples of *Metapenaeopsis provocatoria owstoni* in the bay (shaded areas).

2.4.2.2. *Measurements*

All specimens were classified by sex according to the presence of a petasma in males or a thelycum in females, and only females (*N* = 640) were used. For each female, carapace length (CL) was measured to the nearest 0.01 mm from the posterior margin of the eyestalk to the dorsal midline of the posterior margin of the carapace with slide calipers (CD-15PS; Mitutoyo Corp., Kawasaki, Japan), and body weight was measured to the nearest 0.01 g by electronic balance (EB-430DW; Shimadzu Corp., Kyoto, Japan). The exoskeleton and muscles above the ovary of each female were removed, and the ovary and body widths were measured to the nearest 0.01 mm at the middle of the first abdominal segment (Figure 2.4.2). Ovaries were classified as one of three initial sexual maturity stages based on the ratio of ovary width to body width following Rahman & Ohtomi (2017): Stage I, ovary width < 1/5 body width (Figure 2.4.2A); Stage II, 1/5 body width ≤ ovary width < 1/3 body width (Figure 2.4.2B); and Stage III, ovary width ≥ 1/3 body width (Figure 2.4.2C). The maturity status of these stages was assessed by histological observation (see below). The whole gonad was then removed and weighed to the nearest 0.001 g using the electronic balance. The gonadosomatic index was calculated as: GSI (%) = 100 × [ovary weight / body weight].

2.4.2.3. *Histological analysis of ovary*

Histological observation of the ovary was done using 81 females collected from January 2013 to December 2017 (CL, 9.2 to 21.2 mm; GSI, 0.14 to 7.78%). A small portion of each ovary was dehydrated in an ethanol series, infiltrated with paraffin, and sliced into 6 µm sections. The sections were stained with Mayer's hematoxylin and eosin, cover-slipped with a mounting medium, and examined under a microscope (DMLB; Leica, Wetzlar, Germany). As a preliminary examination, sections from three parts of the ovary (the posterior region of the cephalothoracic lobe and the anterior and middle regions of the abdominal lobe) from one female (CL, 17.3; GSI, 6.78%) were examined to differentiate the maturity stages in relation

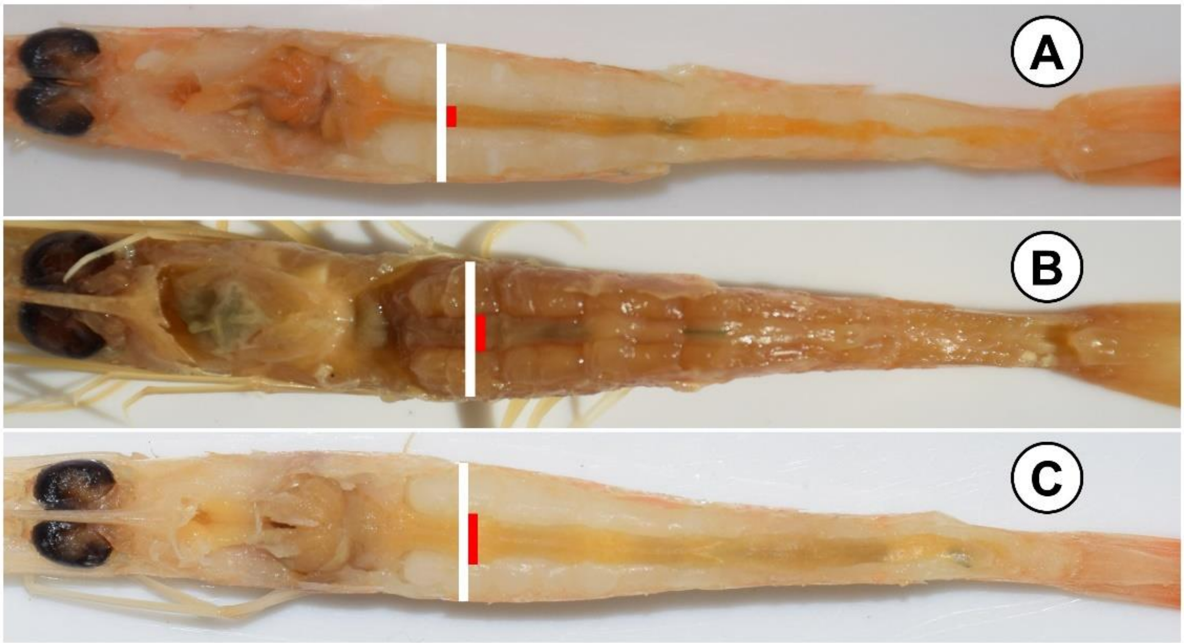


Figure 2.4.2. Macroscopic maturity stages of the ovaries of *Metapenaeopsis provocatoria owstoni*. **A**, Stage I (ovary width < 1/5 body width); **B**, Stage II (1/5 body width ≤ ovary width < 1/3 body width); **C**, Stage III (ovary width ≥ 1/3 body width). White line indicates body width; black line indicates ovary width.

to the position in the ovary. The histological sections showed no difference in maturity stage with respect to ovary position. Tissue samples from the anterior regions of the abdominal lobes were thus used for histological analysis for the rest of the ovaries to determine the oocyte developmental stages following Rahman & Ohtomi (2017). The terminology and criteria for describing oocyte development and ovarian maturation followed Yano (1988), and Rahman & Ohtomi (2017) with few modifications.

2.4.2.4. *Classification of developmental stages of oocytes*

Oocytes were classified into one of five developmental stages based on histological observation: early nucleolus, middle nucleolus, late nucleolus, yolk granule, and mature (Table 2.4.1). Early nucleolus-stage oocytes were small in size, basophilic, and contained thin cytoplasm (Figure 2.4.3A); middle nucleolus-stage oocytes contained strongly basophilic chromatin in the nucleus and a thick layer of follicle cells visible around the oocytes (Figure 2.4.3B); late nucleolus-stage oocytes were stained weakly with hematoxylin in the cytoplasm and the thickness of the follicle layers decreased (Figure 2.4.3C); yolk granule-stage oocytes contained eosin-stained acidophilic yolk granules in the cytoplasm (Figure 2.4.3D); and mature-stage oocytes comprised of acidophilic yolk granules concentrated in and filled the cytoplasm of the oocyte, and germinal vesicle breakdown (GVBD) occurred throughout the cytoplasm of this stage (Figure 2.4.3E).

2.4.2.5. *Assessment of stages of ovarian maturity*

M. provocatoria owstoni had asynchronous ovaries, containing oocytes at various developmental stages (Figure 2.4.3F). The ovarian maturity stages were therefore divided based on the developmental stage of the most advanced oocytes in the ovary into three categories: immature, maturing, and mature (Table 2.4.1). Ovaries with oocytes in the early-, middle-, or late nucleolus-stages (previtellogenic oocytes) were classified as immature.

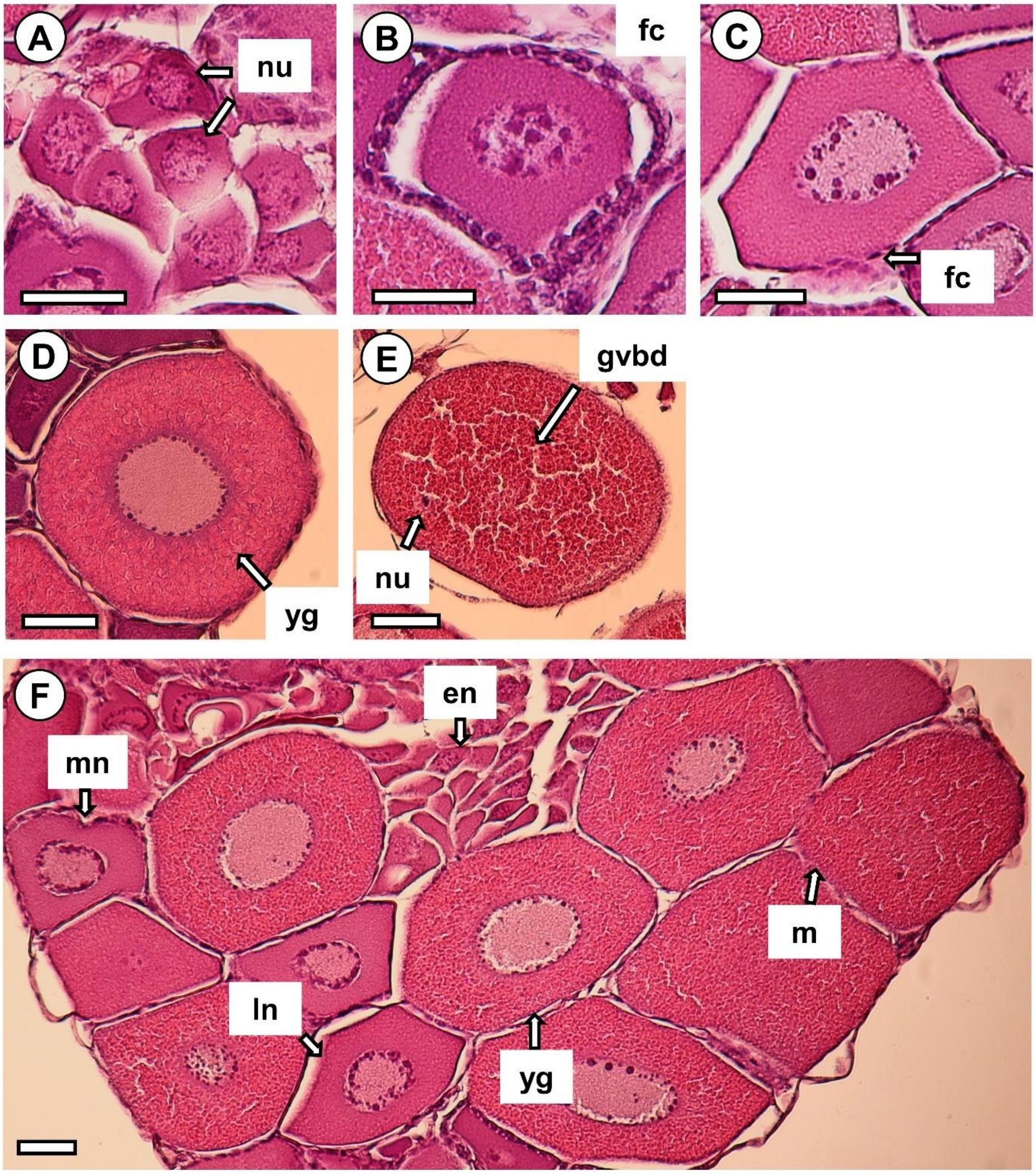


Figure 2.4.3. Histological sections of oocyte development and mature gonad of *Metapenaeopsis provocatoria owstoni*. **a**, early nucleolus stage; **b**, middle nucleolus stage; **c**, late nucleolus stage; **d**, yolk granule stage; **e**, mature stage; **f**, asynchronous oocyte development in the ovary; fc, follicle cells; gbvd, germinal vesicle break down; en, early nucleolus-stage; ln, late nucleolus-stage oocyte; m, mature-stage oocyte; mn, middle nucleolus-stage oocyte; nu, nucleoli; yg, yolk granules. Scale bar = 50 µm.

Table 2.4.1. Histological characteristics and diameter of oocytes in ovaries at different stages of maturity in female *Metapenaeopsis provocatoria owstoni*.

Ovarian maturity stage	Oocyte stage[a]	Histological characteristics
Immature	Early nucleolus Middle nucleolus Late nucleolus	Previtellogenesis
Maturing	Yolk granule	Vitellogenesis
Mature	Mature	Germinal vesicle breakdown

[a] Developmental stage of the most advanced oocytes in the ovary.

Maturing ovaries contained oocytes in the yolk granule-stage (vitellogenic oocytes) in addition to previtellogenic oocytes. Mature ovaries contained mature-stage oocytes as well as previtellogenic and vitellogenic oocytes. In *M. japonicus*, germinal vesicle breakdown (GVBD) can last 5–11 h in the oocytes in the late phases of the prematuration- and maturation-stages (Yano, 1988). Females of *M. provocatoria owstoni* were, therefore, determined to be mature if their oocytes had evidence of GVBD that would lead them to spawn within a few hours.

2.4.2.6. *Size and age at sexual maturity*

The size at sexual maturity was estimated based the relationship of ovarian maturity progression with CL class and the minimum size of macroscopically and histologically determined mature females. Age at sexual maturity was calculated by transforming the CL value to its respective age class following the CL frequency distribution and the estimated growth equation of *M. provocatoria owstoni* (see Chapter 4, Section 4.4).

2.4.2.7. *Spawning season*

The spawning season of *M. provocatoria owstoni* was estimated based on the monthly percent occurrence of mature females.

2.4.3. RESULTS

2.4.3.1. *Change in ovarian maturation with progression of gonadosomatic index*

There was no significant correlation between CL and GSI (R = 0.044, $P = 0.820$) for females determined as sexually mature using histological method. The percent occurrence of females in each histologically determined stage of ovarian maturity gradually moved from most ovaries being immature to a majority being mature as GSI increased (Figure 2.4.4). Females with a GSI < 2% were mostly immature, whereas most females with a GSI of 2 to < 4% were maturing. The mature females first appeared in the GSI 2–3% class. Nearly 50% of the females

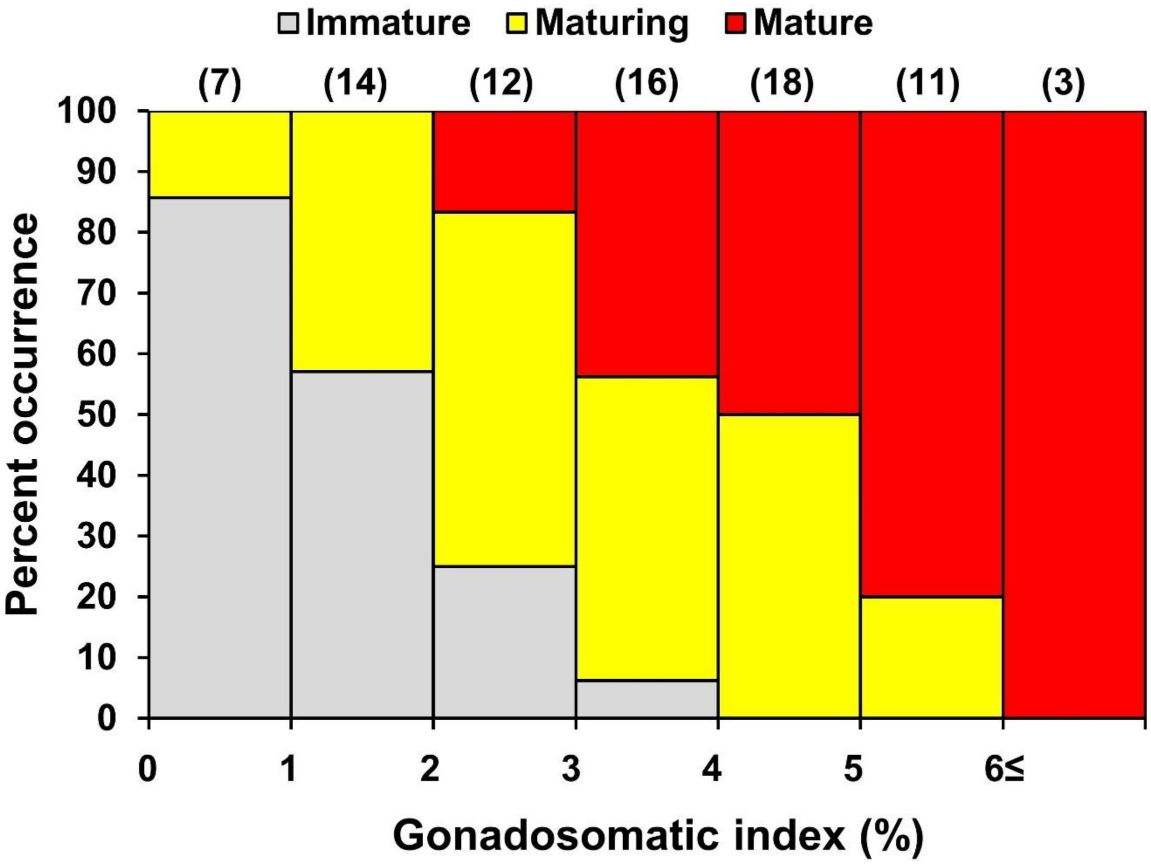


Figure 2.4.4. Percent occurrence of female *Metapenaeopsis provocatoria owstoni* in histologically determined stages of ovarian maturity (immature, maturing, and mature) against the associated gonadosomatic index class. The number of females examined is given in parentheses.

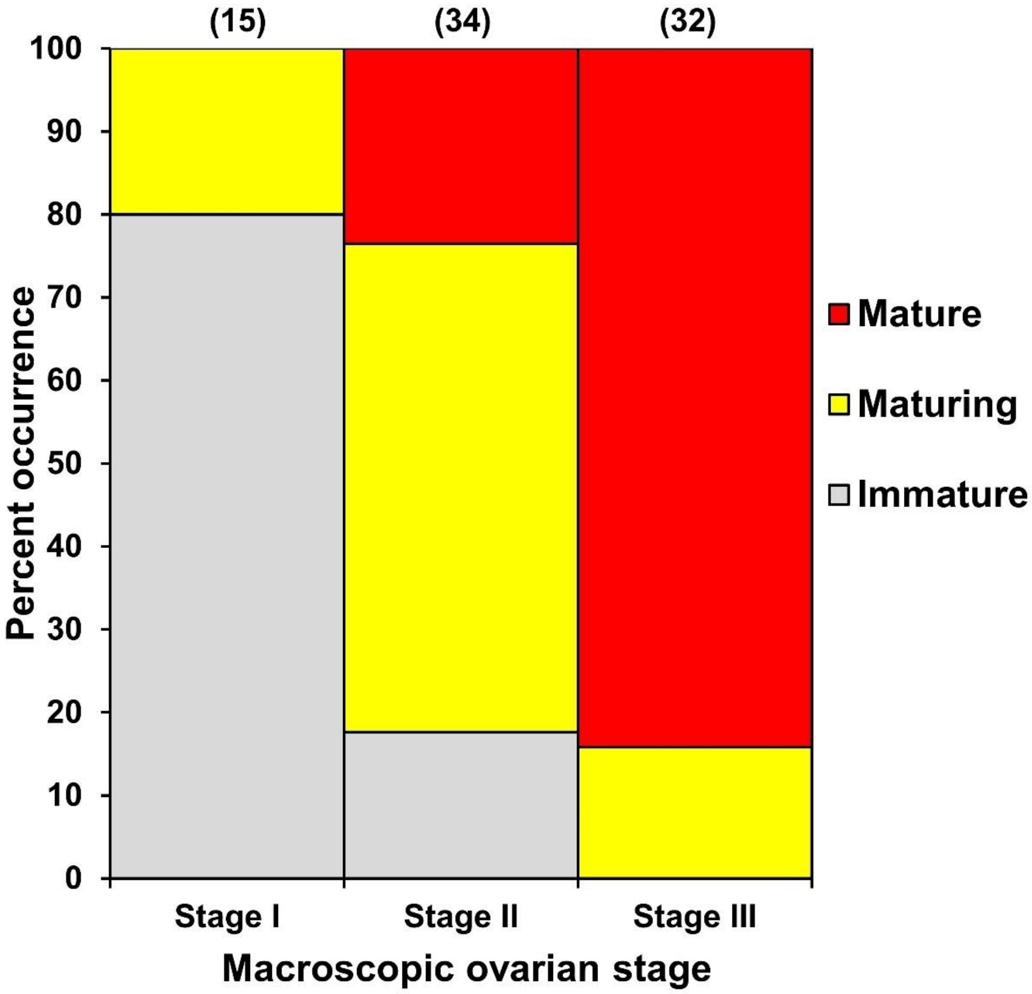


Figure 2.4.5. Percent occurrence of female *Metapenaeopsis provocatoria owstoni* in histologically determined stages of ovarian maturity (immature, maturing, and mature) against the associated macroscopic ovarian stage. The number of females examined is given in parentheses.

with a GSI ≥ 4% were mature, whereas all the females with GSI ≥ 6% were mature.

2.4.3.2. *Histological verification of ovarian stages*

The maturity status of the different ovarian stages that were determined macroscopically was verified by histological analysis (Figure 5). Nearly 85% of Stage I ovaries were immature. Stage II ovaries included all the three maturity stages; however, the dominant stage was maturing (nearly 66%). Of the Stage III ovaries, nearly 80% were mature, and the remaining were maturing. The percentage of mature females steeply increased when classified macroscopically. The macroscopic determination of ovarian maturity based on the ovary and body widths can therefore be used as a simple and reliable index for estimating sexual maturity in *M. provovatoria owstoni*. Females with Stage III ovaries were considered mature.

2.4.3.3. *Size and age at sexual maturity*

The percent occurrence of females with each ovarian maturity stage showed that all the females having a CL < 10.0 mm and ~90% of the female having a CL 10 to <12 mm were immature (Figure 2.4.6). Maturing females first appeared in the 10-11 mm CL class whereas mature females first appeared in the 12-13 mm CL class. The percentage of mature females were low (< 20%) throughout the CL class range. Macroscopic staging and histological observation of the ovaries have confirmed the minimum size of mature females as 12.8 mm CL. I, therefore, considered 12.8 mm CL as the size at sexual maturity of *M. provocatoria owstoni*. Converting the CL to respective age class confirmed the individual to belong to around 1-year-old age class.

2.4.3.4. *Spawning season*

Mature females occurred from April to December with comparatively higher percent occurrence were recorded in May to June. The findings therefore indicated that *M. provocatoria owstoni* spawns April to January in Kagoshima Bay with May-June being the main spawning season.

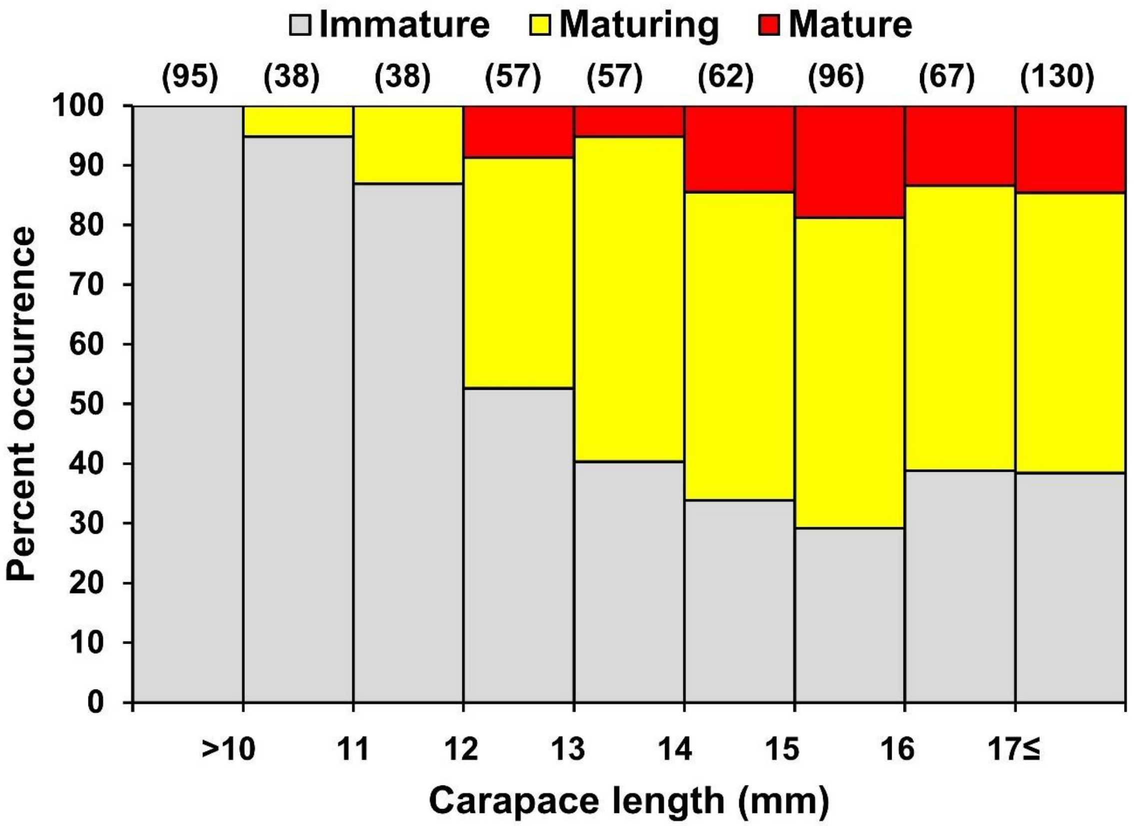


Figure 2.4.6. Percentage occurrence of female *Metapenaeopsis provocatoria owstoni* with each macroscopic ovarian stage against carapace length class.

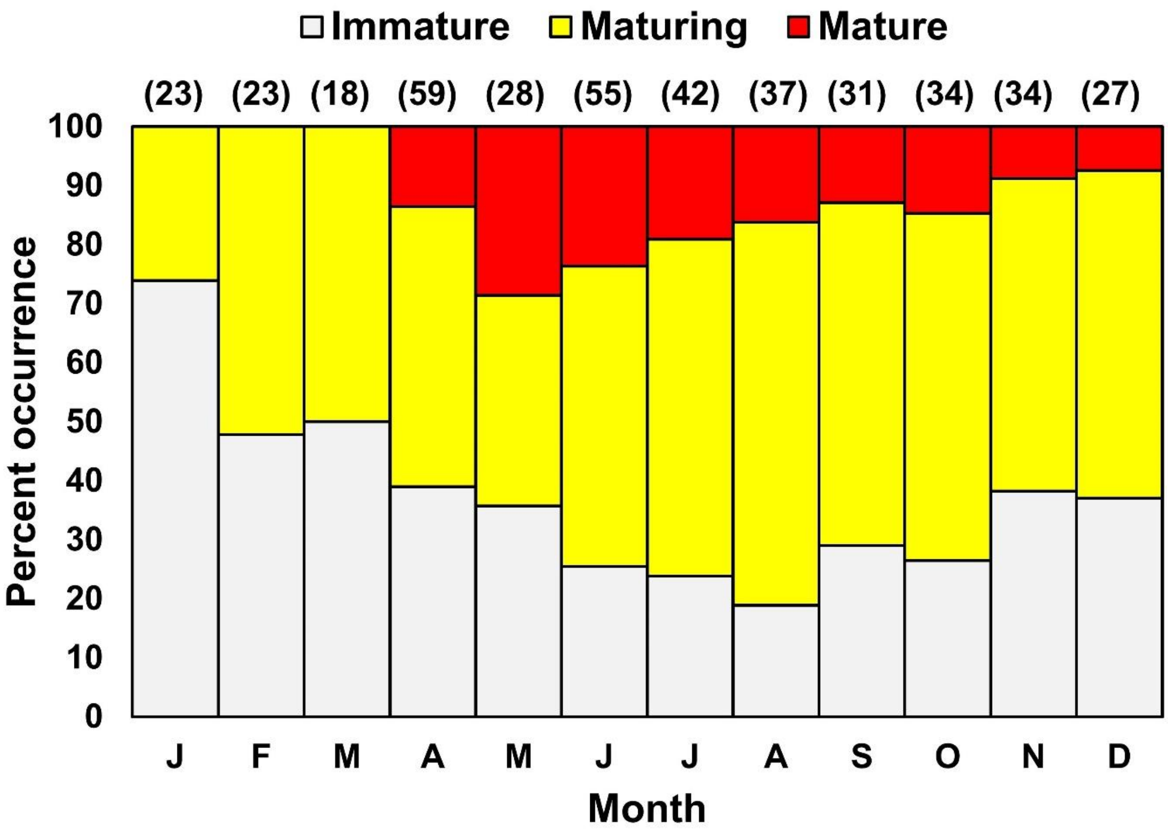


Figure 2.4.7. Monthly change in percent occurrence of females of *Metapenaeopsis provocatoria owstoni* with each ovarian maturity stage during January 2013 to December 2017.

2.4.4. DISCUSSION

For studying the biological aspects of a population, sequential collection of a large number of specimens is of utmost importance (Ohtomi *et al.*, 2000). Samples of *M. provocatoria owstoni* were difficult to obtain, however, it was possible to collect monthly samples over a 5-year-period in Kagoshima Bay. This allowed to examine some important aspects of the reproductive biology of the population, including ovarian maturation, size and age at sexual maturity, and spawning season of this species.

The ovarian maturity status of *M. provocatoria owstoni* was confirmed by histological observation, which is known to be one of the most reliable methods for determining mature females of fishes and shrimps (West, 1990; Ohtomi *et al.*, 2003; Carbonell *et al.*, 2006). This allowed the precise and thorough examination of oocyte development resulting in less ambiguity during ovarian maturity classification (Williams, 2007). Histological observation has confirmed asynchronous oocyte development in the ovary of *M. provocatoria owstoni*. Asynchronous oocyte development is a common phenomenon in *Metapenaeopsis* shrimps such as *M. aegyptia*, *M. barbata*, *M. dalei*, *M. sinica*, *M. palmensis*, and *M. sibogae* (Sakaji *et al.*, 2000; Sakaji, 2001a, 2001b; Chen *et al.*, 2014; Rahman & Ohtomi, 2017). This type of oocyte development is suggestive of multiple spawning in a reproductive season (Bauer, 1989; Yamada *et al.*, 2007; Rahman & Ohtomi, 2017). The ovarian maturity stage was therefore assigned based on the most advanced oocytes present in the ovary (Rahman & Ohtomi, 2017). It was, however, impossible to detect the females with spent-stage ovary because of the difficulty in distinguishing spent-stage ovaries from the developing-stage. Such constraints have also been reported by several authors including Penn (1980); Courtney & Dredge (1988); Ohtomi *et al.* (1998); Farhana & Ohtomi (2016), and Rahman & Ohtomi (2017).

Cortical crypts in the periphery of oocyte cytoplasm, widely recognized as a sign of oocyte pre-maturation in penaeid shrimps (e.g., Bell & Lightner, 1988; Yano, 1988; Ohtomi &

Yamamoto, 1997; Ohtomi *et al.*, 1998, 2003; Farhana & Ohtomi, 2016), were not identifiable in *M. provocatoria owstoni*. Previous studies of species of *Metapenaeopsis*, including *M. aegyptia*, *M. barbata*, *M. sinica*, *M. palmensis*, and *M. sibogae* have also reported the absence of cortical crypts (Sakaji, 2001b; Chen *et al.*, 2014; Rahman & Ohtomi, 2017), and no cortical crypts were found in *M. dalei* even when using samples collected at night (Sakaji *et al.*, 2000). This further confirms the absence of cortical crypts and/or rods in oocyte development to be a general characteristic of genus *Metapenaeopsis* as compared to other penaeid shrimps. Germinal vesicle breakdown was considered to be the characteristic sign of maturity in *M. provocatoria owstoni* as was considered for other species of *Metapenaeopsis* (Sakaji *et al.*, 2000; Sakaji, 2001b; Chen *et al.*, 2014; Rahman & Ohtomi, 2017).

The gonadosomatic index (GSI) has been used extensively in reproductive studies of shrimps (e.g., Gab-Alla *et al.*, 1990; Ohtomi & Yamamoto, 1997; Ohtomi & Matsuoka, 1998; Ohtomi *et al.*, 1998; Hossain & Ohtomi, 2008; Farhana & Ohtomi, 2016), however, not without flaws. Significant relationship between GSI and body size was reported in *M. plebejus*, and GSI was considered as a poor indicator of ovarian maturity (Courtney *et al.*, 1995). No significant relationship was found between CL and GSI in mature females of *M. provocatoria owstoni*; however, the relationship between the two variables was gradual, and similar relationships have been reported for the species of *Metapenaeopsis* including *M. palmensis* (Chen *et al.*, 2014), *M. sibogae* (Rahman & Ohtomi, 2017), and *M. kyushuensis* (see Chapter 2; Section 2.3). Such relationship was also reported for another penaeid shrimp, *M. japonicus* (Ohtomi *et al.*, 2003). As the relationship between GSI and ovarian maturity stages was gradual, GSI was not considered as an appropriate index for estimating the sexual maturity of female *M. provocatoria owstoni*.

Rahman & Ohtomi (2017) proposed a macroscopic index to classify ovarian maturity based on the ratio of ovary width to body width while studying the reproduction biology of *M.*

sibogae. Macroscopic or biological indices could, however, involve high amounts of error due to the difficulties in distinguishing different maturity stages. Any macroscopic determination of maturity or biological index should, therefore, be validated by histology to ensure its accuracy (West, 1990). The macroscopic index proposed by Rahman & Ohtomi (2017) was verified through histology to see its suitability for *M. provocatoria owstoni*. Histological observation revealed a sharp relationship between macroscopically and histologically determined ovarian stages with nearly 80% of the Stage III ovaries being confirmed as mature. The ratio of ovary width to body width can, therefore, also be adopted as a simple and reliable index for estimating ovarian maturity in *M. provocatoria owstoni*. Females with Stage III ovaries were identified as mature.

The estimated size at sexual maturity of *M. provocatoria owstoni* in Kagoshima Bay was 12.8 mm CL. Transforming the value to age indicated that individuals at this size approximately belonged to a 1-year-old age class. For both *M. sibogae* and *M. kyushuensis*, the size at sexual maturity was estimated from the plot of percent occurrence of mature females against length class and a logistic equation fitted to the data following King (2007) (Rahman & Ohtomi, 2017; see Chapter 2, Section 2.3). It was not possible to fit logistic equation to the percentage occurrence of mature females against CL class data due to low percent occurrence of mature females in case of *M. provocatoria owstoni*. The relationship of ovarian maturity progression with CL class and the minimum size of macroscopically and histologically determined mature females was, therefore, used to estimate the size at sexual maturity. There is no information available on the size at sexual maturity of this species in the literature restraining possible comparisons. The provided information would therefore aid to set the cod-end mesh size to restrict catching the first mature shrimp and thus leaving the smallest mature shrimp to spawn (Chen *et al.*, 2014; Rahman & Ohtomi, 2017).

The spawning season of *M. provocatoria owstoni* extended from April to December with May to June being the main spawning season. Extended spawning season is also reported for two *Metapenaeopsis* species from Kagoshima Bay. *M. sibogae* exhibited year-round spawning (Rahman & Ohtomi, 2017), whereas *M. kyushuensis* spawned from April to January (see Chapter 2, section 2.3). Sakaji (2001a) reported year-round spawning in another species of *Metapenaeopsis*, *M. dalei* from Tosa Bay, and several studies have indicated that an extended reproductive period may be a typical feature of deep-water species (George & Menzies, 1967; Harrison, 1988; Gage & Tyler, 1991). Both Kagoshima Bay and Tosa Bay (maximum water depth of over 400 m; Sakaji *et al.*, 2006) are deep-water bays. Seasonality in the reproduction of penaeids is also affected by water temperature, availability of food, and planktonic larvae, among other factors (Thorson, 1950; Giese & Pearse, 1974; Sastry, 1983; Tfyler, 1986; Bauer, 1989, 1992; Bishop & Shalla, 1994; Cha *et al.*, 2004a). The stable bottom-water temperature in Kagoshima Bay in winter is caused by the intrusion of warm Kurushio Current into the bay (Kohno *et al.*, 2004). Bottom-water temperature rarely falls below 15 °C in Tosa Bay (Sakaji, 2001a). Kobari *et al*. (2002) further reported that phytoplankton is predominant throughout the year in Kagoshima Bay. The extended spawning season of *M. provocatoria owstoni* in Kagoshima Bay may therefore influenced by the deep-water habitats, relatively constant bottom-water temperatures, and the availability of larval food (Rahman & Ohtomi, 2017).

Shorter spawning season also has been reported for some Japanese species of *Metapenaeopsis* such as *M. acclivis* and *M. barbata* from Kasaoka Bay in the Ariake Sea and Aki-nada in the Seto Inland Sea is from early summer to autumn (Yasuda, 1949; Ikematsu, 1963), whereas mature females of *M. dalei* were reported to occur in July and August in Suo-nada in the Seto Inland Sea, and Sendai Bay (Yatsuyanagi & Maekawa, 1957; Kosaka, 1977). These are semi-enclosed, shallow water bodies (average water depth ~ 30–50 m) where water temperature falls below 10 °C in winter (Onbe & Kakuda, 1962; Sakaji, 2001a; Tabata *et al.*, 2015) when no spawning was reported. In waters off Taiwan (water depth, 10–40 m), *M. palmensis* spawned from February to June (Chen *et al.*, 2014) whereas in the western sea of Korea (water depth ≤ 30 m) *M. dalei* spawned from July to August (Choi *et al.*, 2005). They noted bottom-water temperatures and latitudinal effects as acting as the drivers of spawning seasonality in these species. The bottom-water temperature in the spawning season of *M. palmensis* ranged from 22.6 to 29.1 °C (Chen *et al.*, 2014), whereas spawning starts when the bottom-water temperature rises to over 23 °C in *M. dalei* (Choi *et al.*, 2005). In contrast, the monthly bottom-water temperature in Kagoshima Bay is reported to be stable throughout the year (Farhana & Ohtomi, 2016; Ohtomi et al., 2018; Rahman & Ohtomi, 2017, 2018a).

A general latitudinal pattern observed in shrimp reproductive strategies characterized by continuous reproduction at low latitudes with increased seasonality towards the poles (Costa & Fransozo, 2004; Costa *et al.*, 2010; van de Kerk *et al.*, 2016). The spawning season of *M. palmensis* could last throughout the year in tropical waters (9°30' S, 10°10' S) (Watson & Keating, 1989), whereas in southwestern Taiwan (22°15' N, 22°45' N) it lasts for close to half a year (February to June) (Chen *et al.*, 2014) but is relatively shorter (June-September) in higher latitude waters (35°20' N, 35°30' N) (Hayashi & Sakamoto, 1978). The spawning season of *M. dalei* along the western coast of Korea is much shorter than that in Japanese waters (Yatsuyanagi & Maekawa, 1957; Kosaka, 1977, Choi *et al.*, 2005). *M. provocatoria owstoni* had an extended spawning season despite the fact that Kagoshima Bay is located in a higher latitude (31°25' N), thus not following the general latitudinal pattern of reproduction. Similar findings were also reported for *M. sibogae* (Rahman & Ohtomi, 2017) and *M. kyushuensis* (See Chapter 2, Section 2.3) in Kagoshima Bay. A general reproductive pattern can be deduced for *Metapenaeopsis* species. Species of *Metapenaeopsis* inhabiting deeper waters where the bottom-water temperatures remains stable throughout the year tends to have an extended spawning season, whereas those inhabiting shallower waters with higher fluctuations in water temperature tends to have shorter reproductive period. Further studies on the spawning frequency and fecundity, as well as a breeding stock assessment, are needed to gain a broader understanding of the reproductive potential of the population, which in turn will form the basis for adequate management regimes.

CHAPTER 3: RECRUITMENT, GROWTH PATTERNS, AND LONGEVITY OF *METAPENAEOPSIS* SPECIES IN KAGOSHIMA BAY

3.1: BACKGROUND OF THE STUDY

Management plans are basic tools for sustainable exploitation of commercially important fisheries (Simon, 2015). Successful fisheries management depends on the delineation of target stocks, permitting sustainable allocation of catch between competing fisheries, recognition and protection of nursery and spawning areas, and formulation of optimal harvest and monitoring strategies (Kutkuhn, 1981; Smith *et al.*, 1990; Begg *et al.*, 1999). Knowledge of various aspects of the life history of a population or stock (recruitment, longevity, growth rate, mortality, and age at sexual maturity) allows modeling of group dynamics and predicting future stock potential through tracking of age classes or cohorts over time. Such modeling and prediction permit assessments of population productivity, reproductive potential, and sustainability, which is essential for decision-making in fisheries management (Campana, 2001; Hutchinson & TenBrink, 2011; Higgins *et al.*, 2015).

Species of the genus *Metapenaeopsis* have a worldwide distribution (De Grave & Fransen, 2011) and its species are important ecologically (Linke *et al.*, 2001; Raymundo-Huizar *et al.*, 2005; De Grave *et al.*, 2009) as well as commercially (Wu, 1985; Watson & Keating, 1989; Sakaji *et al.*, 2000; Sakaji, 2003; De Young, 2006). Studies focusing recruitment, growth patterns and longevity of the species of *Metapenaeopsis* are, however, rare. The few studies that were conducted includes growth and maturation of *M. barbata* in Seto Inland Sea, Japan (Sakaji *et al.*, 1992), growth and mortality of *M. barbata* in northeastern

Taiwan (Tzeng *et al.*, 2005), growth and reproduction of *M. dalei* in western Korea (Choi *et al.*, 2005), and the life history of *M. palmensis* in southwestern Taiwan (Chen *et al.*, 2014). There has, however, been no study conducted on any aspects of growth of either *M. sibogae*, *M. kyushuensis* or *M. provocatoria owstoni*. The absence of information on the recruitment, growth, and longevity of these *Metapenaeopsis* species has limited the formulation of sustainable management protocols.

Growth in crustaceans is difficult to estimate because its exoskeleton is periodically replaced in during ecdysis, making age evaluations based on periodic markings on the body surface impossible (Hartnoll, 2001; Castilho *et al.*, 2015). In a review of age determination in crustaceans, Kilada & Discoll (2017) noted that the most popular methods for age and growth determination of five main crustacean taxa (shrimps, krill, crayfishes, crabs, and lobsters) were length-frequency analysis, lipofuscin analysis, and growth-band counts. Length-frequency analysis was the most frequent method of age determination in crustaceans, featuring in nearly 83% of the studies (Kilada & Discoll, 2017). Length-frequency analysis is classically the most widespread method for age determination of wild crustacean populations (Oh *et al.*, 1999); the other two methods are not convenient enough to be used on a regular basis. The age and growth of wild crustacean populations can confidently be estimated by identifying successive age groups in length-frequency distributions (Pauly & David, 1981; Ohtomi & Irieda, 1997; Tuck *et al.*, 1997; Oh *et al.*, 1999). The von Bertalanffy growth function (VBGF) (von Bertalanffy, 1938) is commonly fitted to the mean length-at-age data within a year class (Parrack, 1979; Garcia & Le Reste, 1981; Frechette & Parsons, 1983; Roa & Ernst, 1996; Tuck *et al.*, 1997). The VBGF appropriately describes growth and it has been extensively used in penaeoid shrimps (e.g., Garcia & Le Reste, 1981; D'Incao & Fonseca; 2000; Peixoto *et al.*, 2001; Papaconstantinou & Kapiris, 2003; Conides *et al.*, 2006; García-Rodríguez *et al.*, 2009; Manasirli *et al.*, 2014). Because of asynchronous molting of individuals within a year class in crustaceans, Pauly & Gaschütz (1979) modified the basic VBGF to incorporate seasonal variations in growth rate. The revised VBGF, also known as the Pauly and Gaschütz growth function (PGGF) is now the most widely used growth model for decapod crustacean stocks (e.g., Pauly *et al.*, 1984; Hopkins & Nilssen, 1990; Bergström, 1992; Ohtomi & Irieda, 1997; Alves & Pezzuto, 1998; Hossain & Ohtomi, 2010; Farhana & Ohtomi, 2017). There is no study available on the age, growth and longevity of *M. sibogae*, *M. kyushuensis, M. provocatoria owstoni* in the literature, which are required for stock assessment and for provide a basis for comparative studies in formulating the sustainable management plans for these rarely studied shrimp populations.

3.2: RECRUITMENT, GROWTH PATTERNS, AND LONGEVITY OF *METAPENAEOPSIS SIBOGAE*

3.2.1. INTRODUCTION

M. sibogae has been reported from relatively deep water (≥ 250 m) from Philippines to New Caledonia (Crosnier, 1987, 2007). The species was first recorded in Japanese waters from Kagoshima Bay by Ohtomi & Nagata (2004), its northernmost and shallowest (~130 m) occurrence. Kagoshima Bay is a semi-enclosed, deep-water bay characterized by the steep slopes of fault scarps, the so-called caldera-walls (Matumoto, 1943). In just over a decade, *M. sibogae* has become one of the five most dominant benthic species of Kagoshima Bay in terms of number of individuals caught per haul, and is gaining in commercial significance (Rahman & Ohtomi, unpublished). The adaptation of *M. sibogae* to this unique habitat and its dominance underline the importance of studies of the population biology of the species. There is, however, no study available on the recruitment, growth patterns, and longevity of *M. sibogae*, which is presenting a stumbling block for aiding the formulation of sustainable management plans for this species. This study therefore aimed at elucidating the recruitment, growth patterns, and longevity of *M. sibogae* for the first time.

3.2.2. MATERIALS AND METHODS

3.2.2.1. *Sampling and measurements*

The study was conducted in Kagoshima Bay, southern Japan, which has a maximum water depth of more than 230 m (Ohtomi, 2001) (Figure 3.2.1). Monthly samples of *M. sibogae* were obtained at 125–230 m using a simple trawl net (LC-VI; Nichimo, Tokyo, Japan) onboard the training vessel *Nansei Maru* (175 t) of the Faculty of Fisheries, Kagoshima University during January 2013 to December 2014 (except September, 2014). The net was 23.5 m long with mesh sizes of 37.9 mm and 20.2 mm in the body and the cod-end, respectively (Ohtomi *et al.*, 2004). The wings of the net were expanded by 1 m^2 canvas kites (Fulanda & Ohtomi, 2011). The net was towed for a preset duration of 10 min at a speed of 2 knots. Specimens of *M. sibogae* were sorted out, counted, and immediately preserved in ice on-board and by 10% formalin upon arrival at the laboratory. A set of compact temperature-depth (Compact-TD, ATD-HR) and temperature-salinity (Compact-CT, ACT-HR) loggers (JFE Advantech, Nishinomiya, Japan) were attached to the headrope of the net for the measurement of bottom-water temperature and salinity, respectively.

All specimens were classified by sex based on the presence of a petasma in males or a thelycum in females. Carapace length (CL) was measured to the nearest 0.01 mm from the posterior margin of the eyestalk to the dorsal midline of the posterior margin of the carapace using slide calipers (CD-15PS; Mitutoyo Corp., Kawasaki, Japan), and wet body weight (BW) was measured to the nearest 0.01 g using an electronic balance (EB-430DW; Shimadzu Corp., Kyoto, Japan).

3.2.2.2. *Length-frequency analysis*

Histograms of CL by sex were constructed for each month using 1 mm class intervals. Hasselblad's maximum-likelihood method (Hasselblad, 1966) was applied to the CL histograms to estimate the parameters for the normal distributions in each histogram. Each identified normal distribution was assumed to represent an age group. The program iteratively computed maximum-likelihood values of the proportions at each age group, mean CL, and standard deviation explained by each component normal distribution. In cases where normal distributions representing two different age groups overlapped, individuals were separated into two age groups using a discriminant function according to Ohtomi & Irieda (1997): $Z_i = (L_m\sigma_n + L_n\sigma_m) / (\sigma_m + \sigma_n) - L_i$, where L_m is the mean CL, σ_m the standard deviation at age m, L_n the mean CL σ_n the standard deviation at age n, and L_i the CL of individual i. If $Z_i > 0$, i belonged

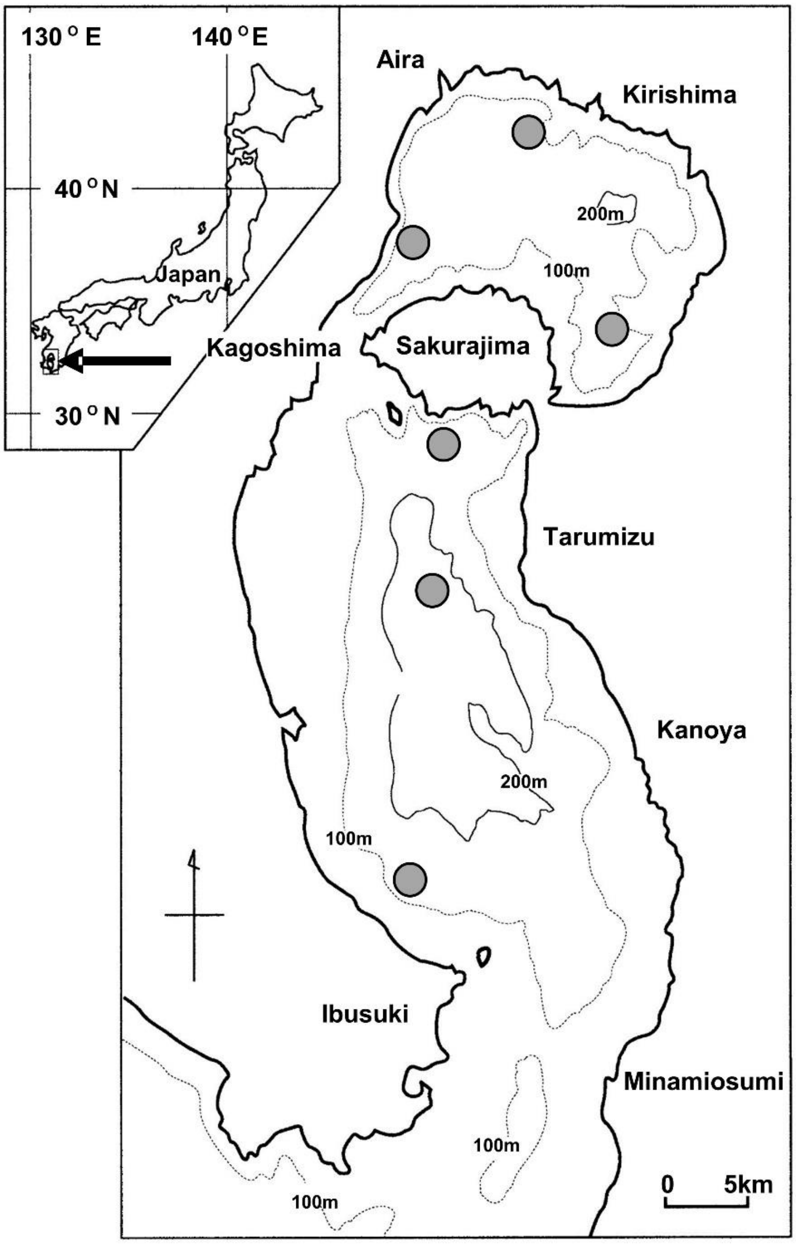


Figure 3.2.1. Kagoshima Bay, southern Japan, with the *Metapenaeopsis sibogae* sampling areas (shaded areas).

to the age m group and i belonged to age n group if $Z_i < 0$. Longevity was estimated from the time series of CL frequency distributions.

3.2.2.3. *Growth analysis*

Age of individuals in months were assigned to the mean CLs belonging to each of the cohorts by arbitrarily assigning 1 October (the approximate midpoint of main spawning season) as the date the shrimp hatched. This assumption was made because the spawning season of *M. sibogae* extends throughout the year with September-October being the main spawning season (Rahman & Ohtomi, 2017). The growth patterns of CL for male and female individuals were modeled by fitting two equations to the mean CLs at ages estimated for each component normal distribution at the various sampling dates. The two equations were the von Bertalanffy growth function (VBGF; von Bertalanffy, 1938): $L_t = L_\infty [1 - \exp\{- K (t - t_0)\}]$ and the Pauly and Gaschütz growth function (PGGF; Pauly & Gaschütz, 1979): $L_t = L_\infty [1 - \exp\{- K (t / 12 - t_0) - (CK / 2\pi) \sin (2\pi (t / 12 - t_s)\}]$,

where L_t is CL (mm) at age t (month), L_∞ the asymptotic CL (mm) corresponding to the CL the shrimp would attain if it grew to an infinite age, K the intrinsic growth rate (per month for VBGF and per year for PGGF) at which L_∞ is approached, C the amplitude of seasonal growth oscillation, t_s the time of the year when growth is the highest, and t_0 the hypothetical age at which CL would be zero.

The numbers of parameters differed in the two growth models (three for VBGF, five for PGGF). We therefore used two information criteria, the Akaike's information criterion (AIC) (Akaike, 1973) and the Bayesian information criterion (BIC) (Schwarz, 1978) to determine the best-fitting model for the CL-at-age data to describe the growth of *M. sibogae*. The AIC and BIC were calculated as:

$$AIC = n (\ln (2\pi RSS / n) + 1) + 2 (r + 1),$$

$$BIC = n (\ln (2\pi RSS / n) + 1) + (r + 1) \ln (n),$$

where n is the number of data, RSS the residual sum of squares, and r the number of estimated parameters. The best-fit model could be chosen based on the lowest value of either AIC or BIC. The values of C were also taken into consideration before selecting the best-fit model describing the growth of the species. An F-test was conducted to compare the growth curves between sexes using the formula provided by Chen *et al.* (1992):

$$F = [(S_b - S_m - S_f) / r] / [(S_m + S_f) / (n_m + n_f - 2r)]$$

where S_m is the residual sum of squares (RSS) for males, S_f the RSS for females, S_b the RSS for both sexes (pooled data), n_m the number of plots for males, n_f the number of plots for females, and r the number of parameters. Once the best-fitting model was selected, the data were divided into two sets (juvenile and adult stages) based on the size at sexual maturity for both males (13.2 mm CL; Rahman & Ohtomi, 2018b) and females (14.3 mm CL; Rahman & Ohtomi, 2017) to estimate the growth rates of juvenile and adult stages.

3.2.2.4. *Length-weight relationship*

The relationships between BW and CL was calculated using the equation log (BW) = log (a) + b log (CL) where, BW is the dependent variable, CL the independent variable, and a and b the regression coefficients. To define the growth type, value of the allometric constant (b) was examined by Student's t-test (Sokal & Rohlf, 1987), using H_0: $b = 3$ ($b = 3$: isometry; $b < 3$: negative allometry; $b > 3$: positive allometry) (Hartnoll, 1982). The analysis of covariance (ANCOVA) was used to analyze the differences of the slopes and intercepts between the regression lines (Zar, 1996).

3.2.2.5. *Statistical analyses*

Microsoft Excel 2013, DeltaGraph 7 and SYSTAT V13.2 software packages were used to conduct the statistical analyses. All statistical analyses were considered significant at a confidence interval of 95%.

3.2.3. RESULTS

A total of 8,787 individuals of *M. sibogae* were examined, of which 55.7% were males and 44.3% were females. The sex ratio differed significantly from a 1:1 ($\chi^2 = 113.12$; $P < 0.001$). The carapace length (CL) ranged from 7.2–18.1 mm in males, and 7.1–21.3 mm in females. The body weight (BW) ranged from 0.28–4.47 g in males, and 0.40–5.84 g in females.

3.2.3.1. *Recruitment, growth pattern, and longevity*

Two normal distributions were detected in both sexes in each month, except in January when three normal distributions were found in females (Figures 3.2.2, 3.2.3). Normal distributions representing newly recruited individuals were first found in winter (January) with a modal size of around 9 mm CL in both years examined. Each cohort could be traced across the months on the CL histograms until December of the following year for males, and until January in the year after that in case of females.

The estimated growth functions for males were as follows:

von Bertalanffy growth function (VBGF): $L_t = 16.79\ [1 - \exp\{-0.181\ (t + 0.867)\}]$

Pauly and Gaschütz growth function (PGGF): $L_t = 16.82\ [1 - \exp\{-2.116\ (t/12 + 0.09) - (0.107/2\pi) \sin (2\pi\ (t/12 - 0.632)\}]$

The estimated growth functions for females were as follows:

VBGF: $L_t = 19.56\ [1 - \exp\{-0.139\ (t + 1.928)\}]$

PGGF: $L_t = 19.61\ [1 - \exp\{-1.629\ (t/12 + 0.184) - (0.125/2\pi) \sin (2\pi\ (t/12 - 0.791)\}]$

Lower values of the Akaike's information criterion (AIC) were obtained for PGGF than for VBGF for both male and female *M. sibogae* (Table 3.2.1). Conversely, lower values of the Bayesian information criterion (BIC) were obtained for VBGF than for PGGF for both sexes. The values of amplitude of seasonal oscillation of growth rate (C) estimated by PGGF were remarkably low for males (0.051) and females (0.077). VBGF was therefore adopted as the best fitting model for describing the growth of both males and females based on BIC and the

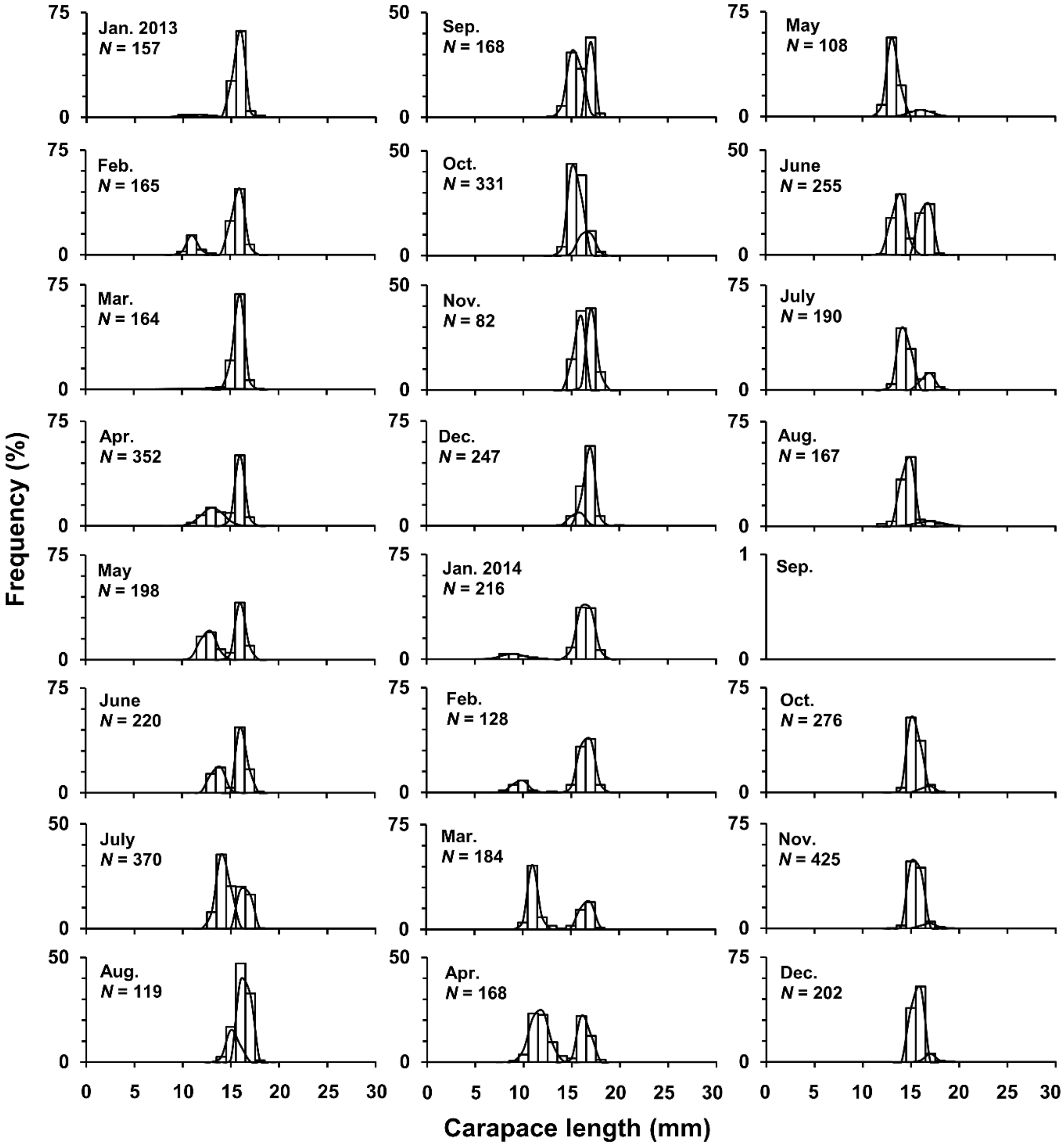


Figure 3.2.2. Length-frequency distributions of male *Metapenaeopsis sibogae* in Kagoshima Bay, southern Japan from January 2013 to December 2014. Curves show the estimated normal distributions of age groups.

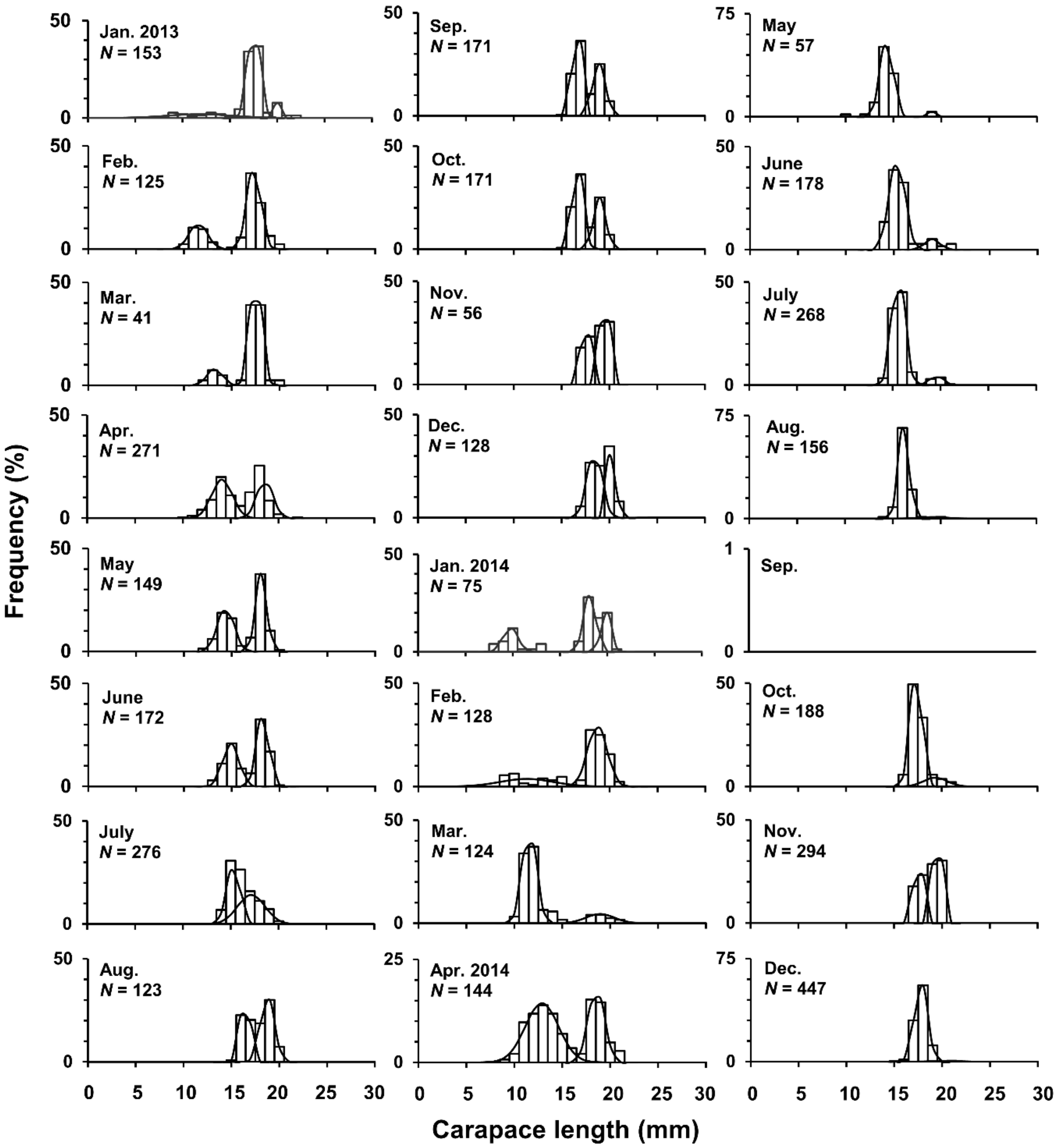


Figure 3.2.3. Length-frequency distributions of female *Metapenaeopsis sibogae* in Kagoshima Bay, southern Japan from January 2013 to December 2014. Curves show the estimated normal distributions of age groups.

lower values of the growth parameter C (Figures 3.2.4, 3.2.5). Females had higher asymptotic length but lower growth rate than males. The F-test revealed a significant difference ($P < 0.01$) between the growths of males and females. The growth rates of juvenile males (0.126 month^{-1}) and females (0.224 month^{-1}) were significantly higher than those of adult males (0.109 month^{-1}) and females (0.106 month^{-1}), respectively. The longevity was estimated to be about 27 months in males and about 28 months in females based on the time series of the CL-frequency distributions (Figures 3.2.2, 3.2.3).

3.2.3.2. *Length-weight relationships*

The relationships between BW and CL indicated negative allometric growths in both sexes (Student's t-test, $P < 0.001$; Figure 3.2.6, Table 3.2.2). Analysis of covariance revealed significant differences between the sexes in both the slope ($P < 0.001$) and intercept ($P < 0.05$) of the BW-CL relationship.

3.2.3.3. *Bottom-water temperature and salinity*

The monthly bottom-water temperature in the sampling areas ranged from 15.2 °C in April to 17.2 °C in December (Figure 3.2.7). The lowest and highest values of salinity were recorded as 29.0 in September and 33.0 in January, respectively (Figure 3.2.7). The monthly bottom-water temperature and salinity in the bay tended to be relatively stable and there were no distinct seasonal trends.

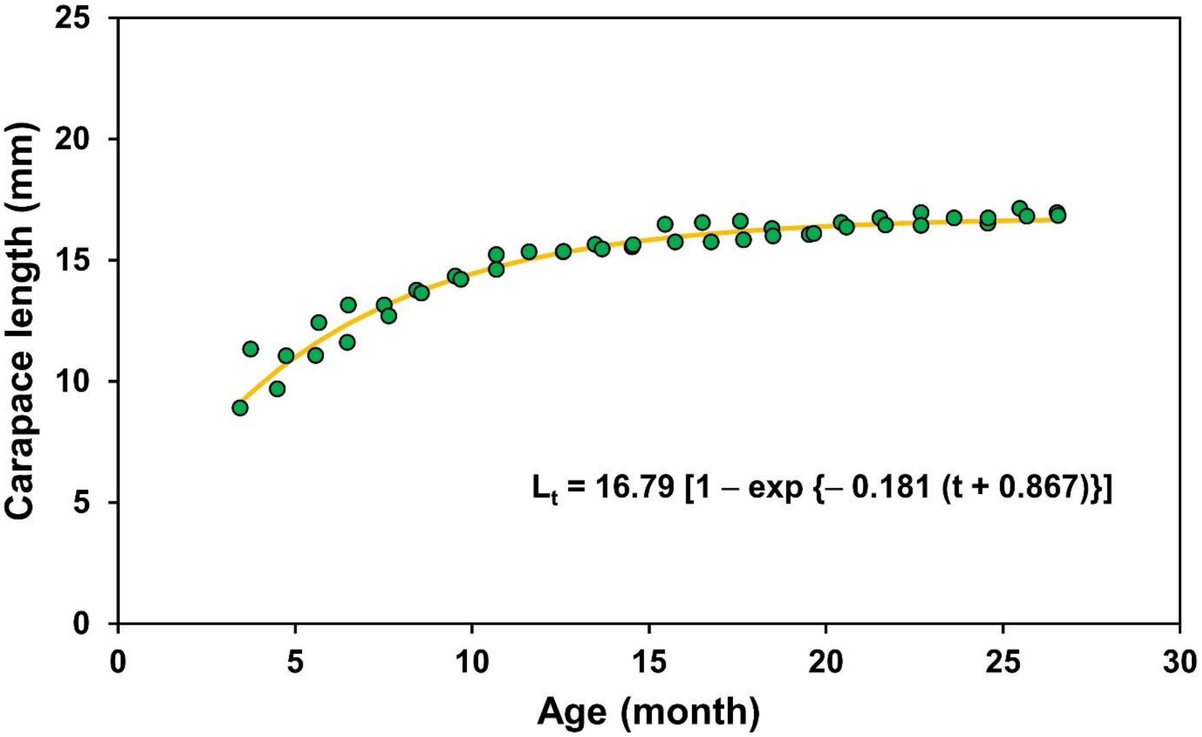


Figure 3.2.4. Growth curve for male *Metapenaeopsis sibogae* in Kagoshima Bay, southern Japan. Black circles show the mean carapace lengths of age groups derived from Figure 3.2.2.

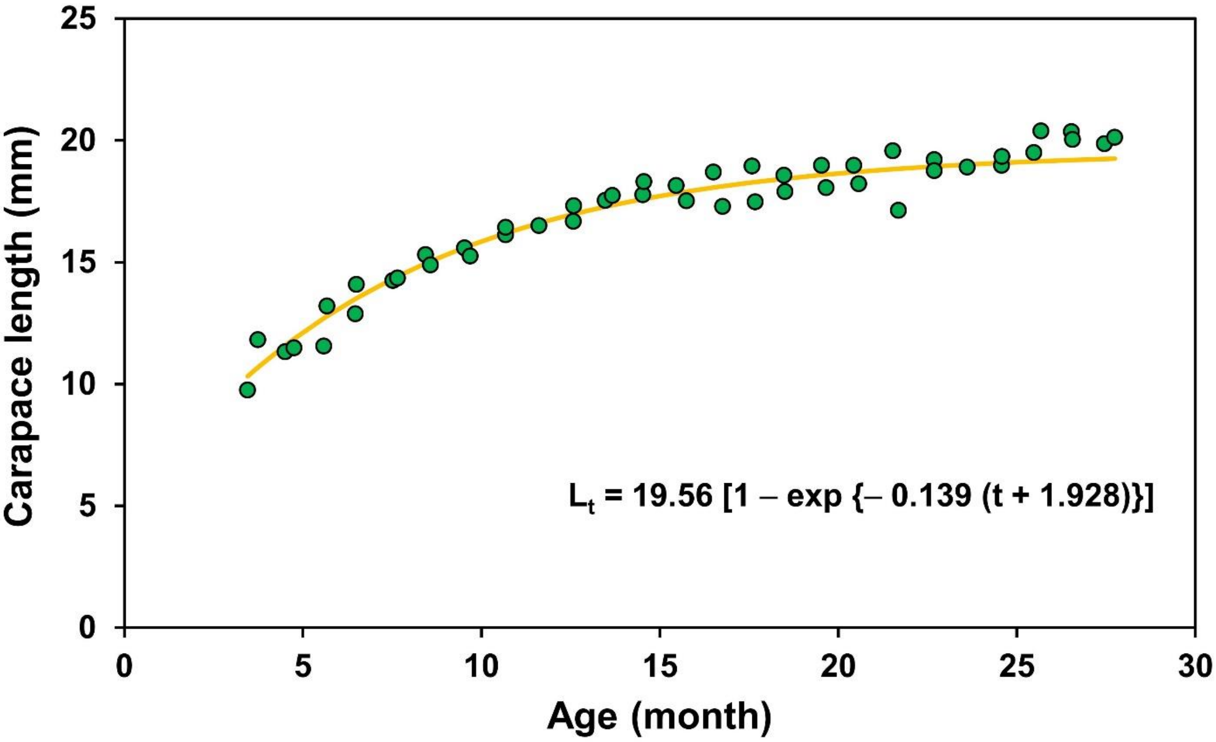


Figure 3.2.5. Growth curve for female *Metapenaeopsis sibogae* in Kagoshima Bay, southern Japan. Black circles show the mean carapace lengths of age groups derived from Figure 3.2.3.

Table 3.2.1. Growth parameters and information criteria estimated for male and female *Metapenaeopsis sibogae* in Kagoshima Bay, southern Japan. L_∞, asymptotic length (mm); K, intrinsic growth rate (month^{-1} for the VBGF and year^{-1} for the PGGF); t_0, hypothetical age at length zero; C, amplitude of seasonal growth oscillation; t_s, summer point, i.e., the time of the year when growth is the highest; *N*, number of observations; AIC, Akaike's information criterion; BIC, Bayesian information criterion; VBGF, von Bertalanffy growth function; PGGF, Pauly and Gaschütz growth function.

Parameters and information criteria	Male		Female	
	VBGF	PGGF	VBGF	PGGF
L_∞	16.79	16.82	19.56	19.61
K	0.181	2.116	0.139	1.629
t_0	−0.867	−0.09	−1.928	−0.184
C	–	0.051	–	0.077
t_s	–	0.632	–	0.791
N	46	46	48	48
AIC	2906.80	2898.04	5821.80	5813.17
BIC	2932.86	2937.01	5846.46	5850.16

3.2.4. DISCUSSION

Males in the studied population of *M. sibogae,* were significantly more abundant than the females in the catchable stock. Greater abundance of females than males is common in populations of penaeoid shrimps (e.g., Costa & Fransozo, 2004; Dhaouadi-Hassen & Boumaïza, 2009; Costa *et al.*, 2010; Grabowski *et al.*, 2014; Lopes *et al.*, 2017). Male-biased sex ratios have nevertheless also been reported for many shrimp populations, as in *Parapenaeus longirostris* (Lucas, 1846) in the Gulf of Alicante, Spain (García-Rodríguez *et al.*, 2009) and *Trachysalambria curvirostris* (Hossain & Ohtomi, 2010) and *Parapenaeus fissuroides* Crosnier, 1986 (Farhana & Ohtomi, 2017) in Kagoshima Bay. Deviations from the 1:1 sex ratio of Fisher (1930) for gonochoristic species are not uncommon and can be a result of the interaction of factors such as differential growth rates, habitat, migration patterns, mortality rates, and longevity differences between the sexes (Wenner, 1972). Because females were larger than the males of the same age and their longevities were similar, we hypothesize that the male-based sex ratio in this population resulted from different migration and distribution patterns and mortality rates between the sexes.

The highest amplitude of body measurements was recorded for females. Sexual dimorphism related to body size is common in penaeoid shrimps, where females are generally larger and heavier than males (Boschi, 1969; Hartnoll, 1982). Similar results were observed in penaeoids such as *H. sibogae* (Ohtomi & Matsuoka, 1998), *Metapenaeus joyneri* (Miers, 1880) (Cha *et al.*, 2004b), *Rimapenaeus constrictus* Stimpson, 1871 (Costa & Fransozo, 2004), *Artemesia longinaris* Spence Bate, 1888 (Semensato & Di Beneditto, 2008), *Pleoticus muelleri* (Castilho *et al.*, 2008, 2012), *P. longirostris* (García-Rodríguez *et al.*, 2009), *T. curvirostris* (Hossain & Ohtomi, 2010), *Xiphopenaeus kroyeri* (Heller, 1862) (Grabowski *et al.*, 2014; Castilho *et al.*, 2015), and *P. fissuroides* (Farhana & Ohtomi, 2017). Such sexual dimorphism

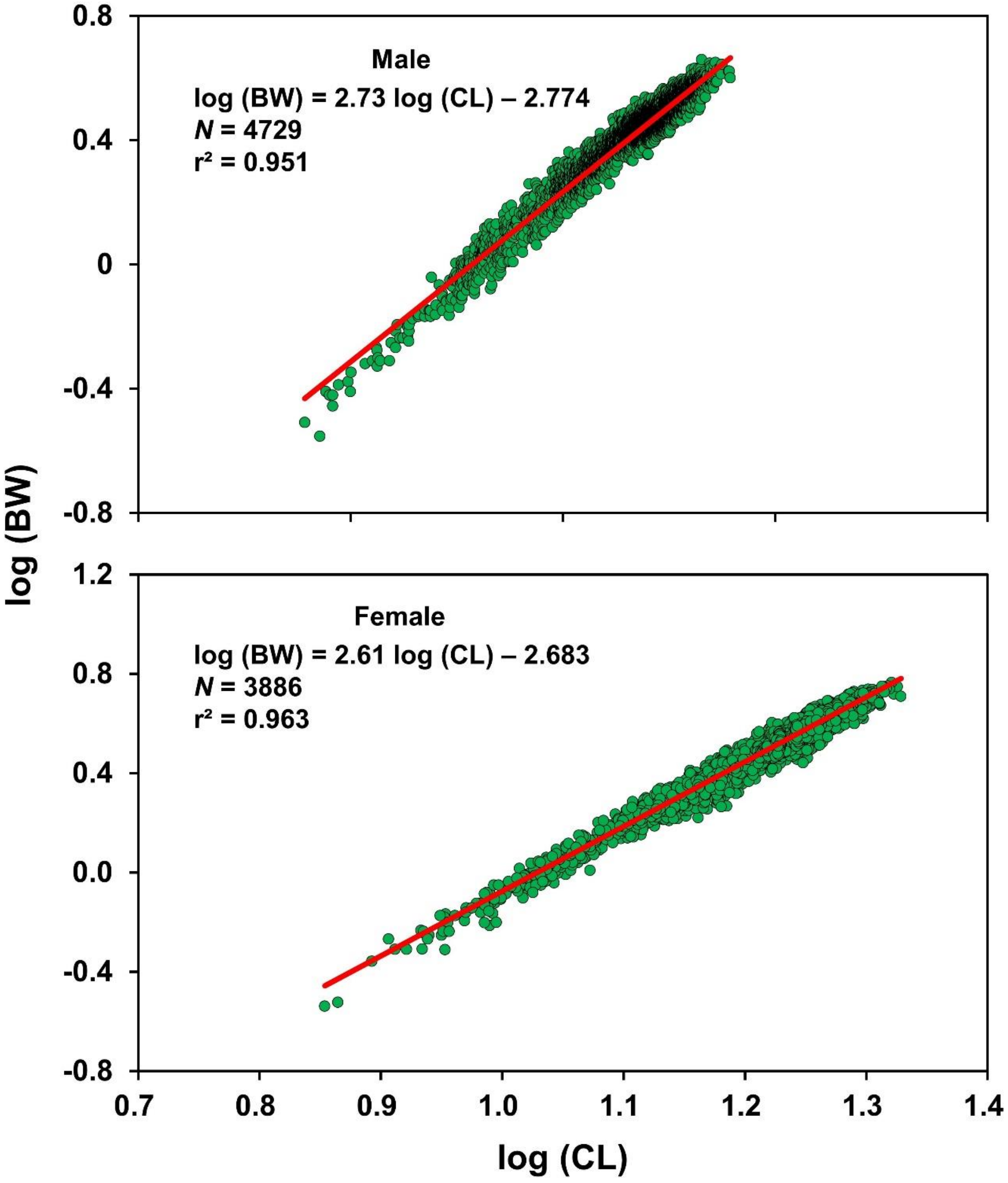


Figure 3.2.6. Relationship between log-transformed carapace length (CL) and body weight (BW) for both sexes of *Metapenaeopsis sibogae* in Kagoshima Bay, southern Japan.

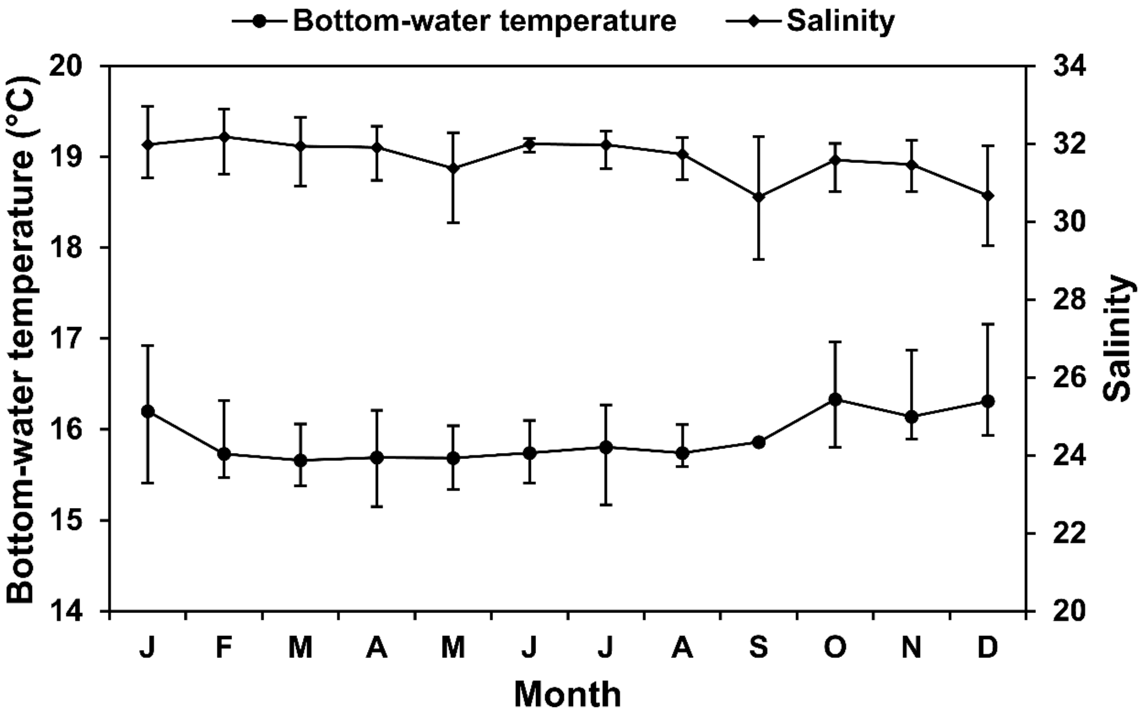


Figure 3.2.7. Monthly changes in mean bottom-water temperature and salinity at the sampling areas in Kagoshima Bay, southern Japan from January 2013 to December 2014. Error bars show the range.

Table 3.2.2. Estimated parameters of the allometric relationships between carapace length (CL) and body weight (BW) of *Metapenaeopsis sibogae* in Kagoshima Bay, southern Kyushu, Japan. The equation is: log (BW) = log (a) + b log (CL). Results of Student's t-test, using H_0: slope = 3; *N*, sample size; a, intercept; b, slope; CI, confidence interval; r^2, coefficient of determination; Allo., allometry; –, negative allometry.

Sex	CL	BW	*N*	a	b	95 % CI of a	95 % Cl	r^2	Allo.	*P*
Male	7.2–18.1	0.28–4.57	4729	0.0017	2.73	0.0016–0.0018	2.72–2.75	0.961	–	<0.001
Female	7.1–21.3	0.40–5.84	3886	0.0021	2.61	0.0019–0.0022	2.59–2.62	0.963	–	<0.001

is considered a general rule among both dendrobranchiate superfamilies, Penaeoidea and Sergestoidea (Boschi, 1989), and is probably an adaptation that increases egg production (Gab-Alla *et al.*, 1990; Yamada *et al.*, 2007).

Criteria have been developed to determine the suitability of length-frequency data to reliably estimate population growth parameters: 1) individuals are short lived; 2) there is significant annual growth; 3) there is a restricted breeding season; and 4) a total sample size of 1,500 or more is collected over a period of six months (Hartnoll, 1982; Hoenig *et al.*, 1987; Wolf, 1989; Kilada *et al.*, 2012). Data used in the present study met almost all of these criteria. The spawning season of *M. sibogae* in Kagoshima Bay extended throughout the year (Rahman & Ohtomi, 2017) but a single, short pulse of recruits was first observed in January and continued until April for both sexes. Recruitment was likely to be synchronized with the main spawning season during September-October, with a time-lag of 3–4 months. Following the main spawning season, recruitment took place within 2–3 months in *S. melantho* (Ohtomi & Irieda, 1997) and *P. fissuroides* (Farhana & Ohtomi, 2017) in Kagoshima Bay and in *M. barbata* on the northeastern coast off Taiwan (Tzeng *et al.*, 2005), and within 3–4 months in *Fenneropenaeus chinensis* (Osbeck, 1765) along the western coast of Korea (Cha *et al.*, 2002). The duration of this time-lag depends upon the time of spawning as well as environmental and hydrological conditions, which may result in a poor relationship between the number of recruits and the size of the spawning stock in penaeids (Garcia & Le Reste, 1981; King, 1995). Detailed studies on this subject are nevertheless rare and inconclusive.

The *M. sibogae* recruitment index was based on the presence of small individuals in the catch, and this could be affected by changes in the reproductive success (spawns), in the pattern of fishing by the fleet (gear-selection and fishing areas), or to biased catch records (Ramírez-Rodríguez & Arreguín-Sánchez, 2000). The sampling procedure used in this study was uniform, which minimized the possibility of biased catch records. The single, short recruitment pulse recorded for *M. sibogae* thus implies that mass mortality may have occurred in the early-spawned cohorts. The presence of a single spawning pulse despite extended spawning season and mass mortality of the early-spawned cohorts have been reported for many penaeid shrimps, e.g., Yamada *et al.* (2007) for *T. curvirostris* in Tokyo Bay, García-Rodríguez *et al.* (2009) for *P. longirostris* in the Gulf of Alicante, Spain, Chen *et al.* (2014) for *M. palmensis* off southwestern Taiwan, and Farhana & Ohtomi (2017) for *P. fissuroides* in Kagoshima Bay. Yamada *et al.* (2007) hypothesized that hypoxia during spring to autumn in Tokyo Bay is a cause of mass mortality of the early spawns of *T. curvirostris*, whereas reduced salinity and increased turbidity could be the reason for unsuccessful recruitment of early spawns of *M. palmensis* in southwestern Taiwan (Chen *et al.*, 2014). Successful recruitment events were observed in winter for *M. sibogae* as well as for *P. fissuroides* in Kagoshima Bay, though Adachi & Kohashi (2011) reported hypoxia in the bay during winter in some years. The fluctuations in bottom-water temperature and salinity were minimal in the bay as also reported by Hossain & Ohtomi (2008) and Rahman & Ohtomi (2017). It was therefore concluded that hypoxia or fluctuations in bottom-water temperature and/or salinity were not responsible for the mass mortality of the early spawns of *M. sibogae*. A variety of factors might influence the larval development and recruitment patterns of a species having continuous reproduction: water currents (Sastry, 1983; Scheltema, 1986; Roughgarden *et al.*, 1988), predation on planktonic larvae (Roughgarden *et al.*, 1988), competitive interactions among larvae of closely related species (Reese, 1968), and seasonality of the larval food supply (Giese & Pearse, 1974; Bauer, 1989). The factors underlying mass mortality of early spawns of *M. sibogae* remains unknown, and it deserve greater attention in future studies.

The Akaike's information criterion (AIC) and the Bayesian information criterion (BIC) were used to find the model that best described the growth of *M. sibogae*. The outcomes of growth model selection based on AIC and BIC were different. Based on the lower values, AIC suggested von Bertalanffy growth function (VBGF) whereas BIC indicated Pauly and Gaschütz growth function (PGGF) as the best-fitting model for both sexes. Bayesian information criterion is sometimes preferred over AIC because BIC is more consistent than AIC, especially when the number of samples is large, and AIC has a finite chance of choosing an unnecessarily complex model (Shono, 2000; Yang, 2005; Nylund *et al.*, 2007; Dziak *et al.*, 2012). Pauly and Gaschütz growth function is a modification of the simple VBGF to fit seasonally oscillating length data. The values of amplitude of seasonal oscillation of growth rate (C) for males (0.051) and females (0.077) were remarkably low representing almost no seasonality in growth rate. VBGF was therefore preferred as the best-fitting model for describing the growth of *M. sibogae* in Kagoshima Bay.

The seasonality in growth rate is likely to be associated with changes in water temperature (Pauly, 1987), as reported in the penaeids *M. joyneri* (Cha *et al.*, 2004b), *M. barbata* (Tzeng *et al.*, 2005), and *T. curvirostris* (Yamada *et al.*, 2007). Crustacean metabolism slows down considerably when the temperature drops below a specific threshold and they do not molt although remaining alive (Conan, 1985). Molting and growth of laboratory-reared *T. curvirostris* occur normally at 15 °C or higher but it does not occur successfully below 12 °C (Imabayashi & Yamada, 1991). The growth rate of *T. curvirostris* was low in Tokyo Bay when bottom-water temperature was less than 15 °C. The growth rate of *M. joyneri* slows down considerably when the bottom-water temperature falls below 10 °C (Cha *et al.*, 2004b). The bottom-water temperature in Kagoshima Bay was relatively constant throughout the study period and never fell below 15° C even during the winter months as a result of the intrusion of the warm Kuroshio Current into the bay in winter (Kohno *et al.*, 2004). Bottom-water temperature did not appear to affect the growth of *M. sibogae*. The growth rate was reported to slow down considerably during the spawning season in *S. melantho* (Ohtomi & Irieda, 1997) and *P. fissuroides* (Farhana & Ohtomi, 2017) in Kagoshima Bay, and in *F. chinensis* in the

Yellow Sea, Korea (Cha *et al.*, 2002). The molting process is reported to stop or slow down during the reproductive period in many crustaceans (Scheer, 1960) as the accumulation of energy required to respond to the development of oocytes may reduce enzyme activities related to stages of molting cycle (Charron *et al.*, 2014). It is noteworthy that in Kagoshima Bay reproduction appeared to affect the growth of *S. melantho* and *P. fissuroides*, whereas it had little or no impact on the growth of *M. sibogae*. The spawning season of *S. melantho* (June to December; Ohtomi *et al.*, 1998) and *P. fissuroides* (July to February; Farhana & Ohtomi, 2016) in Kagoshima Bay is much shorter than the year-long spawning season of *M. sibogae*. It was thus hypothesized that the duration of spawning season might have an influence on the extent of impact that reproduction supposed to have on growth in shrimps.

Males of penaeoids generally show higher growth rates than females, resulting in lower asymptotic lengths and smaller size of males than females of the same age (Hartnoll, 1982; Pauly *et al.*, 1984). Females of *M. sibogae* showed a lower growth rate and greater asymptotic length than those of males, which agrees with earlier studies on *M. sibogae* (Baelde, 1994; Ohtomi & Matsuoka, 1998), *S. melantho* (Ohtomi & Irieda, 1997), *M. dalei* (Choi *et al.*, 2005), *X. kroyeri* (Branco, 2005; Fernandes *et al.*, 2011), *A. longinaris* (Semensato & Di Beneditto, 2008), and *P. longirostris* (García-Rodríguez *et al.*, 2009). In the caridean shrimp *Sclerocrangon boreas* (Phipps, 1774), the earlier onset of sexual maturity in males than females is suggested to contribute to the smaller body size of males (Sainte-Marie *et al.*, 2006), which could also be the case for *M. sibogae* where earlier onset of sexual maturity in males has been reported (Rahman & Ohtomi, 2018b). The growth rates of adult males and females were significantly lower than those of their juvenile counterparts. The reason for this decrease in growth rate in the adult stage is unknown but may result from subsidized energy allocation for somatic growth, because aquatic animals must optimize the use of resources (energy) to grow or reproduce or to perform some combination of these demands (Heino & Kaitala, 1999).

Energy is thought to be allocated entirely for growth in juveniles but to be divided between reproduction and growth in the adults, causing reproduction to impede somatic growth (Paulaj *et al.*, 1982; Taylor & Gabriel, 1992; Pescinelli *et al.*, 2014). Growth rate was more sharply reduced in females than in males as they reached adulthood, which may imply higher energy requirement for reproduction in females than in males. The maximum CLs observed in both sexes were somewhat larger than the estimated asymptotic size, which reflects the high standard deviation of the CLs of the age groups, observed in each of the component normal distributions (Ohtomi, 1997; Ohtomi & Matsuoka, 1998).

The estimated longevity was quite similar for both sexes, 27 months for males and 28 months for females. No study on the longevity of *M. sibogae* is available, although there are a few studies on species of the same genus. Sakaji *et al.* (1992) reported that the longevity of *M. barbata* in the Seto Inland Sea, Japan (average water depth ~ 30 m; Hoshika *et al.*, 1988) was about two years for both sexes, whereas the life span of female *M. dalei* was 15–16 months, and that of males 14–15 months in western Korea (water depth ≤ 30 m) (Choi *et al.*, 2005). These values are considerably lower than those observed in this study. *Metapenaeopsis siboage* is found in deeper water than in *M. barbata* and *M. dale*. The difference between the longevities of the latter two species and *M. sibogae* could be attributed to differences in bathymetric distribution as reported by King & Butler (1985), who observed that deep water shrimp species exhibit longer life cycles compared to shallow water species.

The length-weight relationships of male and female *M. sibogae* showed negative allometric growth; however, the BW of males increased significantly faster with CL than that of the females, which is a common phenomenon in penaeoids such as *H. sibogae* (Baelde, 1994) and *P. fissuroides* (Farhana & Ohtomi, 2017). The asymptotic CL of male and female *M. sibogae* can be further transformed to asymptotic body weights (BW_∞) using the equations $BW_\infty = 3.72$ g for males and $BW_\infty = 4.93$ g for females (Table 2). These outcomes were expected as the BW_∞ of penaeoids are reported to be larger than those of their male counterparts, which makes females economically more viable (e.g., Ohtomi & Irieda, 1997; Hossain & Ohtomi, 2010; Farhana & Ohtomi, 2017).

The present study is the first information on recruitment, growth patterns, and longevity of *M. sibogae*, which will be helpful for sustainable management of this commercially important species. Future research on the planktonic phase and settlement period, together with distribution dynamics of this species, is recommended.

3.3. RECRUITMENT, GROWTH PATTERNS, AND LONGEVITY OF *METAPENAEOPSIS KYUSHUENSIS*

3.3.1. INTRODUCTION

M. kyushuensis is one of the dominant species in the benthic community of Kagoshima Bay and is an emergent fisheries resource (Rahman & Ohtomi, unpublished). There is, however, no study available on the population biology of this species specifically focusing its growth. The present study aims to describe the recruitment, growth patterns and longevity of *M. kyushuensis* for the first time using samples collected from Kagoshima Bay, southern Japan.

3.3.2. MATERIALS AND METHODS

3.3.2.1. *Sampling and measurements*

The present study was conducted in Kagoshima Bay, southern Japan having a maximum water depth of more than 230 m (Ohtomi, 2001) (Figure 3.3.1). Monthly samples of *M. kyushuensis* were obtained at 125–230 m using a simple trawl net (LC-VI; Nichimo, Tokyo, Japan) onboard the training vessel *Nansei Maru* (175 t) of the Faculty of Fisheries, Kagoshima University during January 2016 to December 2017. The net was 23.5 m long with mesh sizes of 37.9 mm and 20.2 mm in the body and the cod-end, respectively (Ohtomi *et al.*, 2004). The wings of the net were expanded by 1 m^2 canvas kites (Fulanda & Ohtomi, 2011). The net was towed for a pre-set duration of 10 min at a speed of 2 knots. Specimens of *M. kyushuensis* were sorted out, counted, and immediately preserved in ice on-board and by 10% formalin upon arrival at the laboratory.

All specimens were classified by sex based on the presence of a petasma in males or a thelycum in females. *M. kyushuensis* is morphologically similar with *M. provocatoria owstoni*.

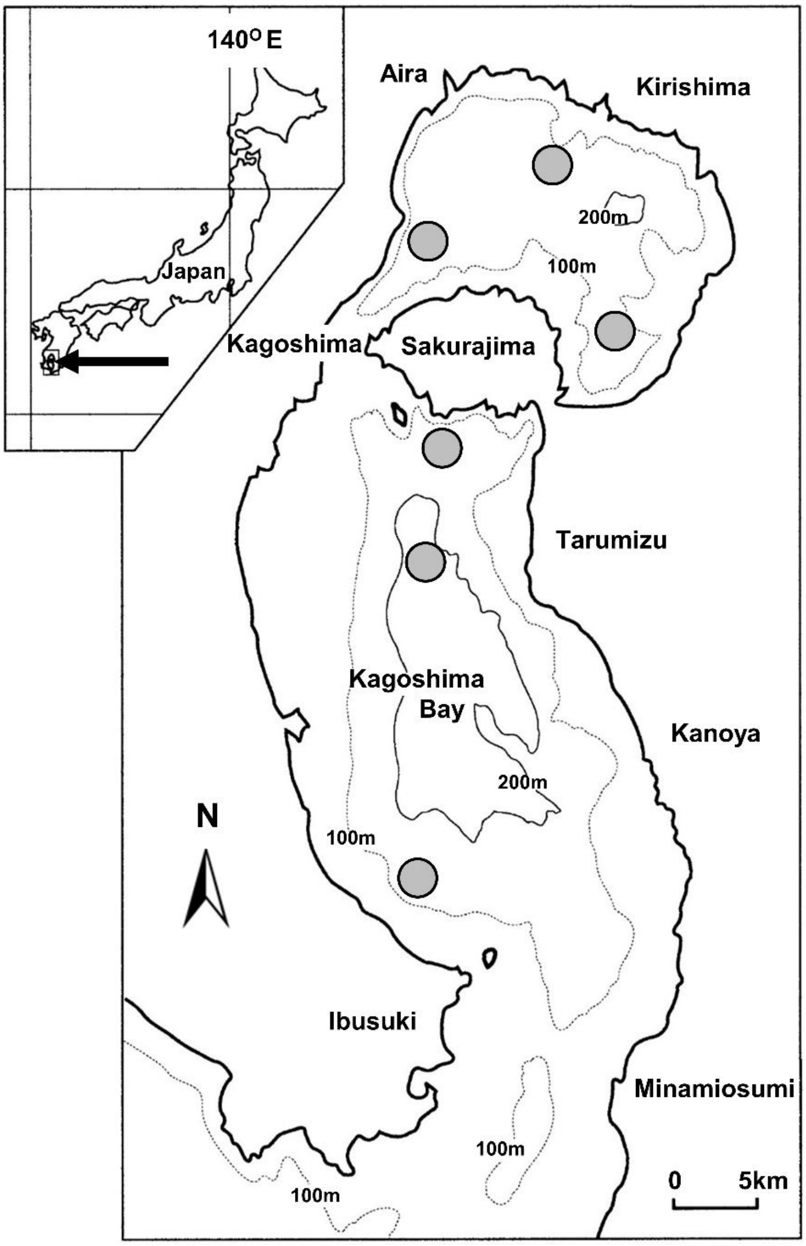


Figure 3.3.1. Kagoshima Bay, southern Japan, with the *Metapenaeopsis kyushuensis* sampling areas (shaded areas).

The females of these two species can be identified based on the differences in thelycum (Figure 3.2.2A, B). However, the shape and size of petasma in males were similar, and it was impossible to distinguish the males of these two species (Figure 3.2.2C). Therefore, only females of *M. kyushuensis* were used in this study. Carapace length (CL) was measured to the nearest 0.01 mm from the posterior margin of the eyestalk to the dorsal midline of the posterior margin of the carapace using slide calipers (CD-15PS; Mitutoyo Corp., Kawasaki, Japan), and wet body weight (BW) was measured to the nearest 0.01 g using an electronic balance (EB-430DW; Shimadzu Corp., Kyoto, Japan).

3.3.2.2. *Length-frequency analysis*

Monthly length-frequency distributions were constructed for females using 1 mm intervals of CL. Hasselblad's maximum-likelihood method (Hasselblad, 1966) was applied to the CL histograms to estimate the parameters for the normal distributions in each histogram. Each identified normal distribution was assumed to represent an age group. The program iteratively computed maximum-likelihood values of the proportions at each age group, mean CL, and standard deviation explained by each component normal distribution. In cases where normal distributions representing two different age groups overlapped, individuals were separated into two age groups using a discriminant function according to Ohtomi & Irieda (1997): $Z_i = (L_m\sigma_n + L_n\sigma_m) / (\sigma_m + \sigma_n) – L_i$, where L_m is the mean CL, σ_m the standard deviation at age m, L_n the mean CL σ_n the standard deviation at age n, and L_i the CL of individual i. If $Z_i > 0$, i belonged to the age m group and i belonged to age n group if $Z_i < 0$. Longevity was estimated from the time series of CL frequency distributions.

3.3.2.3. *Growth analysis*

Age of individuals in months were assigned to the mean CLs belonging to each of the cohorts by arbitrarily assigning 1 August (the approximate midpoint of main spawning season)

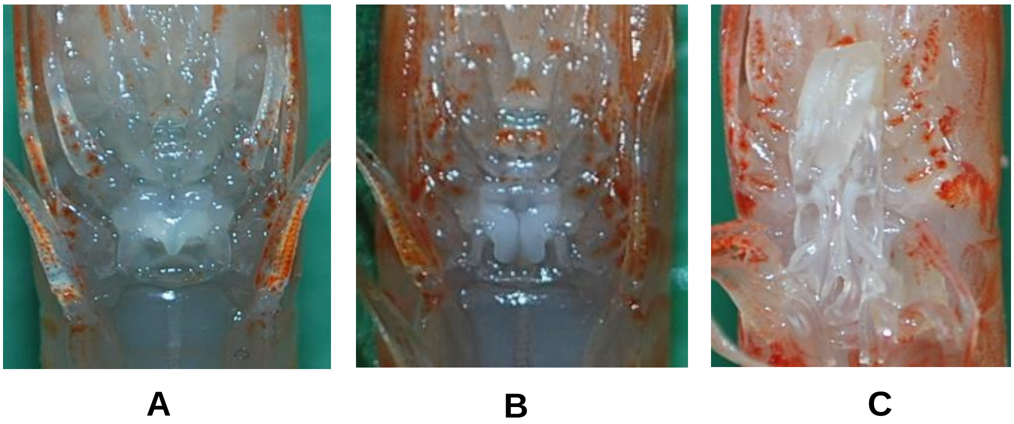


Figure 3.3.2. **A**, thelycum of *Metapenaeopsis kyushuensis*; **B**, thelycum of *Metapenaeopsis provocatoria owstoni*; **C**, petasma of either *Metapenaeopsis kyushuensis* or *Metapenaeopsis provocatoria owstoni*.

as the date the shrimp hatched. This assumption was made because the spawning season of *M. kyushuensis* extends from April to January with June to September being the main spawning season (see Chapter 2, Section 2.3). The growth patterns of CL for female individuals were modeled by fitting two equations to the mean CLs at ages estimated for each component normal distribution at the various sampling dates-

von Bertalanffy growth function (VBGF; von Bertalanffy, 1938):

$$L_t = L_\infty [1 - \exp \{- K (t - t_0)\}], \text{ and}$$

Pauly and Gaschütz growth function (PGGF; Pauly & Gaschütz, 1979):

$$L_t = L_\infty [1 - \exp \{- K (t / 12 - t_0) - (CK / 2\pi) \sin (2\pi (t / 12 - t_s)\}],$$

where L_t is CL (mm) at age t (month), L_∞ the asymptotic CL (mm) corresponding to the CL the shrimp would attain if it grew to an infinite age, K the intrinsic growth rate (per month for VBGF and per year for PGGF) at which L_∞ is approached, C the amplitude of seasonal growth oscillation, t_s the time of the year when growth is the highest (winter point, $t_w = t_s + 0.5$; the time of the year when growth is the lowest), and t_0 the hypothetical age at which CL would be zero.

The numbers of parameters differed in the two growth models (three for VBGF, five for PGGF). We therefore used two information criteria, the Akaike's information criterion (AIC) (Akaike, 1973) and the Bayesian information criterion (BIC) (Schwarz, 1978) to determine the best-fitting model for the CL-at-age data to describe the growth of *M. kyushuensis*. The AIC and BIC were calculated as:

$$AIC = n (\ln (2\pi RSS / n) + 1) + 2 (r + 1),$$

$$BIC = n (\ln (2\pi RSS / n) + 1) + (r + 1) \ln (n),$$

where n is the number of data, RSS the residual sum of squares, and r the number of estimated parameters. The best-fit model could be chosen based on the lowest value of either AIC or BIC.

3.3.2.4. *Length-weight relationship*

The relationships between BW and CL was calculated using the equation log (BW) = log (a) + b log (CL) where, BW is the dependent variable, CL the independent variable, and a and b the regression coefficients. To define the growth type, value of the allometric constant (b) was examined by Student's t-test (Sokal & Rohlf, 1987), using H_0: b = 3 (b = 3: isometry; b < 3: negative allometry; b > 3: positive allometry) (Hartnoll, 1982).

3.3.2.5 *Statistical analyses*

Microsoft Excel 2013, DeltaGraph 7 and SYSTAT V13.2 software packages were used to conduct the statistical analyses. All statistical analyses were considered significant at a confidence interval of 95%.

3.3.3. RESULTS

A total of 1976 females of *M. kyushuensis* with CL ranging from 7.2–19.7 mm and BW from 0.29–4.62 g were used in the present study.

3.3.3.1. *Recruitment, growth pattern, and longevity*

Two normal distributions were detected during December to July (except December 2016) whereas one normal distribution was detected during August to November (Figure 3.3.3). Normal distributions representing newly recruited individuals were first found in winter (December/January) with a modal size of around 10 mm CL in both years examined. Each cohort could be traced across the months on the CL histograms until July of the following year. The estimated growth functions for females were as follows:

VBGF: $L_t = 22.27 [1 - \exp \{- 0.081 (t + 1.38)\}]$

PGGF: $L_t = 24.30 [1 - \exp \{- 0.694 (t/12 + 0.336) - (0.218/2\pi) \sin (2\pi (t/12 - 0.940)\}]$

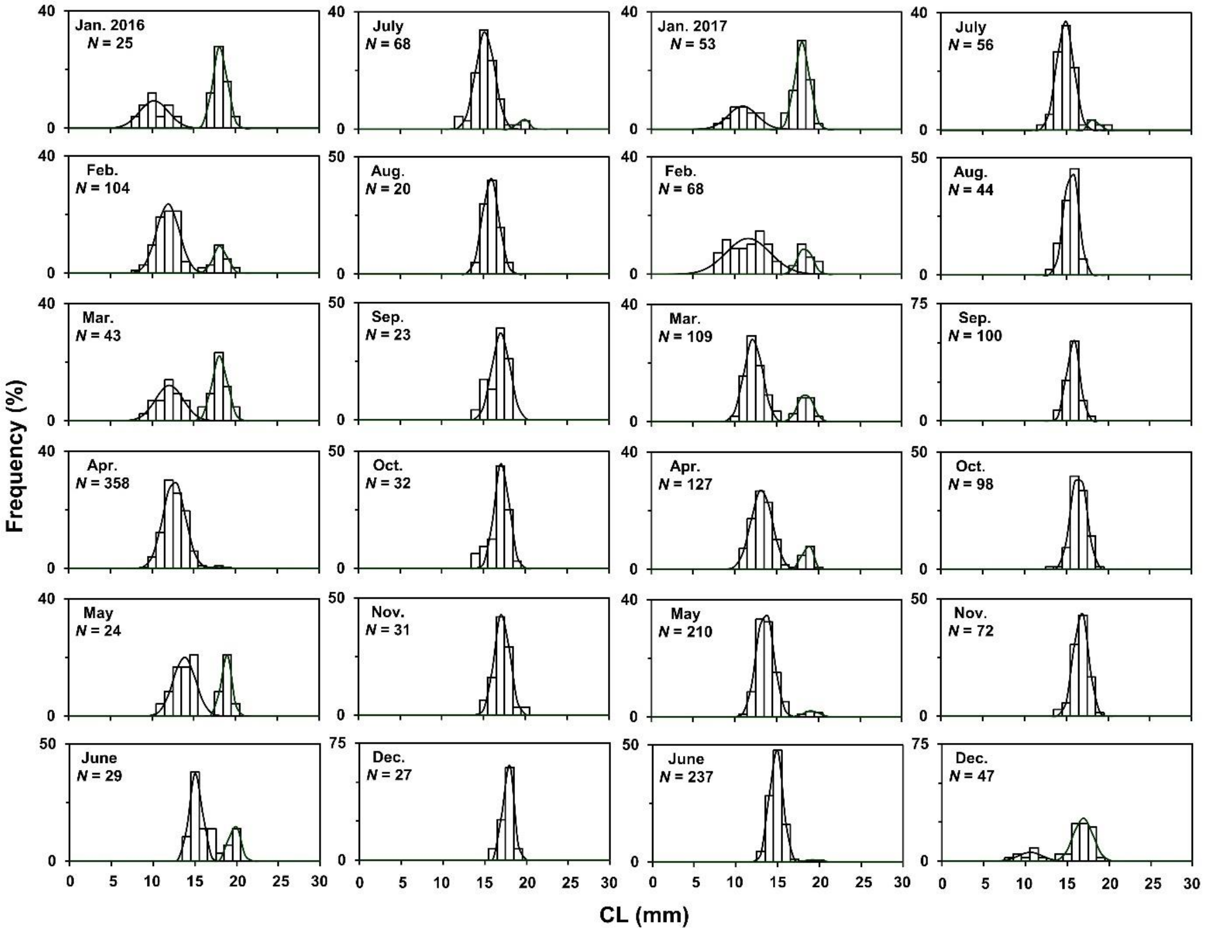


Figure 3.3.3. Length-frequency distributions of female *Metapenaeopsis kyushuensis* in Kagoshima Bay, southern Japan from January 2016 to December 2017. Curves show the estimated normal distributions of age groups.

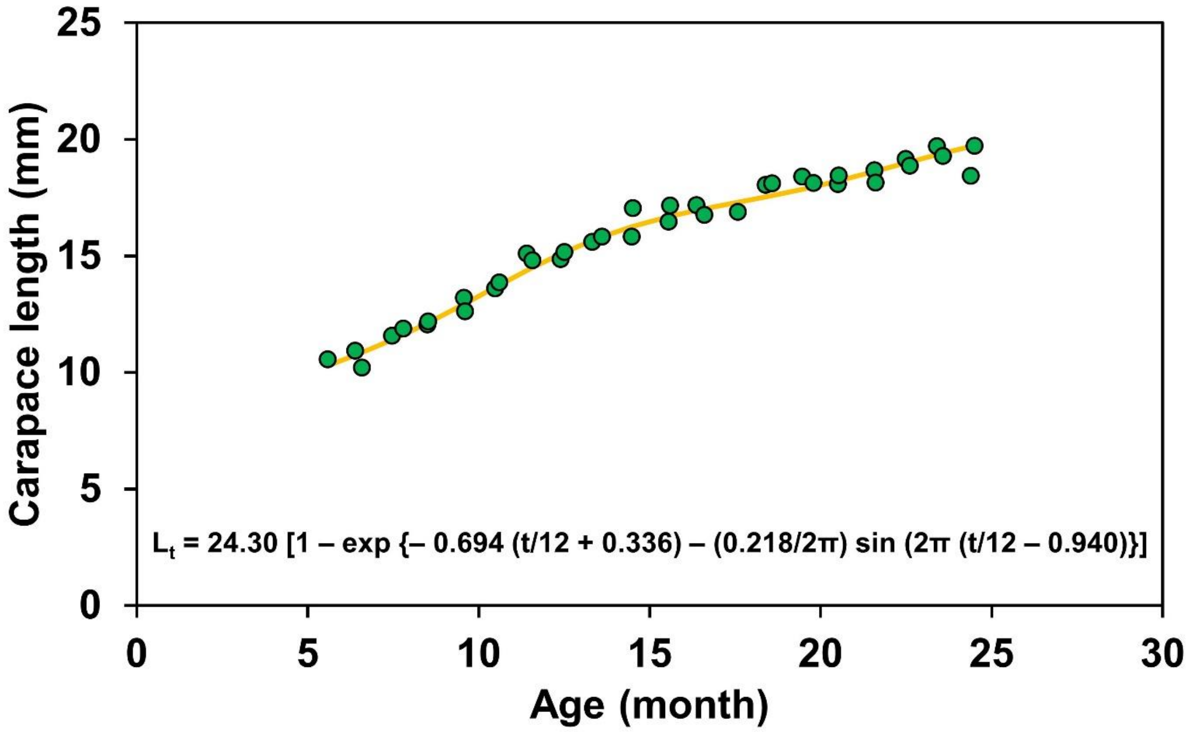


Figure 3.3.4. Growth curve for female *Metapenaeopsis kyushuensis* in Kagoshima Bay, southern Japan. Black circles show the mean carapace lengths of age groups derived from Figure 3.3.3.

Table 3.3.1. Growth parameters and information criteria estimated for female *Metapenaeopsis kyushuensis* in Kagoshima Bay, southern Japan. L_∞, asymptotic length (mm); K, intrinsic growth rate (month^{-1} for the VBGF and year^{-1} for the PGGF); t_0, hypothetical age at length zero; C, amplitude of seasonal growth oscillation; t_s, summer point, i.e., the time of the year when growth is the highest; *N*, number of observations; AIC, Akaike's information criterion; BIC, Bayesian information criterion; VBGF, von Bertalanffy growth function; PGGF, Pauly and Gaschütz growth function.

Parameters and information criteria	Female	
	VBGF	PGGF
L_∞	22.27	24.30
K	0.081	0.694
t_0	−1.379	−0.336
C	–	0.314
t_s	–	0.940
N	38	38
AIC	1343.64	930.51
BIC	1365.99	964.05

The PGGF provided the best fitting model for describing the growth for female *M. kyushuensis* based on the lowest values of both AIC and BIC (Table 3.3.1, Figure 3.3.4). The summer point was estimated to be 0.940 indicating that the growth rate was the highest in November-December and the lowest in May-June. The longevity was estimated to be about 25 months based on the time series of the CL-frequency distributions (Figure 3.3.3).

3.3.3.2. *Length-weight relationships*

The relationships between BW and CL indicated negative allometric growths in female *M. kyushuensis* (Student's t-test, $P < 0.001$; Figure 3.3.5).

3.3.3.3. *Bottom-water temperature*

The monthly bottom-water temperature in the sampling area ranged from 15.1 °C in April to 17.5 °C in January. The monthly bottom-water temperature tended to be relatively stable and never dropped below 15 °C throughout the study period (Figure 3.3.6).

3.3.4. DISCUSSION

Length-frequency analysis is classically the most widespread method for age determination of wild crustacean populations (Oh *et al.*, 1999). There are, however, some criteria developed to determine the suitability of length-frequency data to reliably estimate population growth parameters: 1) individuals are short lived; 2) there is significant annual growth; 3) there is a restricted breeding season; and 4) a total sample size of 1,500 or more is collected over a period of six months (Hartnoll, 1982; Hoenig *et al.*, 1987; Wolf, 1989; Kilada *et al.*, 2012). Data used in the present study met almost all of these criteria. The spawning season of *M. kyushuensis* in Kagoshima Bay extended April to January (See Chapter 2, Section 2.3), however, a single, short pulse of recruits was first observed in December/January and continued until April. Recruitment seems to be synchronized with the main spawning season during June to September, with a time-lag of 5–6 months. Following the main spawning season, recruitment took place within 2–3 months in *S. melantho* (Ohtomi & Irieda, 1997) and *P. fissuroides* (Farhana & Ohtomi, 2017) in Kagoshima Bay and in *M. barbata* on the northeastern coast off Taiwan (Tzeng *et al.*, 2005), and within 3–4 months in *F. chinensis* along the western coast of Korea (Cha *et al.*, 2002) and in *M. sibogae* in Kagoshima Bay (Rahman & Ohtomi, 2018a). The duration of this time-lag depends upon the time of spawning as well as environmental and hydrological conditions, which may result in a poor relationship between the number of recruits and the size of the spawning stock in penaeids (Garcia & Le Reste, 1981; King, 1995). However, the time-lag period between the main spawning season and the first recruitment clearly indicate the mass mortality of early spawns.

The presence of a single spawning pulse despite extended spawning season and mass mortality of the early-spawned cohorts have been reported for many penaeid shrimps, e.g., Yamada *et al.* (2007) for *T. curvirostris* in Tokyo Bay, García-Rodríguez *et al.* (2009) for *P. longirostris* in the Gulf of Alicante, Spain, Chen *et al.* (2014) for *M. palmensis* off southwestern Taiwan, Farhana & Ohtomi (2017) for *P. fissuroides* and Rahman & Ohtomi (2018a) for *M. sibogae* in Kagoshima Bay. Yamada *et al.* (2007) hypothesized that hypoxia during spring to autumn in Tokyo Bay is a cause of mass mortality of the early spawns of *T. curvirostris*, whereas reduced salinity and increased turbidity could be the reason for unsuccessful recruitment of early spawns of *M. palmensis* in southwestern Taiwan (Chen *et al.*, 2014). Successful recruitment events were observed in winter for *M. sibogae* as well as for *P. fissuroides* in Kagoshima Bay, though Adachi & Kohashi (2011) reported hypoxia in the bay during winter in some years. The fluctuations in bottom-water temperature and salinity were minimal in the bay as also reported by Hossain & Ohtomi (2008) and Rahman & Ohtomi (2017, 2018a). Hypoxia or fluctuations in bottom-water temperature and/or salinity were probably not responsible for the mass mortality of the early spawns of *M. kyushuensis* as was also hypothesized for *M. sibogae*. A variety of factors might influence the larval development and

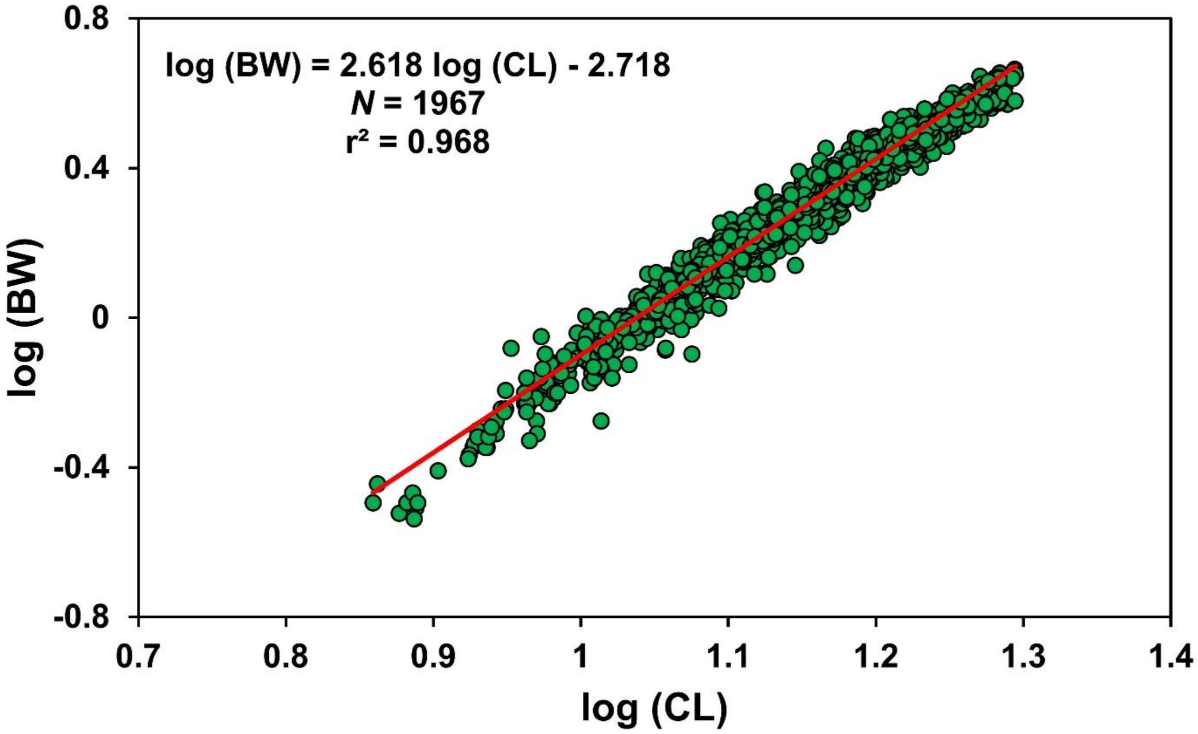


Figure 3.3.5. Relationship between log-transformed carapace length (CL) and body weight (BW) for female *Metapenaeopsis kyushuensis* in Kagoshima Bay, southern Japan.

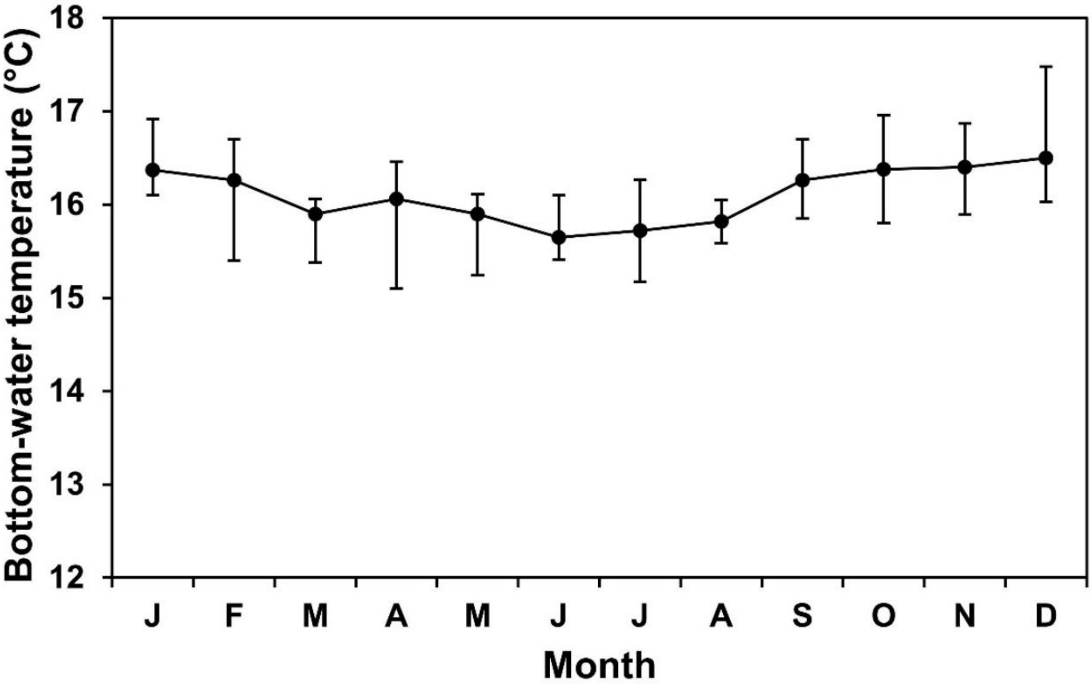


Figure 3.3.6. Monthly changes in bottom-water temperature at the sampling areas in Kagoshima Bay, southern Japan, from January 2016 to December 2017.

recruitment patterns of a species having continuous reproduction: water currents (Sastry, 1983; Scheltema, 1986; Roughgarden *et al.*, 1988), predation on planktonic larvae (Roughgarden *et al.*, 1988), competitive interactions among larvae of closely related species (Reese, 1968), and seasonality of the larval food supply (Giese & Pearse, 1974; Bauer, 1989). The factors underlying mass mortality of early spawns of *M. kyushuensis* remains unknown, and the revelation of this phenomena is recommended for the future studies.

Based on the lowest values of both AIC and BIC, the Pauly and Gaschütz growth function was found to be the best fit to describe the growth of female *M. kyushuensis* suggesting seasonal oscillation in growth rate. The highest growth rate of *M. kyushuensis* (summer point) was estimated to be in November-December and the lowest growth rate was estimated to be in May-June (winter point). The seasonality in growth rate in penaeids is likely to be associated with changes in water temperature (Pauly, 1987) and/or spawning seasonality. Water temperature affected the growth rate of *M. joyneri* (Cha *et al.*, 2004b), *M. barbata* (Tzeng *et al.*, 2005), and *T. curvirostris* (Yamada *et al.*, 2007). Crustacean metabolism slows down considerably when the temperature drops below a specific threshold and they do not molt although remaining alive (Conan, 1985). Molting and growth of laboratory-reared *T. curvirostris* occur normally at 15 °C or higher but it does not occur successfully below 12 °C (Imabayashi & Yamada, 1991). The growth rate of *T. curvirostris* was low in Tokyo Bay when bottom-water temperature was less than 15 °C. The growth rate of *M. joyneri* slows down considerably when the bottom-water temperature falls below 10 °C (Cha *et al.*, 2004b). The bottom-water temperature in Kagoshima Bay was relatively constant throughout the study period and never fell below 15° C even during the winter months as a result of the intrusion of the warm Kuroshio Current into the bay in winter (Kohno *et al.*, 2004). Bottom-water temperature did not appear to affect the growth of *M. kyushuensis* as was also reported for *M. sibogae* (Rahman & Ohtomi, 2018a). Spawning season, however, might have influenced the growth rate as the slower growth rate period coincides with the main spawning season of *M. kyushuensis*. Similar results were also reported for other penaeid shrimps. The growth rate was reported to slow down considerably during the spawning season in *S. melantho* (Ohtomi & Irieda, 1997) and *P. fissuroides* (Farhana & Ohtomi, 2017) in Kagoshima Bay, and in *F. chinensis* in the Yellow Sea, Korea (Cha *et al.*, 2002). The molting process is reported to stop or slow down during the reproductive period in many crustaceans (Scheer, 1960) as the accumulation of energy required to respond to the development of oocytes may reduce enzyme activities related to stages of molting cycle (Charron *et al.*, 2014). It is to be noted that in Kagoshima Bay reproduction appeared to affect the growth of *S. melantho* and *P. fissuroides*, whereas it had little or no impact on the growth of *M. sibogae*. The duration of spawning season was reported to have an influence on the extent of impact that reproduction supposed to have on growth in shrimps (Rahman & Ohtomi, 2018a). The spawning season of *S. melantho* (June to December; Ohtomi *et al.*, 1998) and *P. fissuroides* (July to February; Farhana & Ohtomi, 2016) in Kagoshima Bay is much shorter than the year-long spawning season of *M. sibogae*. The spawning season of *M. kyushuensis* was from April to January, shorter than that of *M. sibogae*. This outcome further supported the hypothesis of the influence of spawning season duration on the growth of shrimps.

The growth rate of female *M. kyushuensis* (0.694 year^{-1}) was much lower than that of female *M. sibogae* (1.667 year^{-1}) resulting in higher asymptotic length in *M. kyushuensis* (24.3 mm) than that of *M. sibogae* (19.6 mm) (Rahman & Ohtomi, 2018a). The estimated longevity was 25 months for female *M. kyushuensis*. No study on the longevity of *M. kyushuensis* is available, although there are a few studies on species of the same genus. Sakaji *et al.* (1992) reported that the longevity of *M. barbata* in the Seto Inland Sea, Japan (average water depth ~ 30 m; Hoshika *et al.*, 1988) was about two years for both sexes, whereas the life span of female *M. dalei* was 15–16 months, and that of males 14–15 months in western Korea (water depth ≤

30 m) (Choi *et al.*, 2005). These values are lower than those observed in this study. *Metapenaeopsis kyushuensis* is found in deeper waterss than that of *M. barbata* and *M. dalei*. The difference between the longevities of the latter two species and *M. kyushuensis* could be attributed to differences in their bathymetric distribution. The hypothesis of deep-water shrimp species to exhibit longer life cycles compared to shallow water species is further supported by the fact that the longevity of *M. sibogae* was estimated to be 27 months in males and 28 months in females in Kagoshima Bay (Rahman & Ohtomi, 2018a) similar to the finding of the present study. The length-weight relationship of female *M. kyushuensis* showed negative allometric growth; which seems to be a general phenomenon in most of the penaeid shrimps as was also reported for the females of *M. sibogae* (Rahman & Ohtomi, 2018a).

The present study is the first information on recruitment, growth patterns, and longevity of female *M. kyushuensis,* which will be helpful for sustainable management of this commercially important species. It was not possible to distinguish between the males of *M. kyushuensis* and *M. provocatoria owstoni*, and thereby the growth pattern of male M. kyushuensis was not possible to estimate. Even though the growth often reported to vary between sexes, generally same growth function fit the data best for both sexes of a given species (see Ohtomi & Irieda, 1997; Hossain & Ohtomi, 2010; Farhana & Ohtomi, 2017; Rahman & Ohtomi, 2018a). Growth pattern and seasonal oscillation of growth rate of male *M. kyushuensis* are hypothesized to follow the similar pattern with that of the females. Future research on the identification of male *M. kyushuensis* followed by their growth together with planktonic phase, settlement period, and distribution dynamics of this species, is recommended.

3.4: RECRUITMENT, GROWTH PATTERNS, AND LONGEVITY OF *METAPENAEOPSIS PROVOCATORIA OWSTONI*

3.4.1. INTRODUCTION

Growth studies are essential for the analysis of exploited stocks, because these results will define the age structure of the catch, estimates of mortality and exploitation rates, and available biomass (Ramírez-Rodríguez & Arreguín-Sánchez, 2000). *M. provocatoria owstoni* is a small penaeid shrimp distributed in the waters of Japan, Korea and Taiwan (Shinomiya & Sakai, 2000). It is one of the dominant *Metapenaeopsis* species in Kagoshima and is commercially important as it is marketed together with other species of the same genus (Rahman & Ohtomi, unpublished). There is no information available on any aspects of population biology specifically focusing the growth of this species in the literature, which represents an obstacle for formulating the proper management strategies for this important shrimp fishery. The main goal of the present study was to study the recruitment, growth patterns, and longevity of *M. provocatoria owstoni* for the first time.

3.4.2. MATERIALS AND METHODS

3.4.2.1. *Sampling and measurements*

The study was conducted in Kagoshima Bay, southern Japan, which has a maximum water depth of more than 230 m (Ohtomi, 2001) (Figure 3.4.1). Monthly samples of *M. provocatoria owstoni* were obtained at ~80–230 m using a simple trawl net (LC-VI; Nichimo, Tokyo, Japan) onboard the training vessel *Nansei Maru* (175 t) of the Faculty of Fisheries, Kagoshima University during January 2013 to December 2017. The net was 23.5 m long with mesh sizes of 37.9 mm and 20.2 mm in the body and the cod-end, respectively (Ohtomi *et al.*,

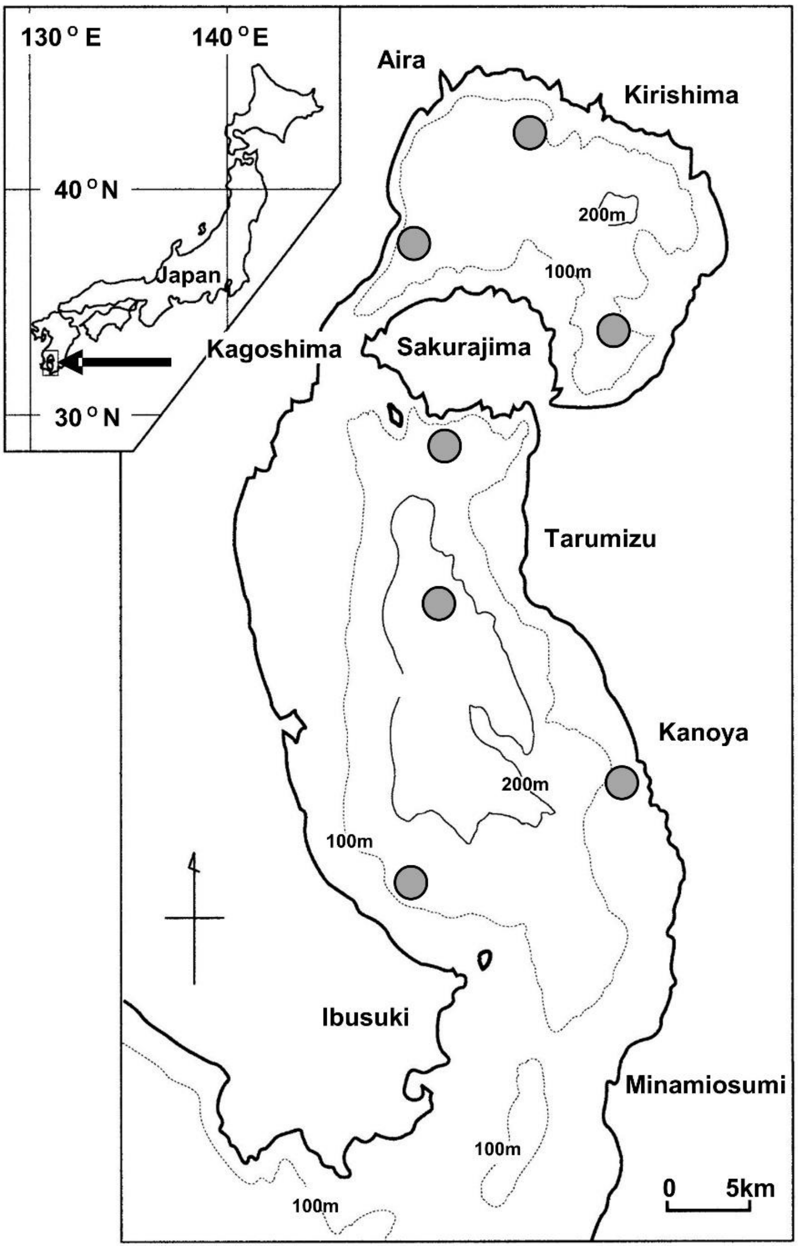


Figure 3.4.1. Kagoshima Bay, southern Japan, with the *Metapenaeopsis provocatoria owstoni* sampling areas (shaded areas).

2004). The wings of the net were expanded by 1 m^2 canvas kites (Fulanda & Ohtomi, 2011). The net was towed for a preset duration of 10 min at a speed of 2 knots. Specimens of *M. provocatoria owstoni* were sorted out, counted, and immediately preserved in ice on-board and by 10% formalin upon arrival at the laboratory.

All specimens were classified by sex based on the presence of a petasma in males or a thelycum in females. *M. provocatoria owstoni* is morphologically similar with *M. kyushuensis*. The females of these two species can be identified based on the differences in thelycum (Figure 3.2.2A, B). However, the shape and size of petasma in males were similar, and it was impossible to distinguish the males of these two species (Figure 3.2.2C). Therefore, only females of *M. provocatoria owstoni* were used in this study. Carapace length (CL) was measured to the nearest 0.01 mm from the posterior margin of the eyestalk to the dorsal midline of the posterior margin of the carapace using slide calipers (CD-15PS; Mitutoyo Corp., Kawasaki, Japan), and wet body weight (BW) was measured to the nearest 0.01 g using an electronic balance (EB-430DW; Shimadzu Corp., Kyoto, Japan).

3.4.2.2. ***Length-frequency analysis***

A total of 690 females of *M. provocatoria owstonia* was sampled over the five years sampling period. Due to the fewer number of individuals that were possible to sample per month, the date was merged for each month. Histograms of CL for females were constructed for each month using 1 mm class intervals. Hasselblad's maximum-likelihood method (Hasselblad, 1966) was applied to the CL histograms to estimate the parameters for the normal distributions in each histogram. Each identified normal distribution was assumed to represent an age group. The program iteratively computed maximum-likelihood values of the proportions at each age group, mean CL, and standard deviation explained by each component normal distribution. In cases where normal distributions representing two different age groups

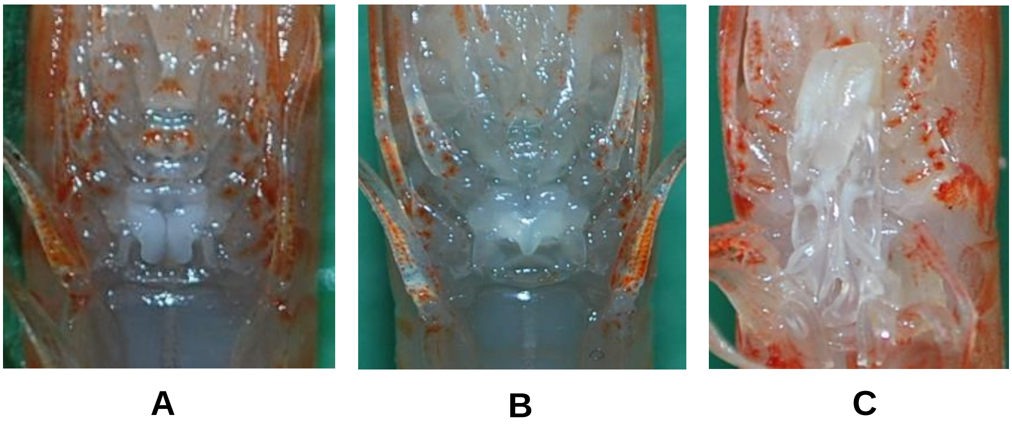


Figure 3.4.2. **A**, thelycum of *Metapenaeopsis provocatoria owstoni*; **B**, thelycum of *Metapenaeopsis kyushuensis*; **C**, petasma of either *Metapenaeopsis provocatoria owstoni* or *Metapenaeopsis kyushuensis.*

overlapped, individuals were separated into two age groups using a discriminant function according to Ohtomi & Irieda (1997): $Z_i = (L_m\sigma_n + L_n\sigma_m) / (\sigma_m + \sigma_n) - L_i$, where L_m is the mean CL, σ_m the standard deviation at age m, L_n the mean CL σ_n the standard deviation at age n, and L_i the CL of individual i. If $Z_i > 0$, i belonged to the age m group and i belonged to age n group if $Z_i < 0$. Longevity was estimated from the time series of CL frequency distributions.

3.4.2.3. *Growth analysis*

Age of individuals in months were assigned to the mean CLs belonging to each of the cohorts by arbitrarily assigning 1 June (the approximate midpoint of main spawning season) as the date the shrimp hatched. This assumption was made because the spawning season of *M. provocatoria owstoni* extends from April-December with May-June being the main spawning season (see Chapter 2, Section 2.3). The growth patterns of CL for female individuals were modeled by fitting two equations to the mean CLs at ages estimated for each component normal distribution at the various sampling dates. The two equations were the von Bertalanffy growth function (VBGF; von Bertalanffy, 1938): $L_t = L_\infty [1 - \exp \{- K (t - t_0)\}]$ and the Pauly and Gaschütz growth function (PGGF; Pauly & Gaschütz, 1979): $L_t = L_\infty [1 - \exp \{- K (t / 12 - t_0) - (CK / 2\pi) \sin (2\pi (t / 12 - t_s)\}]$,

where L_t is CL (mm) at age t (month), L_∞ the asymptotic CL (mm) corresponding to the CL the shrimp would attain if it grew to an infinite age, K the intrinsic growth rate (per month for VBGF and per year for PGGF) at which L_∞ is approached, C the amplitude of seasonal growth oscillation, t_s the time of the year when growth is the highest (winter point, $t_w = t_s + 0.5$; the time of the year when growth is the lowest), and t_0 the hypothetical age at which CL would be zero.

The numbers of parameters differed in the two growth models (three for VBGF, five for PGGF). Two information criteria, the Akaike's information criterion (AIC) (Akaike, 1973) and the Bayesian information criterion (BIC) (Schwarz, 1978), were therefore used to

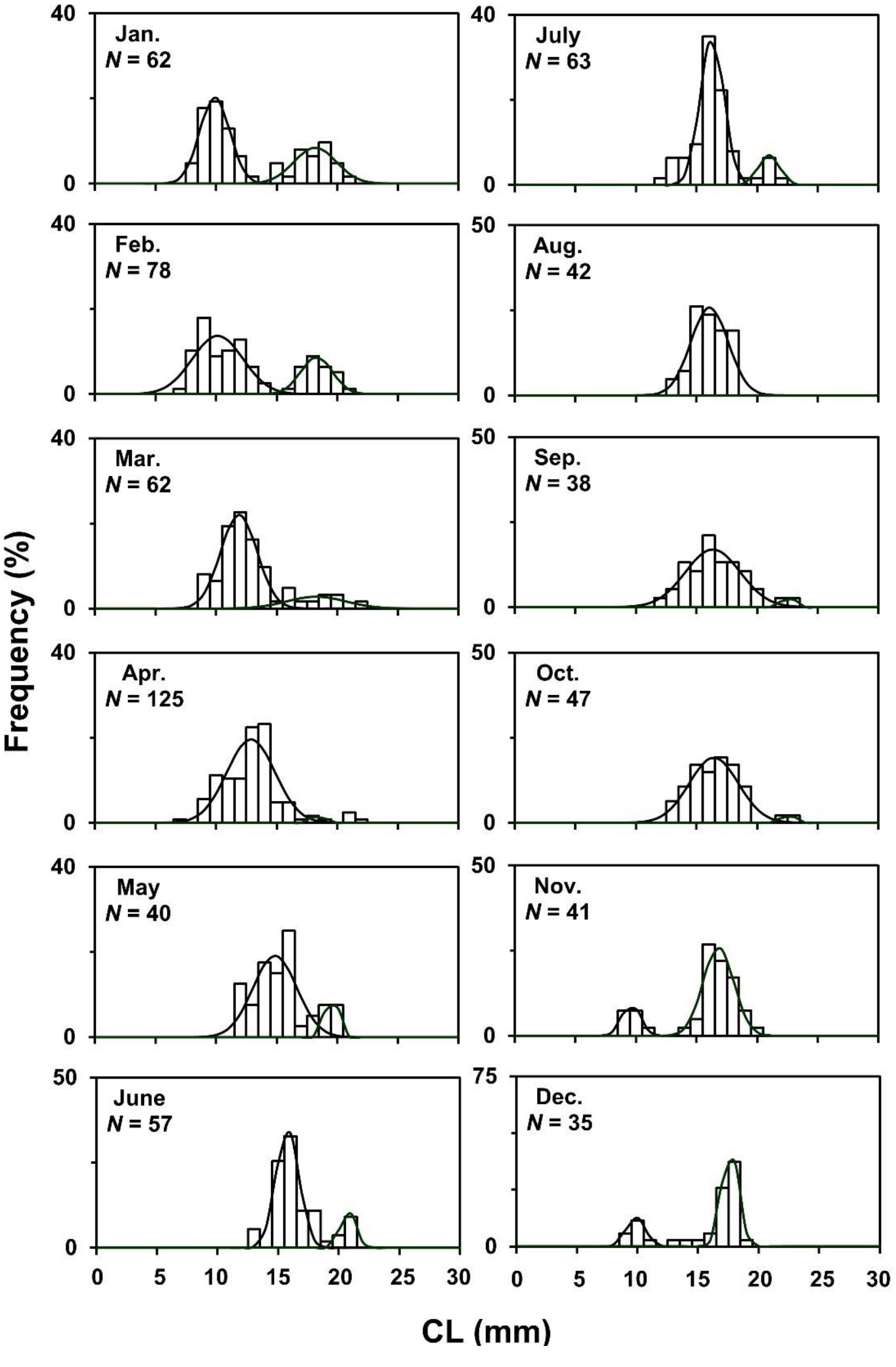


Figure 3.4.3. Length-frequency distributions of female *Metapenaeopsis provocatoria owstoni* in Kagoshima Bay, southern Japan from January 2013 to December 2017. Curves show the estimated normal distributions of age groups.

determine the best-fitting model for the CL-at-age data to describe the growth of *M. provocatoria owstoni*.

The AIC and BIC were calculated as:

$$AIC = n\,(\ln(2\pi RSS / n) + 1) + 2\,(r + 1),$$

$$BIC = n\,(\ln(2\pi RSS / n) + 1) + (r + 1)\ln(n),$$

where n is the number of data, RSS the residual sum of squares, and r the number of estimated parameters. The best-fit model could be chosen based on the lowest value of either AIC or BIC.

3.4.2.4. *Length-weight relationship*

The relationships between BW and CL was calculated using the equation log (BW) = log (a) + b log (CL) where, BW is the dependent variable, CL the independent variable, and a and b the regression coefficients. To define the growth type, value of the allometric constant (b) was examined by Student's t-test (Sokal & Rohlf, 1987), using H_0: b = 3 (b = 3: isometry; b < 3: negative allometry; b > 3: positive allometry) (Hartnoll, 1982).

3.4.2.5. *Statistical analyses*

Microsoft Excel 2013, DeltaGraph 7 and SYSTAT V13.2 software packages were used to conduct the statistical analyses. All statistical analyses were considered significant at a confidence interval of 95%.

3.4.3. RESULTS

3.4.3.1. *Recruitment, growth pattern, and longevity*

A total of 690 females of *M. provocatoria owstoni* with CL ranging from 6.0–22.6 mm and BW ranged from 0.18–6.70 g were used in the present study. Two normal distributions were detected in each month except for August (Figure 3.4.3). Normal distributions representing newly recruited individuals were first found in autumn (November) with a modal size of around

Table 3.4.1. Growth parameters and information criteria estimated for female *Metapenaeopsis provocatoria owstoni* in Kagoshima Bay, southern Japan. L_∞, asymptotic length (mm); K, intrinsic growth rate (month^{-1} for the VBGF and year^{-1} for the PGGF); t_0, hypothetical age at length zero; C, amplitude of seasonal growth oscillation; t_s, summer point, i.e., the time of the year when growth is the highest; *N*, number of observations; AIC, Akaike's information criterion; BIC, Bayesian information criterion; VBGF, von Bertalanffy growth function; PGGF, Pauly and Gaschütz growth function.

Parameters and information criteria	Female	
	VBGF	PGGF
L_∞	22.25	28.77
K	0.095	0.521
t_0	1.221	−0.297
C	–	0.871
t_s	–	0.891
N	23	23
AIC	1464.67	716.87
BIC	1482.81	744.09

10 mm CL. Each cohort could be traced across the months on the CL histograms until July of the following year.

The estimated growth functions for females were as follows:

von Bertalanffy growth function (VBGF): $L_t = 22.25 [1 – \exp \{– 0.095 (t – 1.221)\}]$

Pauly and Gaschütz growth function (PGGF): $L_t = 28.77 [1 – \exp \{– 0.521 (t/12 + 0.297) – (0.454/2\pi) \sin (2\pi (t/12 – 0.891)\}]$

The lowest values of both AIC and BIC were obtained for PGGF (Table 3.4.1), suggesting that the PGGF is the best-fit model to describe the growth of female *M. provocatoria owstoni* (Figure 3.4.4). The summer point was estimated to be 0.891 indicating that the growth rate was the highest in October-November and the lowest in April-May. The longevity was estimated to be about 27 months based on the time series of the CL-frequency distributions (Figure 3.4.3).

3.4.3.2. *Length-weight relationships*

The relationships between BW and CL indicated negative allometric growths in female *M. provocatoria owstoni* (Student's t-test, $P < 0.001$; Figure 3.4.5).

3.4.4. DISCUSSION

The monthly collection of a large number of specimens is essential to examine the biological aspects of a population but is difficult due to various circumstances (Ohtomi & Irieda, 1997). This situation was further complicated by the fact that the population of *M. provocatoria owstoni* is in a dwindling condition (see Chapter 5, Section 5.4). Even though it was possible to collect monthly samples over 5 years period, the number of individuals per month was few. Therefore, the data was merged for each month, which allowed estimation of recruitment and growth patterns, and longevity of *M. provocatoria owstoni* using length-frequency analysis. A single, short pulse of recruits was first observed in November/December and continued until April. Recruitment seems to be synchronized with the main spawning season during May to

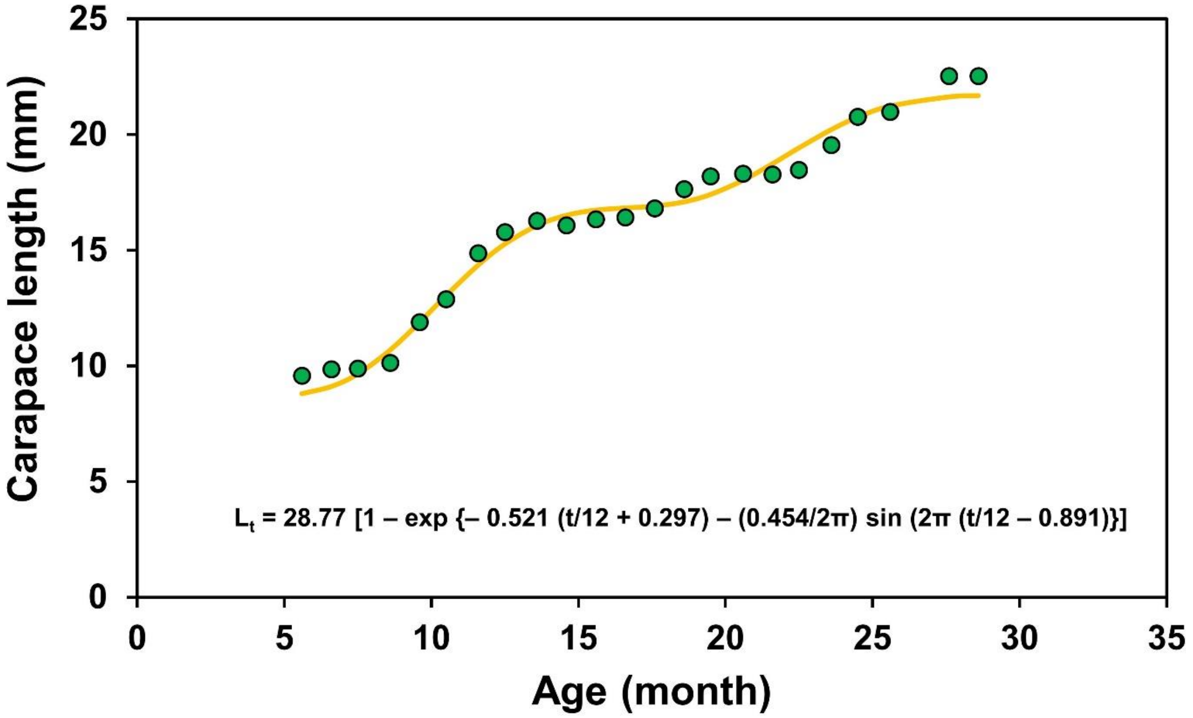


Figure 3.4.4. Growth curves for female *Metapenaeopsis provocatoria owstoni* in Kagoshima Bay, southern Japan. Black circles show the mean carapace lengths of age groups derived from Figure 3.3.3.

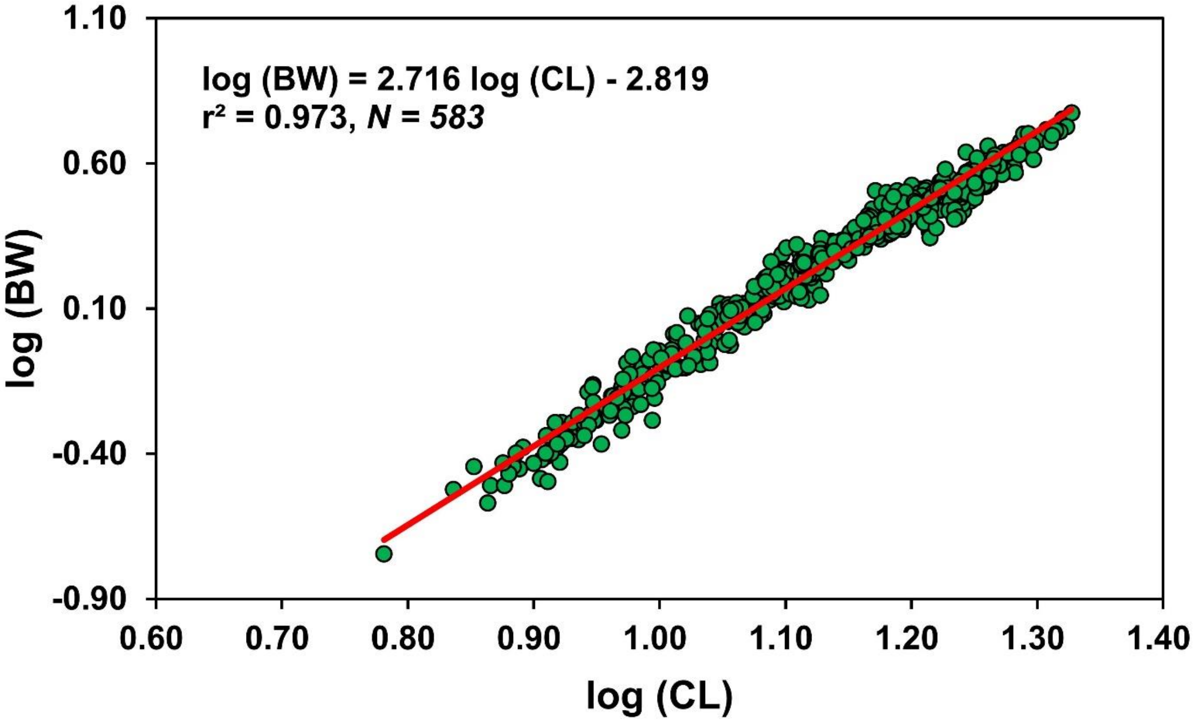


Figure 3.4.5. Relationship between log-transformed carapace length (CL) and body weight (BW) for female *Metapenaeopsis provocatoria owstoni* in Kagoshima Bay, southern Japan.

July (See Chapter 2, Section 2.4), with a time-lag of 5–6 months. Following the main spawning season, recruitment took place within 2–3 months in *S. melantho* (Ohtomi & Irieda, 1997) and *P. fissuroides* (Farhana & Ohtomi, 2017) in Kagoshima Bay and in *M. barbata* on the northeastern coast off Taiwan (Tzeng *et al.*, 2005), within 3–4 months in *F. chinensis* along the western coast of Korea (Cha *et al.*, 2002) and in *M. sibogae* in Kagoshima Bay (Rahman & Ohtomi, 2018a), and within 5–6 months in *M. kyushuensis* in Kagoshima Bay (see Chapter 3, Section 3.3). The duration of this time-lag depends upon the time of spawning as well as environmental and hydrological conditions (Garcia & Le Reste, 1981; King, 1995). The short recruitment pulse despite having extended spawning season indicates mass mortality of the early spawns. The poor relationship between the number of recruits and the size of the spawning stock in penaeids is often reported, e.g., Yamada *et al.* (2007) reported for *T. curvirostris* in Tokyo Bay, García-Rodríguez *et al.* (2009) for *P. longirostris* in the Gulf of Alicante, Spain, Chen *et al.* (2014) for *M. palmensis* off southwestern Taiwan, Farhana & Ohtomi (2017) for *P. fissuroides* and Rahman & Ohtomi (2018a) for *M. sibogae* in Kagoshima Bay, however, the exact reason is yet to be singled out.

A variety of factors might affect the survival rate of the early spawns. Yamada *et al.* (2007) hypothesized that hypoxia during spring to autumn in Tokyo Bay is a cause of mass mortality of the early spawns of *T. curvirostris,* whereas reduced salinity and increased turbidity could be the reason for unsuccessful recruitment of early spawns of *M. palmensis* in southwestern Taiwan (Chen *et al.*, 2014). Successful recruitment events were observed in winter for *M. sibogae* as well as for *P. fissuroides* and *M. kyushuensis* in Kagoshima Bay, though Adachi & Kohashi (2011) reported hypoxia in the bay during winter in some years. The fluctuations in bottom-water temperature and salinity were minimal in the bay as also reported by Hossain & Ohtomi (2008) and Rahman & Ohtomi (2017, 2018a). Hypoxia or fluctuations in bottom-water temperature and/or salinity were probably not responsible for the mass mortality of the early spawns of *M. provocatoria owstoni* as was also hypothesized for *M. sibogae* and *M. kyushuensis* in the bay. Several other factors might also influence the larval development and recruitment patterns of a species having continuous reproduction: water currents (Sastry, 1983; Scheltema, 1986; Roughgarden *et al.*, 1988), predation on planktonic larvae (Roughgarden *et al.*, 1988), competitive interactions among larvae of closely related species (Reese, 1968), and seasonality of the larval food supply (Giese & Pearse, 1974; Bauer, 1989). The factors underlying mass mortality of early spawns of *M. provocatoria owstoni* remains unknown, and the revelation of this phenomena is recommended for future studies.

The Pauly and Gaschütz growth function was found to be the best-fit model to describe the growth of female *M. provocatoria owstoni* based on the lowest values of both AIC and BIC. This suggests seasonal oscillation in growth rate with the highest growth rate of *provocatoria owstoni* (summer point) was estimated to be in October-November and the lowest growth rate was estimated to be in April-May (winter point). Both water temperature (Pauly, 1987) and spawning seasonality have been reported to influence the growth rate in penaeids. Water temperature affected the growth rate of *M. joyneri* (Cha *et al.*, 2004b), *M. barbata* (Tzeng *et al.*, 2005), and *T. curvirostris* (Yamada *et al.*, 2007). Crustacean metabolism slows down considerably when the temperature drops below a specific threshold and they do not molt although remaining alive (Conan, 1985). Molting and growth of laboratory-reared *T. curvirostris* occur normally at 15 °C or higher but it does not occur successfully below 12 °C (Imabayashi & Yamada, 1991). The growth rate of *T. curvirostris* was low in Tokyo Bay when bottom-water temperature was less than 15 °C. The growth rate of *M. joyneri* slows down considerably when the bottom-water temperature falls below 10 °C (Cha *et al.*, 2004b). The bottom-water temperature in Kagoshima Bay was relatively constant throughout the study period and never fell below 15° C even during the winter months as a result of the intrusion of the warm Kuroshio Current into the bay in winter (Kohno *et al.*, 2004). Bottom-water temperature did not appear to affect the growth of *M. kyushuensis* (see Chapter 3, Section 3.3) as was also reported for *M. sibogae* (Rahman & Ohtomi, 2018a). It was therefore hypothesized that bottom-water temperature might not affect the growth of *M. provocatoria owstoni* in Kagoshima Bay. Spawning season, on the other hand, might have influenced the growth rate as the slower growth rate period (April-May) coincides with the main spawning season (May-July) of *M. provocatoria owstoni*. Similar results were also reported for other penaeid shrimps. The growth rate was reported to slow down considerably during the spawning season in *S. melantho* (Ohtomi & Irieda, 1997), *P. fissuroides* (Farhana & Ohtomi, 2017) and *M. kyushuensis* (see Chapter 3, Section 3.3) in Kagoshima Bay, and in *F. chinensis* in the Yellow Sea, Korea (Cha *et al.*, 2002). To achieve growth, crustaceans go through a process of successive molts separated by intermolts (Hartnoll, 1982, 2001). During the intermolt, a crustacean's integument is hard, thereby restricting growth, and at each molt, the integument is shed, which is followed by a period of rapid and extensive growth over a short time lapse prior to the hardening of a new integument (Hartnoll, 1982).The molting process is, however, reported to stop or slow down during the reproductive period in many crustaceans (Scheer, 1960) as the accumulation of energy required to respond to the development of oocytes may reduce enzyme activities related to stages of molting cycle (Charron *et al.*, 2014). It is to be noted that in Kagoshima Bay reproduction appeared to affect the growth of *S. melantho* and *P. fissuroides*, whereas it had little or no impact on the growth of *M. sibogae*. The duration of spawning season was reported to have an influence on the extent of impact that reproduction supposed to have on growth in shrimps (Rahman & Ohtomi, 2018a). The spawning season of *S. melantho* (June to December; Ohtomi *et al.*, 1998) and *P. fissuroides* (July to February; Farhana & Ohtomi, 2016) in Kagoshima Bay is much shorter than the year-long spawning season of *M. sibogae*. The spawning season of *M. provocatoria owstoni* was from April to December, shorter than that of *M. sibogae*. This outcome further supported the hypothesis of the influence of spawning season duration on the growth of shrimps. The variation in food availability can be an important factor for seasonal growth patterns shrimps as was reported for *P. fissuroides* in West Africa (Yacouba *et al.*, 2014), which could not be verified in the present study. The food habit of *M. provocatoria owstoni* needs to be explored in future research.

The growth rate of female *M. provocatoria owstoni* (0.521 year^{-1}) was much lower than that of female *M. kyushuensis* (0.694 year^{-1}) and female *M. sibogae* (1.667 year^{-1}) resulting in higher asymptotic length in *M. provocatoria owstoni* (28.8 mm) than that of *M. kyushuensis* (24.3 mm) and *M. sibogae* (19.61 mm) in the bay (Rahman & Ohtomi, 2018a). The estimated longevity was 27 months for female *M. provocatoria owstoni*. No study on the longevity of *M. provocatoria owstoni* is available, although there are a few studies on species of the same genus. Sakaji *et al.* (1992) reported that the longevity of *M. barbata* in the Seto Inland Sea, Japan (average water depth ~ 30 m; Hoshika *et al.*, 1988) was about two years for both sexes, whereas the life span of female *M. dalei* was 15–16 months, and that of males 14–15 months in western Korea (water depth ≤ 30 m) (Choi *et al.*, 2005). These values are lower than those observed in this study. *Metapenaeopsis provocatoria owstoni* is found in deeper waters than that of *M. barbata* and *M. dalei*. The difference between the longevities of the latter two species and *M. provocatoria owstoni* could be attributed to differences in their bathymetric distribution. King & Butler (1985) observed that deep-water shrimp species exhibit longer life cycles compared to shallow-water species. This hypothesis is further supported by the fact that the longevity of *M. sibogae* was estimated to be 27 months in males and 28 months in females (Rahman & Ohtomi, 2018a) whereas it was estimated to be 25 months in female *M. kyushuensis* (see Chapter 3, Section 3.3) in Kagoshima Bay. The length-weight relationship of female *M. provocatoria owstoni* showed negative allometric growth; which seems to be a general phenomenon in most of the penaeid shrimps as was also reported for the females of *M. sibogae*

(Rahman & Ohtomi, 2018a) and *M. kyushuensis* in Kagoshima Bay (see Chapter 3, Section 3.3).

The present study is the first information on recruitment, growth patterns, and longevity of female *M. provocatoria owstoni*, which will be helpful for sustainable management of this important species. It was impossible to distinguish the males of *M. provocatoria owstoni* from that of *M. kyushuensis*, and thereby the growth pattern of male *M. provocatoria owstoni* was not possible to estimate. Generally, the same growth function provided the best-fit for both sexes of a given species, although the growth often varies between sexes (see Ohtomi & Irieda, 1997; Hossain & Ohtomi, 2010; Farhana & Ohtomi, 2017; Rahman & Ohtomi, 2018a). Growth pattern and seasonal oscillation of growth rate of male *M. provocatoria owstoni* are therefore hypothesized to follow the similar patterns with that of the females. Future research on the identification of male *M. provocatoria owstoni* followed by their growth together with planktonic phase, settlement period, and distribution dynamics, is recommended.

CHAPTER 4: RELATIVE GROWTH AND MORPHOLOGICAL SEXUAL MATURITY OF *METAPENAEOPSIS* SPECIES

4.1: BACKGROUND OF THE STUDY

Species of *Metapenaeopsis* are widely distributed and are ecologically and commercially important (Linke *et al.*, 2001; Raymundo-Huizar *et al.*, 2005; De Young, 2006). Twelve species and two subspecies of *Metapenaeopsis* inhabit Japanese waters (Shinomiya & Sakai, 2000; Ohtomi & Nagata, 2004). In Kagoshima Bay, southern Japan, three species and one subspecies of *Metapenaeopsis* have been recorded of which *M. kyushuensis*, *M. provocatoria owstoni* and *M. sibogae* are morphologically similar with a slender body without a stridulating organ (Ohtomi & Nagata, 2004). Among these species, *M. sibogae* was recorded in the bay in 2004, the first time it was found in Japanese waters (Ohtomi & Nagata, 2004). This species is also reported to inhabit the deep waters of Indonesia, New Caledonia, and the Philippines (Crosnier, 1987, 2007; De Grave & Fransen, 2011). Kagoshima Bay is the northernmost and shallowest habitat of *M. sibogae* and is a unique territory, forming a semi-enclosed deep-water bay within which two gigantic calderas are proposed to exist, and is characterized by steep slopes on either side as a result of fault scarps, the so-called caldera walls (Matumoto, 1943). However, in just over a decade since being first recorded, *M. sibogae* has become one of the most dominant species in the benthic community of Kagoshima Bay surpassing two native species, *M. kyushuensis* and *M. provocatoria owstoni*. All these three species are commercially important as they are marketed as *Metapenaeopsis* spp. complex (Rahman & Ohtomi, unpublished). The adaptation and dominance of *M. sibogae* in such a unique habitat together with the dwindling population status of the other two species in the bay render population biology studies of these species more significant.

The growth of a body part or organ in relation to the entire body is known as relative growth (Teissier, 1960; Hartnoll, 1982). The allometric relationships are powerful tools that are used by taxonomists and ecologists to analyze intraspecific and interspecific variation between populations (Williams, 1984; Hines, 1989; Costa & Soares-Gomes, 2008) and can be helpful in identification of closely related species. Relative growth studies of *Metapenaeopsis* shrimps are thus significant, because they are morphologically similar (Dall *et al.*, 1990) and because taxonomic groupings of this genus have been based primarily on morphological and ecological properties (see Crosnier, 1987, 2007). Furthermore, in many decapod crustaceans, the onset of sexual maturity is characterized by critical morphological changes (Hartnoll, 1982; Lovett & Felder, 1989; Petriella & Boschi, 1997; Sampedro *et al.*, 1999), some of which can be detected by analyzing relative growth (Haley, 1969). *Metapenaeopsis* is the most diversified genus of the family Penaeidae (De Grave & Fransen, 2011), but few studies have been conducted on the population biology of *Metapenaeopsis* species (Kosaka, 1977; Hayashi & Sakamoto, 1978; Watson & Keating, 1989; Sakaji *et al.*, 1992, 2000; Sakaji, 2001a, 2001b; Tzeng *et al.*, 2005; Choi *et al.*, 2005; Chen *et al.*, 2014; Rahman & Ohtomi, 2017), and none has addressed the morphological changes related to sexual maturity. Rahman & Ohtomi (2017) studied the reproductive biology of female *M. sibogae* where they estimated the size at sexual maturity by verifying this by histological observation of the ovaries. However, we did not investigate the relative growth and its possible association with sexual maturity. Relative growth studies of *M. sibogae*, *M. kyushuensis* and *M. provocatoria owstoni* would therefore be helpful to identify intraspecific and interspecific variations while estimation of morphological sexual maturity would aid the regular monitoring of the stock.

4.2: RELATIVE GROWTH AND MORPHOLOGICAL SEXUAL MATURITY OF *METAPENAEOPSIS SIBOGAE*

4.2.1. INTRODUCTION

M. sibogae is a newly recorded species in Japanese waters, reported for the first time in 2004 in Kagoshima Bay (Ohtomi & Nagata, 2004). This shrimp has become one of the dominant species in the benthic community of the bay and gaining in commercial significance. There is, however, no study available on the relative growth of *M. sibogae*, which would be helpful to identify intraspecific and interspecific variations among different populations and closely related species while estimation of morphological sexual maturity would aid the regular monitoring of the stock. Thus, the present study examined the relative growth of *M. sibogae* to facilitate comparative studies and to explain the size at sexual maturity based on critical morphological changes to aid its sustainable management practices.

4.2.2. MATERIALS AND METHODS

4.2.2.1. *Sampling*

Monthly samples of *M. sibogae* were obtained from Kagoshima Bay, southern Kyushu, Japan (31°25' N, 130°38' E) at depths of 125–230 m (Figure 4.2.1) using the training vessel Nansei-Maru (175 t) of the Faculty of Fisheries, Kagoshima University, from January 2013 to December 2014. A simple trawl net (LC-VI; Nichimo Corp., Tokyo, Japan), 23.5 m long, 37.9 mm mesh size in the body, 20.2 mm in the cod-end (Ohtomi *et al.*, 2004), carrying canvas kites on the tips of the wings was used for sampling. The net was towed for a pre-set tow duration of 10 min at a velocity of 2 knots. *M. sibogae* was sorted out of each haul, counted and placed immediately in ice on-board and then preserved in formalin on arrival at the laboratory.

4.2.2.2. *Measurements*

All specimens were sexed according to the presence of a petasma in males or a thelycum in females. For each individual, carapace length (CL), the distance between the posterior margin of the eyestalk to the dorsal midline of the posterior margin of the carapace and body length (BL), the distance from the posterior margin of the eyestalk to the tip of the telson (Figure 4.2.2) were measured to the nearest 0.01 mm with a digital slide caliper. Individual wet body weight (BW) was measured to the nearest 0.01 g by an electronic balance.

4.2.2.3. *Relative growth and determination of morphological sexual maturity*

Changes in the growth patterns of BL and BW in relation to the independent variable (CL) were verified by relative growth analysis using least squares regression. The morphological sexual maturity was estimated from the relationship of BL vs. CL. These two parameters were chosen as in penaeid shrimps the ovaries are contained in the dorsal side of the cephalothorax and abdomen, whereas the testes are contained within the cephalothorax and any change in the gonads during maturation is likely to have an impact on the relationship between these two parameters. The BL vs. CL relationship was obtained based on the linear function described by Huxley (1950): BL = a + bCL, using untransformed data, where, BL is the dependent variable, CL is the independent variable, *a* and *b* are the regression coefficients. Data for each sex were repeatedly partitioned into two size-delimited subsets based on a hypothesized transition point—(I) early phase: individuals with a CL < a hypothesized transition point; and (II) late phase: individuals with a CL ≥ a hypothesized transition point. The hypothesized transition point was sequentially relocated at 0.1 mm intervals of CL throughout the dataset for both sexes. A separate regression function was calculated for each of the two subsets after each relocation of the hypothesized transition point. The transition point indicating morphological sexual maturity was selected based on the lowest combined sum of squared residuals (RSS) in the resulting two subsets of data (Somerton, 1980; Lovett & Felder,

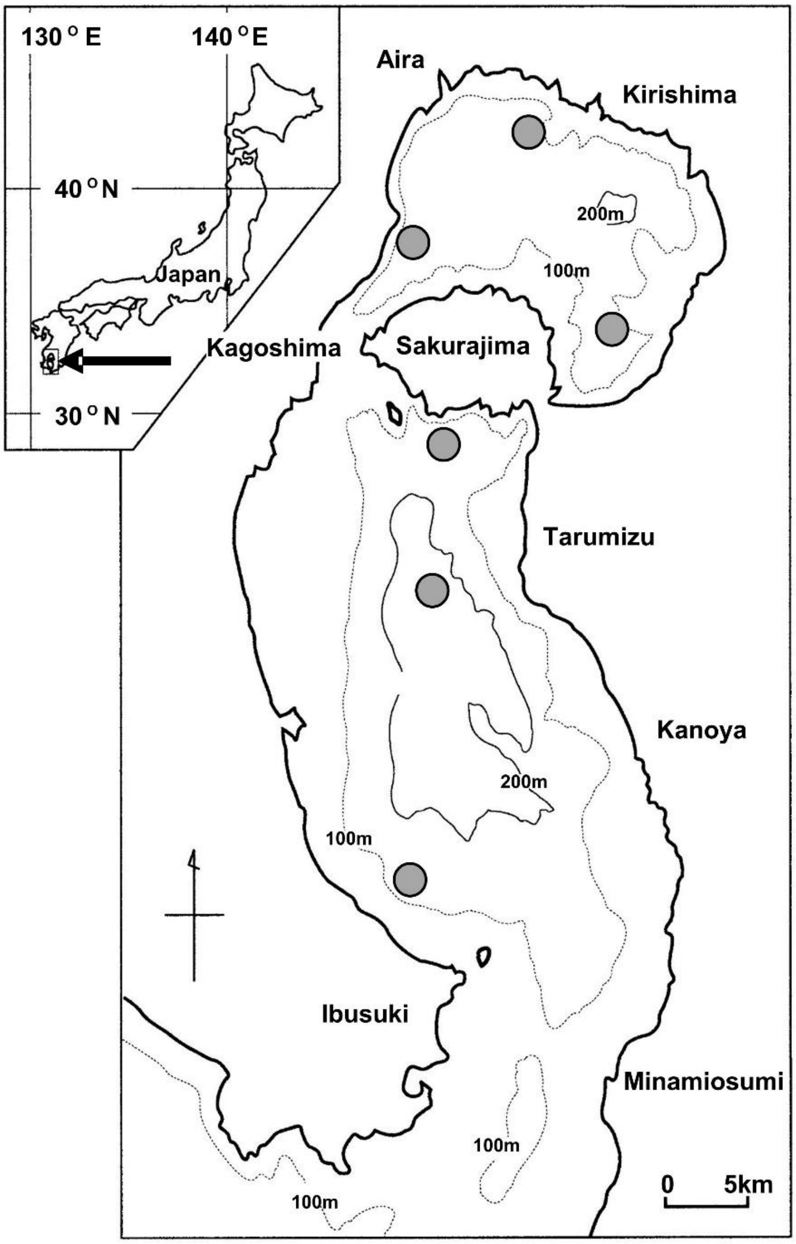


Figure 4.2.1. Kagoshima Bay, southern Japan, with sampling sites for collection of *Metapenaeopsis sibogae* in the bay (shaded areas).

1989; Ohtomi *et al.*, 2005; Ahamed & Ohtomi, 2014). Once the transition points for both sexes were established, the relative growth of BW in relation to CL for both phases was calculated based on the nonlinear power function $BW = aCL^b$, using untransformed data, where, BW is the dependent variable, CL is the independent variable, *a* and *b* are the regression coefficients. To define the growth type, further regression was performed for all cases using log-transformed data. Values of the allometric constant (b) were examined by Student's t-test (Sokal & Rohlf, 1987), using H_0: $b = 1$ in the case of BL-CL relationship ($b = 1$: isometry; $b < 1$: negative allometry; $b > 1$: positive allometry) or H_0: $b = 3$ in the case of BW vs. CL relationship ($b = 3$: isometry; $b < 3$: negative allometry; $b > 3$: positive allometry) (Hartnoll, 1982). Analysis of covariance (ANCOVA) was used to analyze the differences of the slopes and intercepts between the regression lines (Zar, 1996). Microsoft Excel 2013, PAST 3 and SYSTAT V13.2 software packages were used to conduct the statistical analyses. All statistical analyses were considered significant at a confidence interval of 95%.

4.2.3. RESULTS

A total of 2822 individuals of *M. sibogae* consisting of 1255 males and 1567 females we examined examined. In males, CL ranged from 9.2 to 17.1 mm, and BW ranged from 0.55 to 3.88 g. In females, CL varied between 7.9 and 21.2 mm, whereas BW varied from 0.35 to 5.31 g. The relative growth of BL to CL in male and female *M. sibogae* using early and late phases, separated by a specific CL value as a hypothesized transition point, and the RSS values of the regression analyses are shown in Tables 4.2.1 and 4.2.2. Comparisons of RSS indicated that 2 separate linear BL vs. CL models, one for each life phase (Figures 4.2.3 & 4.2.4), fit the data better than a single linear model that was applied over the entire CL range for both sexes (Tables 4.2.1 & 4.2.2). Based on the lowest value of the total RSS, clear transition points were noted in both sexes. In males, the transition point in the BL vs. CL relationship possibly

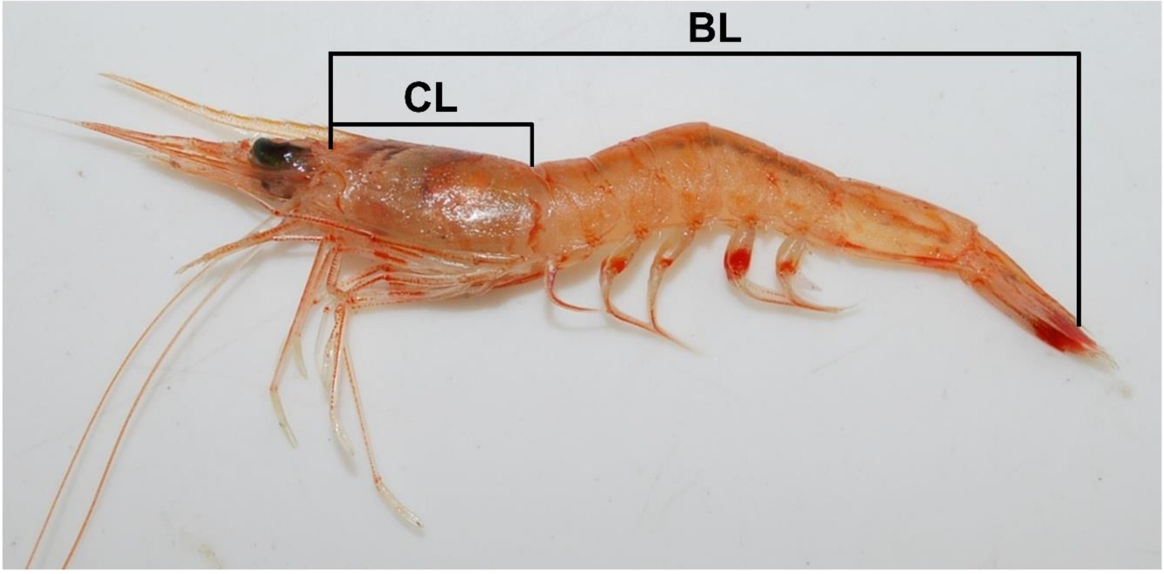


Figure 4.2.2. Lateral view of *Metapenaeopsis sibogae*. Notes: CL, carapace length; BL, body length.

reflecting examined sexual maturity was estimated to be 13.2 mm CL. We observed a significant decrease in the slope and a corresponding increase in the intercept (ANCOVA, $P < 0.001$) for late-phased males (CL $\geq$ 13.2 mm) compared with early-phased males (CL $<$ 13.2 mm). In early-phased males, BL increased isometrically with CL (Student's t-test, $P = 0.213$), but in late-phased males, the growth rate slowed significantly, resulting in negative allometric growth (Student's t-test, $P < 0.001$; Table 4.2.3). On the other hand, in females, an abrupt change in the BL vs. CL relationship likely indicating morphological sexual maturity was estimated to be 15.0 mm CL. Similar to males, a significant decrease in the slope and a corresponding increase in the intercept (ANCOVA, $P < 0.001$) occurred for late-phased females (CL $\geq$ 15.0 mm) versus early-phased females (CL $<$ 15.0 mm). The growth rate of BL in relation to CL was negative allometric in both phases (Student's t-test, $P < 0.001$; Table 4.2.3), but slowed significantly in the late phase (ANCOVA, $P < 0.001$; Figure 4.2.4). Analysis of covariance revealed significant differences in both slope and intercept of the BL vs. CL relationship between sexes for both phases ($P < 0.01$).

The growth rate of BL in relation to CL was negative allometric in both phases (Student's t-test, $P < 0.001$; Table 4.2.3), but slowed significantly in the late phase (ANCOVA, $P < 0.001$; Figure 4.2.4). Analysis of covariance revealed significant differences in both slope and intercept of the BL vs. CL relationship between sexes for both phases ($P < 0.01$).

The relative growth rate of BW to CL for early-phased males varied significantly versus that of late-phased males (ANCOVA, $P < 0.001$). In early-phased males, positive allometric growth was found between BW and CL (Student's t-test, $P < 0.001$), but the rate declined abruptly thereafter, yielding negative allometric growth in late phased-males (Student's t-test, $P < 0.001$; Figure 4.2.5, Table 4.2.3). In case of females, the relative growth rate of BW to CL for early-phased individuals also varied significantly versus that of late-phased individuals (ANCOVA, $P < 0.001$). Negative allometric growth was recorded in both size groups

Table 4.2.1. Estimated slope and intercept of the linear regression representing the relationship of body length (BL, mm) with carapace length (CL, mm) in male *Metapenaeopsis sibogae* and sum of squared residuals (RSS) in different sets of early and late phases. *N*, number of individuals. The CL value at which the lowest total RSS was found is marked in bold format.

Relationship	Transition point (CL)	Early phase				Late phase				Total RSS
		Slope	Intercept	*N*	RSS	Slope	Intercept	*N*	RSS	
BL vs. CL	10.0	4.07	4.39	14	3.12	4.16	5.27	1241	2370.14	2373.26
	11.0	5.06	-4.88	27	13.73	4.14	5.55	1228	2357.11	2370.84
	11.5	5.03	-4.57	34	16.48	4.14	5.58	1221	2354.31	2370.79
	12.0	4.25	3.22	47	48.75	4.09	6.16	1208	2300.06	2348.81
	12.5	4.41	1.58	150	211.33	3.93	8.51	1105	2052.33	2263.66
	13.0	4.57	-0.26	314	538.33	3.75	11.21	941	1660.73	2199.06
	13.1	4.59	-0.47	356	674.59	3.71	11.82	899	1511.83	2186.42
	13.2	**4.63**	**-0.91**	**404**	**788.43**	**3.68**	**12.25**	**851**	**1395.29**	**2183.72**
	13.3	4.69	-1.62	462	907.39	3.67	12.34	793	1285.63	2193.02
	13.4	4.73	-2.02	526	1066.47	3.66	12.49	729	1128.31	2194.78
	13.5	4.75	-2.35	578	1160.04	3.67	12.32	677	1038.07	2198.11
	13.6	4.72	-1.92	639	1289.27	3.64	12.80	616	907.69	2196.96
	13.7	4.69	-1.56	717	1428.26	3.59	13.50	538	766.05	2194.31
	13.8	4.66	-1.26	798	1552.25	3.55	14.13	457	643.67	2195.92
	13.9	4.69	-1.66	862	1663.19	3.62	13.09	393	534.68	2197.87
	14.0	4.64	-0.93	956	1848.79	3.55	14.18	299	352.40	2201.19
	14.5	4.60	-0.48	1105	2074.88	3.59	13.56	150	128.18	2203.06
	15.0	4.50	0.80	1165	2174.75	3.48	15.37	90	73.81	2248.56
Single line using all data		4.24	4.27	1255	2407.63	-	-	-	-	2407.63

Table 4.2.2. Estimated slope and intercept of the linear regression representing the relationship of body length (BL, mm) with carapace length (CL, mm) in female *Metapenaeopsis sibogae* and sum of squared residuals (RSS) in different sets of early and late phases. *N*, number of individuals. The CL value at which the lowest total RSS was found is marked in bold format.

Relationship	Transition point (CL)	Early phase				Late phase				Total RSS
		Slope	Intercept	*N*	RSS	Slope	Intercept	*N*	RSS	
BL vs. CL	10.0	3.97	4.92	39	46.67	3.35	13.40	1528	3683.48	3730.15
	11.0	4.09	3.92	98	182.46	3.29	14.44	1469	3384.99	3567.45
	11.5	4.13	3.60	147	296.14	3.24	15.18	1420	3173.59	3469.73
	12.0	4.11	3.69	210	385.54	3.19	16.03	1357	2984.88	3370.42
	12.5	4.12	3.73	280	495.57	3.14	16.5	1287	2796.76	3292.33
	13.0	4.11	3.68	356	592.83	3.10	17.41	1211	2666.63	3259.46
	13.5	3.97	5.34	489	885.43	2.99	19.13	1078	2247.85	3133.28
	14.0	3.99	5.04	658	1173.93	2.93	20.30	909	1909.53	3083.46
	14.2	4.01	4.81	734	1305.84	2.92	20.38	833	1779.24	3085.08
	14.4	4.02	4.72	807	1485.96	2.93	20.27	760	1595.73	3081.69
	14.6	3.99	5.11	894	1715.62	2.90	20.77	673	1374.89	3090.51
	14.8	3.98	5.20	985	1934.15	2.94	20.13	582	1154.31	3088.46
	14.9	3.96	5.40	1035	2058.75	2.95	19.93	532	1038.40	3097.15
	15.0	**3.96**	**5.45**	**1090**	**2180.66**	**3.11**	**17.17**	**477**	**892.32**	**3072.98**
	15.1	3.95	5.49	1092	2189.54	3.09	17.34	475	889.56	3079.10
	15.2	3.94	5.54	1097	2205.24	3.09	17.35	470	880.99	3086.23
	15.4	3.94	5.69	1103	2252.27	3.05	18.12	464	859.01	3111.28
	15.6	3.93	5.74	1107	2264.01	3.06	18.06	460	852.72	3116.73
	15.8	3.92	5.88	1115	2287.47	3.06	18.02	452	844.44	3131.91
	16.0	3.91	6.02	1124	2325.68	3.08	17.70	443	819.38	3145.06
	17.0	3.67	9.11	1276	3031.18	2.97	19.56	291	532.11	3563.29
Single line using all data		3.43	12.19	1567	4011.28	-	-	-	-	4011.28

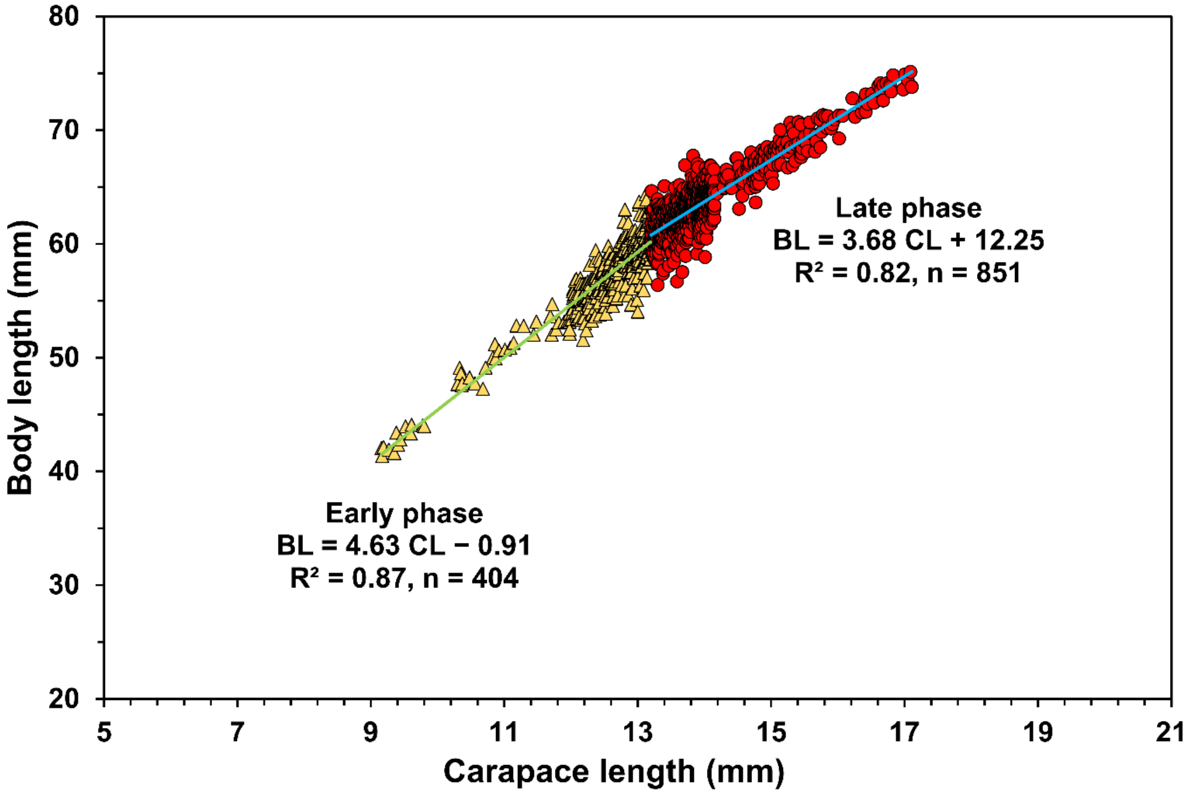


Figure 4.2.3. Relationship of body length (BL) with carapace length (CL), as estimated by the least squares regression with untransformed data of male *Metapenaeopsis sibogae*.

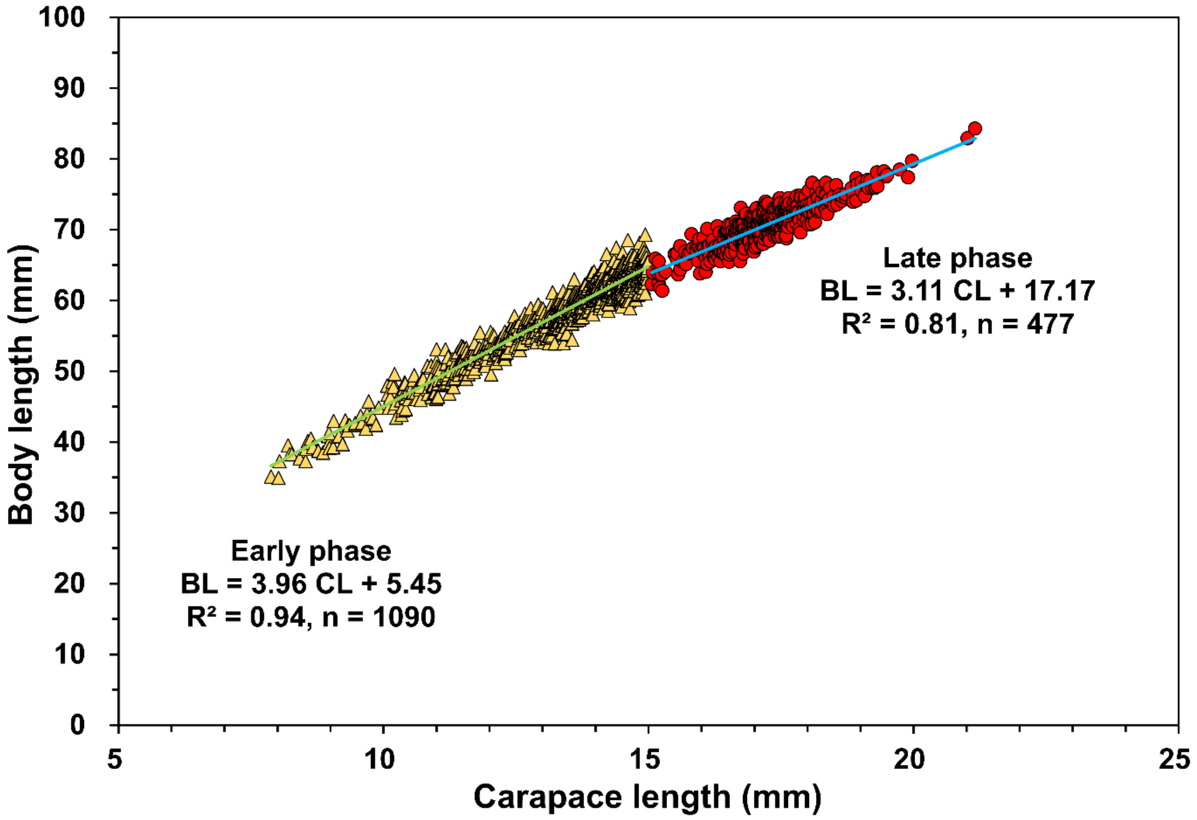


Figure 4.2.4. Relationship of body length (BL) with carapace length (CL), as estimated by the least squares regression with untransformed data of female *Metapenaeopsis sibogae*.

(Student's t-test, $P < 0.001$), but this rate was significantly lower in the late-phased individuals (ANCOVA, $P < 0.001$; Figure 4.2.6, Table 4.2.3). Analysis of covariance further detected significant differences in the intercept and slope of the BW vs. CL relationship between early-phased males and females ($P < 0.001$) but only between the intercepts of late-phased males and females ($P < 0.001$) not the slopes ($P = 0.154$).

4.2.4. DISCUSSION

The growth of crustaceans is characterized by ontogenetic changes that occur during the development of their body structure and is widely applied as tool for making morphological observations of the transition between the juvenile and adult stages (Wenner *et al.*, 1974; Hartnoll, 1978, 1982; Vaninni & Gherardi 1988; Dalabona *et al.*, 2005; Herrera *et al.*, 2017). The present investigation of the relationship between BL and CL revealed the existence of dimorphic growth in both sexes of *M. sibogae*. Analysis of RSS showed clear transition points in both sexes, favoring two separate linear BL vs. CL models that fit the data better than a single linear model that was applied over the entire CL range, indicating two life phases (Somerton, 1980). A change in allometry between early- and late-phased males was indicated by a 20.5% decrease in the slope of the BL vs. CL relationship (Figure 4.2.3). Similarly, in females, a change in allometry between early- and late-phased individuals was reflected by a 21.5% decrease in the slope of the BL vs. CL relationship (Figure 4.2.4).

A decline in the slope of the BL vs. CL relationship indicates that the carapace grows faster relative to the abdomen in late-phased individuals versus early-phased individuals, because BL is essentially the sum of CL and the abdomen length (Diaz *et al.*, 2001). In penaeids, the cephalothorax houses most internal organs, including major portions of the ovaries of fully mature females (Solis, 1988) and the whole testes, whereas, the abdomen only contains a minor portion of the ovaries (Diaz *et al.*, 2001). The shift in the BL vs. CL relationship in *M. sibogae*

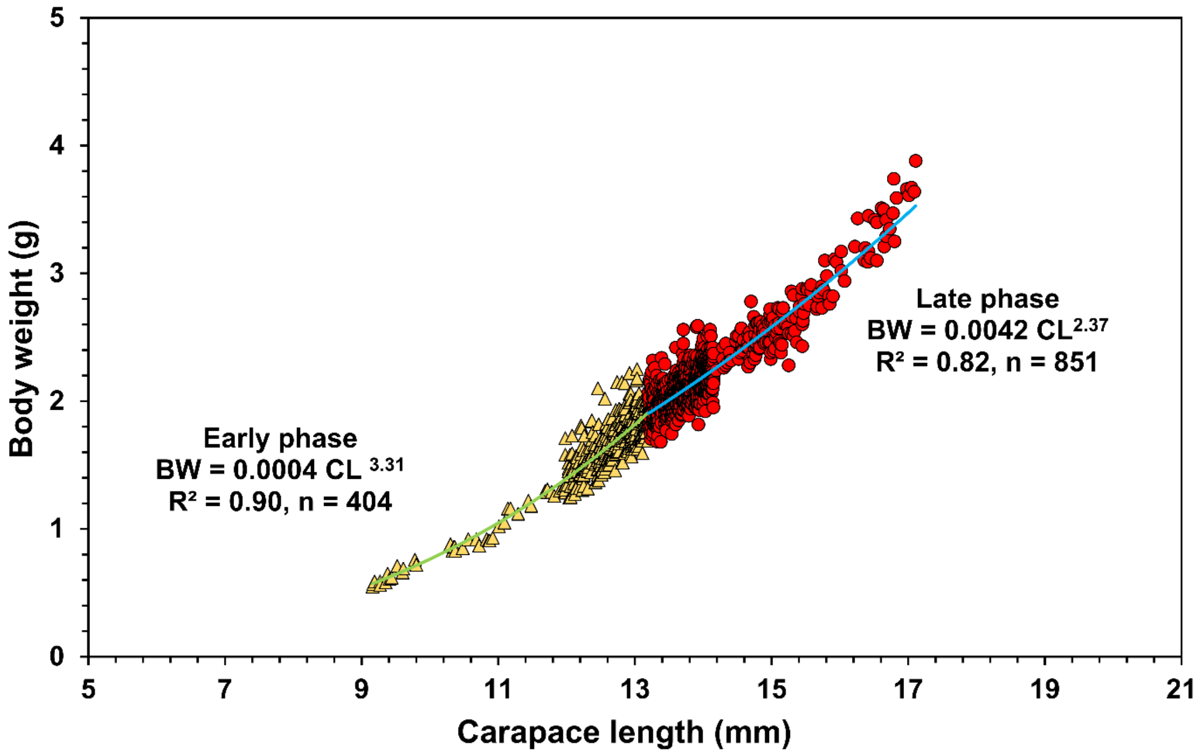


Figure 4.2.5. Relationship of body weight (BW) with carapace length (CL), of male *Metapenaeopsis sibogae*, as estimated by the least squares regression with untransformed data.

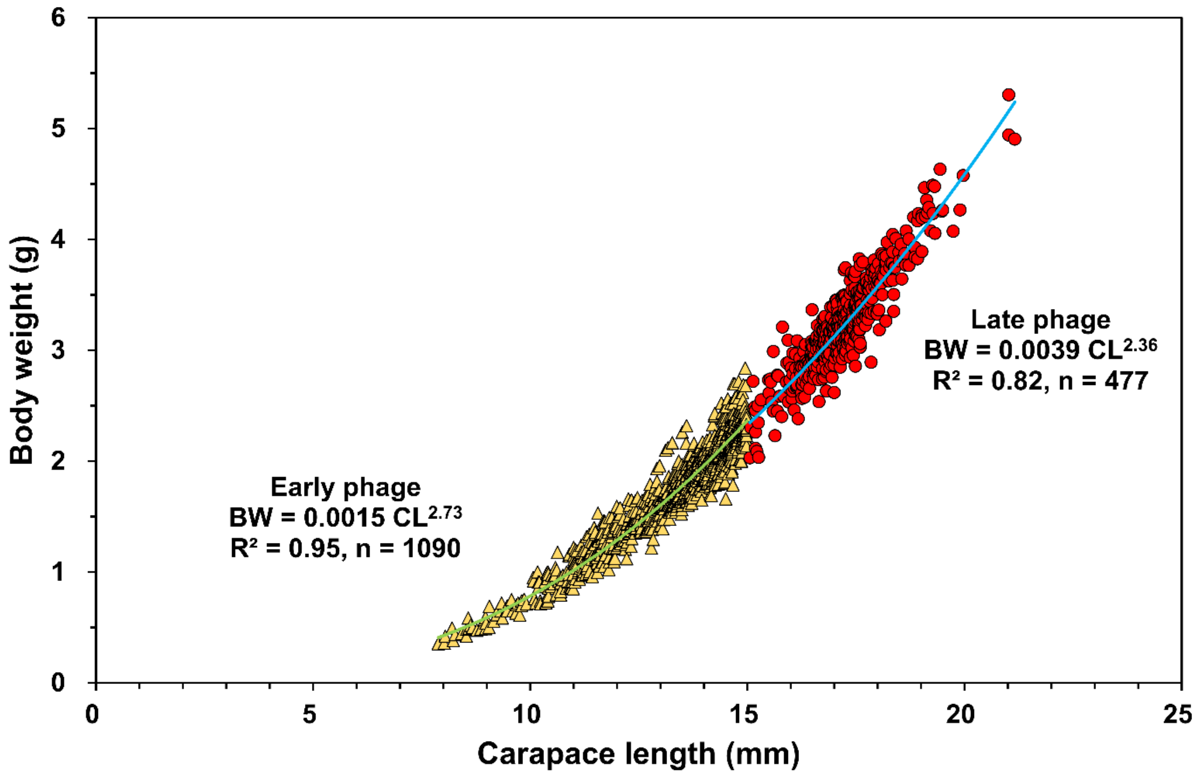


Figure 4.2.6. Relationship of body weight (BW) with carapace length (CL) of female *Metapenaeopsis sibogae*, as estimated by the least squares regression with untransformed data.

Table 4.2.3. Regression coefficients for the estimation of body length (BL) and body weight (BW) from carapace length (CL) for early and late phases of male and female *Metapenaeopsis sibogae* (De Man, 1907) (by least square estimate, with log-transformed data). Results of Student's t-test, using H_0: slope = 1 in the case of BL-CL relationship or H_0: slope = 3 in the case of BW-CL relationship. *N*, sample size; r^2, coefficient of determination; *P*, probability; 0, isometry; –, negative allometry; +, positive allometry.

Relationship	Sex, phase	*N*	Intercept	Slope	r^2	Allometry	*P*
BL vs. CL	Male, early phase	404	4.47	1.01	0.89	0	0.213
	Male, late phase	851	7.41	0.82	0.80	–	<0.001
	Female, early phase	1090	5.62	0.90	0.95	–	<0.001
	Female, late phase	477	8.13	0.76	0.80	–	<0.001
BW vs. CL	Male, early phase	404	0.0004	3.31	0.90	+	<0.001
	Male, late phase	851	0.0042	2.37	0.82	–	<0.001
	Female, early phase	1090	0.0015	2.73	0.95	–	<0.001
	Female, late phase	477	0.0039	2.36	0.82	–	<0.001

thus appears to reflect the changing biological requirements of individuals that reach sexual maturity, as described by Diaz *et al.* (2001) for the penaeid shrimp *Penaeus duorarum* Burkenroad, 1939. The transition points in the BL vs. CL relationships (13.2 mm CL in males and 15.0 mm CL in females) therefore possibly indicating sexual maturation, because abrupt shifts in allometry are usually related with sexual maturation, as reported for many types of crustaceans (Hartnoll, 1982; Lovett & Felder, 1989; Dalabona *et al.*, 2005; Pantaleão *et al.*, 2012; Ahamed & Ohtomi, 2014; Pescinelli *et al.*, 2014, 2015), including the penaeids (Diaz *et al.*, 2001). Rahman & Ohtomi (2017) estimated the CL at 50% sexual maturity of female *M. sibogae* verified by histological analysis to be 14.3 mm, which approximates the findings of this study. There is, however, no study on the functional sexual maturity size in male *M. sibogae*, limiting the validation of morphological sexual maturity size. Though allometry analysis is an easily applicable field method, additional histological studies on testis maturation in *M. sibogae* are recommended to verify the morphological sexual maturity size.

According to Watters & Hobday (1998), it is imperative to know the size at sexual maturity of the target species for successful management practices, and this value should be regularly re-evaluated. Defining mature individuals through histological observation of gonads and subsequent estimation of size at sexual maturity is a widely used and reliable method in penaeoid shrimp reproductive studies (eg. Ohtomi *et al.*, 1998, 2003; Chen *et al.*, 2014; Farhana & Ohtomi, 2016; Rahman & Ohtomi, 2017). This procedure, however, is expensive and time consuming. It is therefore highly convenient for regular monitoring of size at sexual maturity of a species through observing critical morphological changes by analyzing relative growth once validated by histological observation. In the studied population of *M. sibogae*, females reached morphological sexual maturity at larger sizes when compared with males, perhaps owing to the size difference between sexes, females are predominantly larger than males in many penaeid shrimps, as reported for *T. curvirostris* (Hossain & Ohtomi, 2010) and *P. fissuroides* (Farhana & Ohtomi 2017).

The growth rate of BL in relation to CL in females was significantly lower than that for males in both life phases. Segura & Delgado (2012) also reported significantly lower growth rate of total length (TL) to CL in females than males for both juvenile and adult *Pleoticus muelleri* (Spence Bate, 1888). In the case of *P. duorarum*, both Kutkuhn (1966) and McCoy (1972) reported slower growth rate of TL to CL in females than males. This phenomenon, however, remains mostly undiscussed. On the other hand, Diaz *et al.* (2001) reported no sex differences in the TL vs. CL relationship of *P. duorarum* by life stage, suggesting that sampling constraints might lead to such an outcome. The ovary of penaeid shrimp is comparatively larger than the testis at the same developmental stage. As the cephalothorax contains most portions of testes and ovaries in penaeid shrimps (Hartnoll, 1982; Solis, 1988), it was thereby hypothesized that the slower growth rate of BL to CL in females could be the natural phenomena of faster growth of carapace in relation to BL in females than males to accommodate the relatively larger ovary.

The life-stage-specific analysis for the BW vs. CL relationship provides further insights into the differential growth in *M. sibogae*. The growth rate of BW in relation to CL slowed significantly after morphological sexual maturity was reached in both sexes, demonstrating the existence of dimorphic growth. The exact reason for this decrease in growth rate in the late phases is unknown, but it is often hypothesized that it results from subsidized energy allocation for somatic growth, because aquatic animals must optimize the use of resources (energy) to grow or reproduce, or perform some combination of these demands (Heino & Kaitala, 1999). Whereas energy is supposed to be allocated entirely for growth in early-phased individuals, it has been hypothesized to be divided for reproduction and growth in late-phased individuals, causing reproduction to impede somatic growth (Paulraj *et al.*, 1982; Taylor & Gabriel, 1992;

Pescinelli *et al.*, 2015). Furthermore, in early-phased individuals, the intercept and slope of the log-transformed BW vs. CL model differed between sexes, whereas only the intercept differed between late-phased male and female individuals. These results also suggest that although sex-specific disparities in the BW vs. CL relationship occur during the early phase, these differences only become apparent after the onset of morphological sexual maturity, consistent with the findings of Diaz *et al.* (2001) for *P. duorarum*.

The present study provided for the first time, fairly subtle biological aspects of *M. sibogae* allometry, revealing the existence of dimorphic growth, which is possibly associated with sexual maturity. Abrupt shifts in allometry in association with sexual maturation have been reported for many types of crustaceans, and results of the present study indicate that this phenomenon extends to *Metapenaeopsis* shrimps. The present study will facilitate comparative studies between populations of *M. sibogae* and will be helpful in distinguishing closely related species. Furthermore, the findings will be the baseline for regular re-evaluation of the size at sexual maturity of *M. sibogae* for vigorous monitoring of the stock to aid its sustainable management in the future.

4.3: RELATIVE GROWTH AND MORPHOLOGICAL SEXUAL MATURITY OF *METAPENAEOPSIS KYUSHUENSIS*

4.3.1. INTRODUCTION

M. kyushuensis is one of the dominant species in the benthic community of Kagoshima Bay (Rahman & Ohtomi, unpublished). This species is morphologically similar to two other *Metapenaeopsis* species, *M. sibogae* and *M. provocatoria owstoni*, inhabiting the bay. These three species can be separated via the shape and size of petasma in males and thelycum in females. Relative growth studies of these *Metapenaeopsis* shrimps are thus significant (Dall *et al.*, 1990), and such studies of *M. kyushuensis* would be extremely helpful to identify intraspecific and interspecific variations and to estimate the morphological sexual maturity to aid the regular monitoring of the stock. The present study, therefore, aims to estimate the relative growth of *M. kyushuensis* to facilitate comparative studies and to explain the size at sexual maturity based on critical morphological changes.

4.3.2. MATERIALS AND METHODS

4.3.2.1. *Sampling*

Monthly samples of *M. kyushuensis* were obtained from Kagoshima Bay, southern Japan (31°25' N, 130°38' E) at depths of 125–230 m (Figure 4.3.1) using the training vessel Nansei-Maru (175 t) of the Faculty of Fisheries, Kagoshima University, from January 2016 to December 2017. A simple trawl net (LC-VI; Nichimo Corp., Tokyo, Japan), 23.5 m long, 37.9 mm mesh size in the body, 20.2 mm in the cod-end (Ohtomi *et al.*, 2004), carrying canvas kites on the tips of the wings was used for sampling. The net was towed for a preset tow duration of 10 min at a speed of 2 knots. *M. kyushuensis* was sorted out of each haul, counted and placed immediately in ice on-board and then preserved in formalin on arrival at the laboratory.

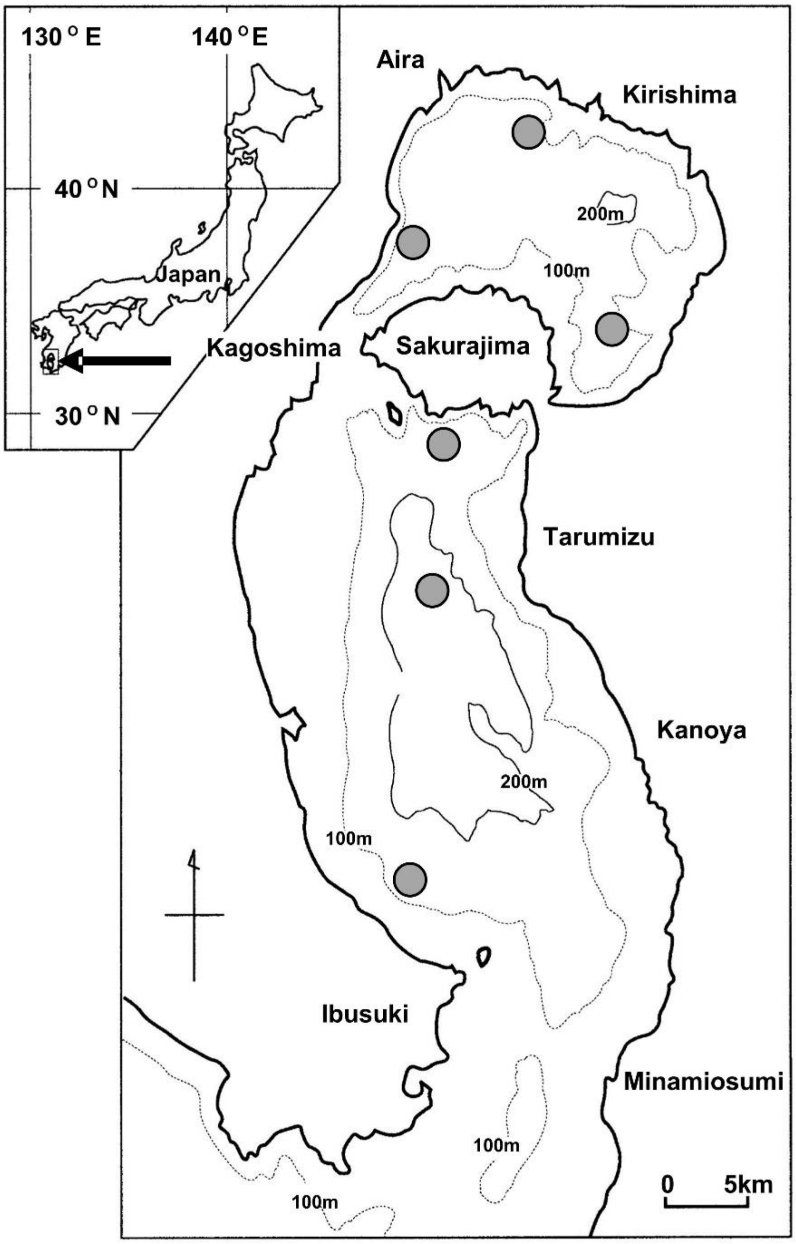


Figure 4.3.1. Kagoshima Bay southern Japan, with sampling sites for collection of *Metapenaeopsis kyushuensis* in the bay (shaded areas).

4.3.2.2. *Measurements*

All specimens were sexed according to the presence of a petasma in males or a thelycum in females. Due to the difficulty of distinguishing the males of *M. kyushuensis* from the males of *M. provocatoria owstoni*, only female individuals were used in the present study. For each individual, carapace length (CL), the distance between the posterior margin of the eyestalk to the dorsal midline of the posterior margin of the carapace and body length (BL), the distance from the posterior margin of the eyestalk to the tip of the telson were measured to the nearest 0.01 mm with a digital slide caliper. Individual wet body weight (BW) was measured to the nearest 0.01 g by an electronic balance.

4.3.2.3. *Relative growth and determination of morphological sexual maturity*

Changes in the growth patterns of BL and BW in relation to the independent variable (CL) were verified by relative growth analysis using least squares regression. The morphological sexual maturity was estimated from the relationship of BL vs. CL. These two parameters were chosen as in penaeid shrimps, the ovaries are contained in the dorsal side of the cephalothorax and abdomen whereas testes are contained within the cephalothorax and any change in the gonads during maturation is likely to have an impact on the relationship between these two parameters (Rahman & Ohtomi, 2018b). The BL vs. CL relationship was obtained based on the linear function described by Huxley (1950): $BL = a + bCL$, using untransformed data, where, BL is the dependent variable, CL is the independent variable, a and b are the regression coefficients. Data were repeatedly partitioned into two size-delimited subsets based on a hypothesized transition point—(I) early phase: individuals with a CL < a hypothesized transition point; and (II) late phase: individuals with a CL ≥ a hypothesized transition point. The hypothesized transition point was sequentially relocated at 0.1 mm intervals of CL throughout the dataset. A separate regression function was calculated for each of the two subsets after each relocation of the hypothesized transition point. The transition point indicating morphological sexual maturity was selected based on the lowest combined sum of squared residuals (RSS) in the resulting two subsets of data (Somerton, 1980; Lovett & Felder, 1989; Ohtomi *et al.*, 2005; Ahamed & Ohtomi, 2014; Rahman & Ohtomi, 2018b). Once the transition point was established, the relative growth of BW in relation to CL for both phases was calculated based on the nonlinear power function $BW = aCL^b$, using untransformed data, where, BW is the dependent variable, CL is the independent variable, *a* and *b* are the regression coefficients. To define the growth type, further regression was performed for all cases using log-transformed data. Values of the allometric constant (b) were examined by Student's t-test (Sokal & Rohlf, 1987), using H_0: b = 1 in the case of BL-CL relationship (b = 1: isometry; b < 1: negative allometry; b > 1: positive allometry) or H_0: b = 3 in the case of BW vs. CL relationship (b = 3: isometry; b < 3: negative allometry; b > 3: positive allometry) (Hartnoll, 1982). Analysis of covariance (ANCOVA) was used to analyze the differences of the slopes and intercepts between the regression lines (Zar, 1996). Microsoft Excel 2013, PAST 3 and SYSTAT V13.2 software packages were used to conduct the statistical analyses. All statistical analyses were considered significant at a confidence interval of 95%.

4.3.3. RESULTS

A total of 1999 females of *M. kyushuensis* having CL varied between 7.2 and 19.7 mm and BW varied from 0.29 to 4.62 g, were used in the present study. The relative growth of BL to CL in female *M. kyushuensis* using early and late phases, separated by a specific CL value as a hypothesized transition point, and the RSS values of the regression analyses are shown in Table 4.3.1. Comparisons of RSS indicated that two separate linear BL vs. CL models, one for each life phase (Figure 4.3.2), fit the data better than a single linear model that was applied over the entire CL range for both sexes (Table 4.3.1). Based on the lowest value of the total RSS, clear transition points were noted. The transition point in the BL vs. CL relationship

Table 4.3.1. Estimated slope and intercept of the linear regression representing the relationship between carapace length (CL, mm) and body length (BL, mm) in female *Metapenaeopsis kyushuensis* and residual sum of square (RSS) in different sets of two phages. *N*, number of individuals. The CL value at which the lowest total RSS was found is marked in bold format.

Relationship	Breakpoint (CL, mm)	Early phase				Late phase				Total RSS
		Slope	Intercept	*N*	RSS	Slope	Intercept	*N*	RSS	
BL vs. CL	8.5	3.26	8.38	13	16.47	3.56	9.80	1986	3480.38	3496.85
	9.0	4.09	2.08	28	50.42	3.54	10.15	1971	3318.02	3368.44
	9.5	5.33	-7.95	50	121.72	3.53	10.32	1949	3235.64	3357.36
	10.0	5.14	-6.37	71	146.52	3.51	10.52	1928	3177.05	3323.57
	10.1	5.22	-7.06	78	159.82	3.51	10.52	1921	3164.49	3324.31
	10.3	5.13	-6.26	90	186.87	3.51	10.58	1909	3133.27	3320.14
	10.5	4.84	-3.76	120	253.15	3.49	10.80	1879	3048.34	3301.49
	10.7	4.70	-2.49	144	273.82	3.48	10.98	1855	3010.07	3283.89
	11.0	4.49	-0.60	179	330.29	3.46	11.28	1820	2919.13	3249.42
	11.2	4.52	-0.83	217	371.53	3.46	11.38	1782	2874.22	3245.75
	11.4	4.48	2.72	252	423.25	3.45	11.49	1747	2817.99	3241.24
	11.6	4.39	0.43	293	499.42	3.43	11.74	1706	2727.34	3226.76
	11.8	4.32	1.09	342	565.99	3.42	11.99	1657	2646.92	3212.91
	12.0	4.20	2.24	403	698.13	3.39	12.49	1596	2477.66	3175.79
	12.1	4.19	2.26	423	728.33	3.38	12.58	1576	2442.40	3170.73
	12.2	4.23	1.98	455	761.68	3.38	12.59	1544	2411.76	3173.44
	12.3	4.22	2.06	484	791.63	3.38	12.69	1515	2378.14	3169.77
	12.4	4.21	2.18	515	834.59	3.37	12.81	1484	2330.61	3165.20
	12.5	4.17	2.55	556	918.64	3.35	13.09	1443	2234.97	3153.61
	12.6	4.16	2.72	577	930.70	3.34	13.24	1422	2216.59	3147.29
	12.7	4.14	2.85	601	950.57	3.33	13.38	1398	2191.14	3141.71
	12.8	4.14	2.92	624	972.86	3.33	13.47	1375	2166.33	3139.19
	12.9	4.11	3.20	658	1006.20	3.31	13.72	1341	2123.91	3130.11
	13.0	**3.51**	**4.08**	**683**	**1037.85**	**3.30**	**13.99**	**1316**	**2081.29**	**3119.14**
	13.1	4.09	3.40	710	1062.71	3.30	13.99	1289	2057.95	3120.66
	13.2	4.10	3.27	743	1119.90	3.30	13.95	1256	2002.45	3122.35
	13.3	4.10	3.32	776	1165.19	3.30	14.00	1223	1957.35	3122.54
	13.4	4.11	3.24	813	1244.62	3.30	13.92	1186	1876.94	3121.56
	13.5	4.09	3.38	843	1293.20	3.30	13.99	1156	1830.82	3124.02
	13.6	4.08	3.57	878	1319.67	3.29	14.11	1121	1807.62	3127.29
	13.7	4.06	3.73	908	1351.14	3.28	14.23	1091	1779.42	3130.56
	13.8	4.03	4.06	942	1415.69	3.26	14.57	1057	1721.07	3136.76
	13.9	4.02	4.18	972	1466.99	3.26	14.67	1027	1671.58	3138.57
	14.0	3.99	4.45	998	1556.09	3.24	15.01	1001	1587.35	3143.44
	14.1	4.01	4.34	1024	1623.47	3.25	14.78	974	1515.82	3139.29
	14.2	4.01	4.31	1059	1656.46	3.26	14.55	940	1478.70	3135.16
	14.3	4.00	4.36	1088	1692.80	3.27	14.42	911	1441.53	3134.33
	14.4	4.00	4.44	1112	1720.99	3.27	14.35	887	1414.40	3135.39
	14.5	3.97	4.73	1146	1790.74	3.26	14.60	853	1364.05	3154.79
	14.6	3.96	4.85	1179	1873.83	3.26	14.52	820	1283.87	3157.70
	14.7	3.95	4.95	1209	1916.46	3.27	14.40	790	1244.54	3161.00
	14.8	3.94	5.13	1242	1965.02	3.27	14.42	757	1207.33	3172.35
	14.9	3.92	5.31	1283	2036.79	3.28	14.27	716	1144.27	3181.06
	15.0	3.91	5.47	1310	2088.23	3.27	14.36	689	1106.58	3194.81
	15.5	3.87	6.06	1449	2289.43	3.35	12.89	550	937.94	3227.37
	16.0	3.77	7.05	1568	2654.17	3.25	14.88	431	709.10	3363.27
	16.5	3.73	7.55	1658	2907.37	3.18	16.03	341	510.73	3418.10
Single line using all data		3.59	9.39	1999	3675.18	—	—	—	—	3675.18

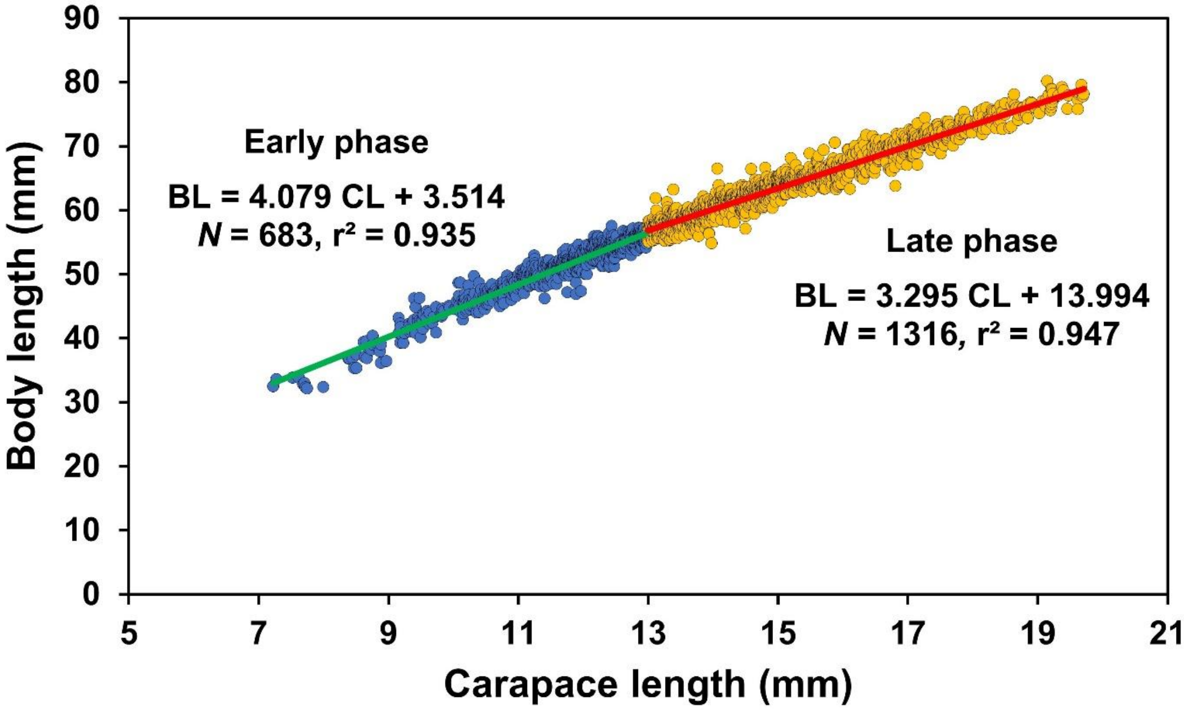


Figure 4.3.2. Relationship of body length (BL) with carapace length (CL), as estimated by the least squares regression with untransformed data of female *Metapenaeopsis kyushuensis*.

possibly reflecting morphological sexual maturity was estimated to be 13.0 mm CL. A significant decrease in the slope and a corresponding increase in the intercept (ANCOVA, $P < 0.001$) for late-phased females (CL ≥ 13.0 mm) compared with early-phased females (CL < 13.0 mm). BL showed negative allometric growth with CL in both early-phased (Student's t-test, $P < 0.001$) and late-phased females (Student's t-test, $P < 0.001$), but in late-phased males, the growth rate slowed significantly (ANCOVA, $P < 0.001$; Table 4.3.2).

The relative growth rate of BW to CL for early-phased females varied significantly versus that of late-phased females (ANCOVA, $P < 0.001$). In early-phased females, negative allometric growth was found between BW and CL (Student's t-test, $P < 0.01$). Negative allometric growth (Student's t-test, $P < 0.001$) was also reported for late-phased females but the rate declined abruptly (ANCOVA, $P < 0.001$; Figure 4.3.3, Table 4.3.2).

4.3.4. DISCUSSION

The present study of the relationship between BL and CL revealed the existence of dimorphic growth in female *M. kyushuensis*. Analysis of RSS showed clear transition points, favoring two separate linear BL vs. CL models that fit the data better than a single linear model that was applied over the entire CL range, indicating two life phases (Somerton, 1980; Rahman & Ohtomi, 2018b). A change in allometry between early- and late-phased females was indicated by a 19.2% decrease in the slope of the BL vs. CL relationship (Figure 4.3.2). Similar results were observed in females of *M. sibogae*, where a change in allometry between early- and late-phased individuals was reflected by a 21.5% decrease in the slope of the BL vs. CL relationship (Rahman & Ohtomi, 2018b).

The abrupt reduction in the slope of the BL vs. CL relationship indicates that the carapace grows faster relative to the abdomen in late-phased individuals than that in the early-phased individuals, because BL is essentially the sum of CL and the abdomen length (Diaz *et*

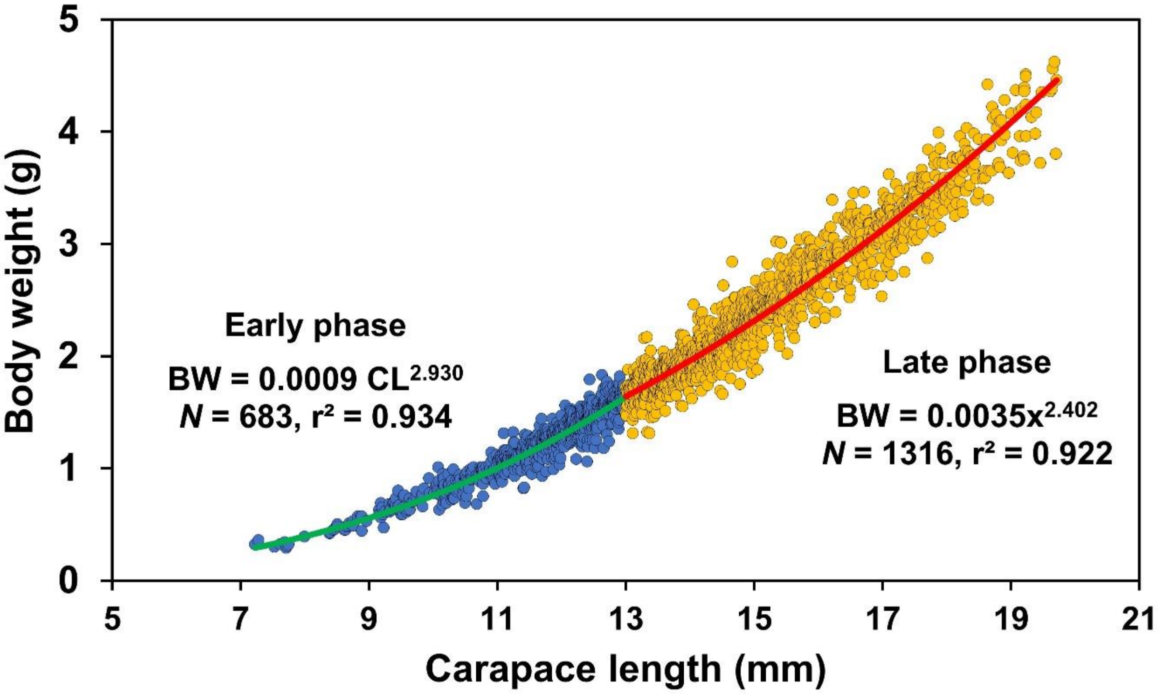


Figure 4.3.3. Relationship of body weight (BW) with carapace length (CL) of female *Metapenaeopsis kyushuensis*, as estimated by the least squares regression with untransformed data.

Table 4.3.2. Regression coefficients for the estimation of body length (BL) and body weight (BW) from carapace length (CL) for early and late phases of female *Metapenaeopsis kyushuensis* (by least square estimate, with log-transformed data). Results of Student's t-test, using H_0: slope = 1 in the case of BL-CL relationship or H_0: slope = 3 in the case of BW-CL relationship. *N*, sample size; r^2, coefficient of determination; *P*, probability; 0, isometry; –, negative allometry; +, positive allometry.

Relationship	Sex, phase	*N*	Intercept	Slope	r^2	Allometry	*P*
BL vs. CL	Female, early phase	683	5.07	0.94	0.94	–	<0.01
	Female, late phase	1316	7.54	0.79	0.94	–	<0.001
BW vs. CL	Female, early phase	683	0.0009	2.93	0.93	–	<0.01
	Female, late phase	1316	0.0035	2.40	0.92	–	<0.001

al., 2001; Rahman & Ohtomi, 2018b). The cephalothorax in penaeid shrimps houses most internal organs, including major portions of the ovaries of fully mature females (Solis, 1988) and the whole testes, whereas, the abdomen only contains a minor portion of the ovaries (Diaz *et al.*, 2001; Rahman & Ohtomi, 2018b). The shift in the BL vs. CL relationship in female *M. kyushuensis* thus reflects the changing biological requirements of individuals that reach sexual maturity, as described by Rahman & Ohtomi (2018b) for *M. sibogae* and by Diaz *et al.* (2001) for the penaeid shrimp *P. duorarum*. The transition points in the BL vs. CL relationships (13.0 mm CL) therefore possibly indicating sexual maturation, because abrupt shifts in allometry are usually related with sexual maturation, as reported for many types of crustaceans (Hartnoll, 1982; Lovett & Felder, 1989; Dalabona *et al.*, 2005; Pantaleão *et al.*, 2012; Ahamed & Ohtomi, 2014; Pescinelli *et al.*, 2014, 2015), including the penaeids (Diaz *et al.*, 2001; Rahman & Ohtomi, 2018b, 2018c). The CL at 50% sexual maturity of female *M. kyushuensis* was estimated to be 13.7 mm (see Chapter 2, Section 2.3), which approximates the findings of this study further validates the hypothesis. Defining mature individuals through histological observation of gonads and subsequent estimation of size at sexual maturity the most accurate method but is expensive and time consuming (Rahman & Ohtomi, 2018b). It is therefore highly convenient for regular monitoring of size at sexual maturity of a species through observing critical morphological changes by analyzing relative growth once validated by histological observation.

The life-stage-specific analysis for the BW vs. CL relationship provides further insights into the differential growth in female *M. kyushuensis*. The growth rate of BW in relation to CL slowed significantly after morphological sexual maturity was reached, demonstrating the existence of dimorphic growth. The exact reason for this decrease in growth rate in the late-phased females is unknown, but it is often hypothesized that it results from subsidized energy allocation for somatic growth, because aquatic animals must optimize the use of resources (energy) to grow or reproduce or perform some combination of these demands (Heino & Kaitala, 1999; Rahman & Ohtomi, 2018b, 2018c). While energy is supposed to be allocated entirely for growth in early-phased individuals, it has been hypothesized to be divided for reproduction and growth in late-phased individuals, causing reproduction to impede somatic growth (Paulraj *et al.*, 1982; Taylor & Gabriel, 1992; Pescinelli *et al.*, 2015; Rahman & Ohtomi, 2018b, 2018c).

The present study provided for the first time information on the allometry of female *M. kyushuensis*, revealing the existence of dimorphic growth, which is possibly associated with sexual maturity. Abrupt shifts in allometry in association with sexual maturation have been reported for many types of crustaceans, and results of the present study indicate that this phenomenon extends to *Metapenaeopsis* shrimps. The present study will facilitate comparative studies between populations of *M. kyushuensis* and will be helpful in distinguishing closely related species including that of *M. sibogae* and *M. provocatoria owstoni*. Future research on the identification of male *M. kyushuensis* through morphological analysis and supported by genetic markers is highly recommended.

4.4: RELATIVE GROWTH AND MORPHOLOGICAL SEXUAL MATURITY OF *METAPENAEOPSIS PROVOCATORIA OWSTONI*

4.4.1. INTRODUCTION

M. provocatoria owstoni is one of the dominant *Metapenaeopsis* species in the benthic community of Kagoshima Bay (Rahman & Ohtomi, unpublished). This species is morphologically similar to the two other species of *Metapenaeopsis* inhabiting the bay, *M. kyushuensis* and *M. sibogae*. In many decapod crustaceans, the onset of sexual maturity is characterized by critical morphological changes (Hartnoll, 1982; Lovett & Felder, 1989; Petriella & Boschi, 1997; Sampedro *et al.*, 1999), some of which can be detected by analyzing relative growth (Haley, 1969). In shrimps, the use of relative growth to estimate morphological sexual maturity is, however, rare and mostly restricted to the caridean shrimps (Ahamed & Ohtomi, 2014; Rahman & Ohtomi, 2018b). Taxonomic groupings of the genus *Metapenaeopsis* have been based primarily on morphological and ecological properties (see Crosnier 1987, 2007), and thus making the relative growth studies of *Metapenaeopsis* shrimps significant. The present study, thus, aims to explain the relative growth of *M. provocatoria owstoni* to facilitate comparative studies and to explain the size at sexual maturity based on critical morphological changes.

4.4.2. MATERIALS AND METHODS

4.4.2.1. *Sampling*

Monthly samples of *M. provocatoria owstoni* were obtained from Kagoshima Bay, southern Japan (31°25' N, 130°38' E) at depths of ~80–230 m (Figure 4.4.1) using the training vessel Nansei-Maru (175 t) of the Faculty of Fisheries, Kagoshima University, from January 2013 to December 2017. A simple trawl net (LC-VI; Nichimo Corp., Tokyo, Japan), 23.5 m

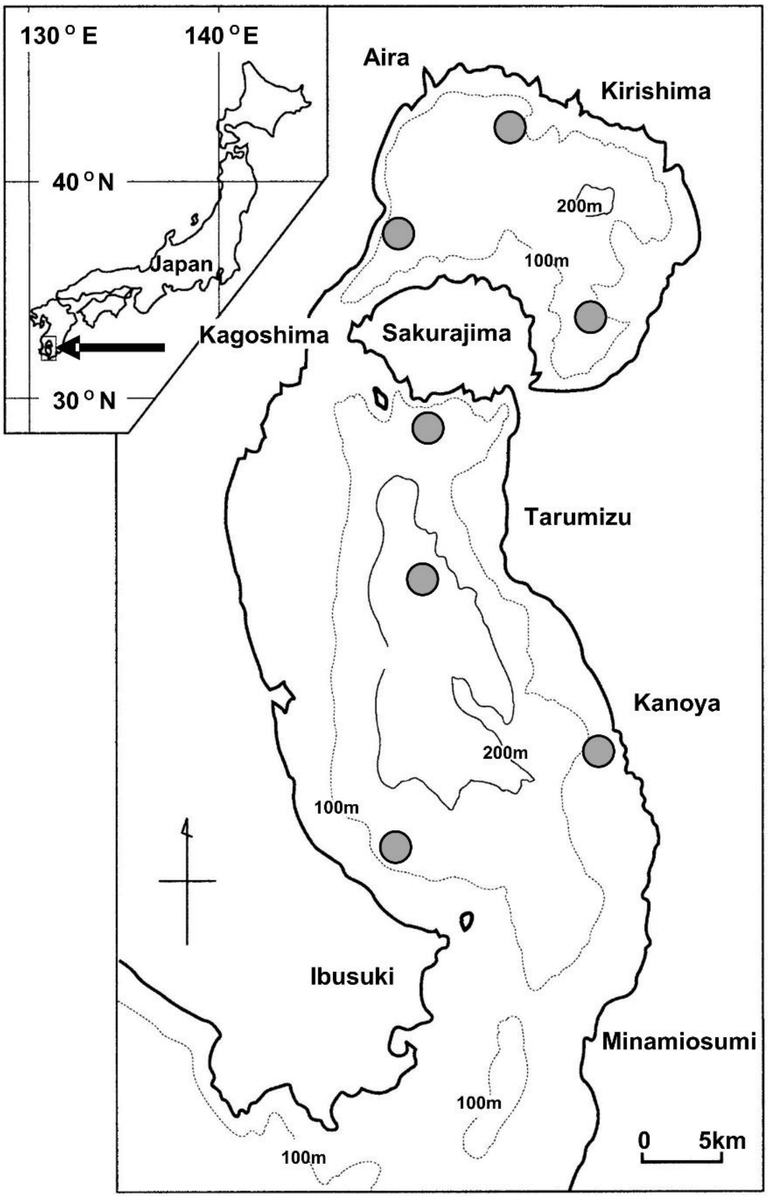


Figure 4.4.1. Kagoshima Bay southern Japan, with sampling sites for collection of *Metapenaeopsis provocatoria owstoni* in the bay (shaded areas).

long, 37.9 mm mesh size in the body, 20.2 mm in the cod-end (Ohtomi *et al.*, 2004), carrying canvas kites on the tips of the wings was used for sampling. The net was towed for a pre-set tow duration of 10 min at a speed of 2 knots. *M. provocatoria owstoni* was sorted out of each haul, counted and placed immediately in ice on-board and then preserved in formalin on arrival at the laboratory.

4.4.2.2. *Measurements*

All specimens were sexed according to the presence of a petasma in males or a thelycum in females. Due to the difficulty of distinguishing the males of *M. provocatoria owstoni* from the males of *M. kyushuensis*, only female individuals were used in the present study. For each individual, carapace length (CL), the distance between the posterior margin of the eyestalk to the dorsal midline of the posterior margin of the carapace and body length (BL), the distance from the posterior margin of the eyestalk to the tip of the telson were measured to the nearest 0.01 mm with a digital slide caliper. Individual wet body weight (BW) was measured to the nearest 0.01 g by an electronic balance.

4.4.2.3. *Relative growth and determination of morphological sexual maturity*

The growth patterns of BL and BW in relation to the independent variable (CL) were studied by relative growth analysis using least squares regression. The morphological sexual maturity was estimated from the relationship of BL vs. CL. These two parameters were chosen as in penaeid shrimps, the ovaries are contained in the dorsal side of the cephalothorax and abdomen whereas testes are contained within the cephalothorax and any change in the gonads during maturation is likely to have an impact on the relationship between these two parameters (Rahman & Ohtomi, 2018b). The BL vs. CL relationship was obtained based on the linear function described by Huxley (1950): $BL = a + bCL$, using untransformed data, where, BL is the dependent variable, CL is the independent variable, a and b are the regression coefficients. Data were repeatedly partitioned into two size-delimited subsets based on a hypothesized transition point—(I) early phase: individuals with a CL < a hypothesized transition point; and (II) late phase: individuals with a CL ≥ a hypothesized transition point. The hypothesized transition point was sequentially relocated at 0.1 mm intervals of CL throughout the dataset. A separate regression function was calculated for each of the two subsets after each relocation of the hypothesized transition point. The transition point indicating morphological sexual maturity was selected based on the lowest combined sum of squared residuals (RSS) in the resulting two subsets of data (Somerton, 1980; Lovett & Felder, 1989; Ohtomi *et al.*, 2005; Ahamed & Ohtomi, 2014; Rahman & Ohtomi, 2018b). Once the transition point was established, the relative growth of BW in relation to CL for both phases was calculated based on the nonlinear power function $BW = aCL^b$, using untransformed data, where, BW is the dependent variable, CL is the independent variable, a and b are the regression coefficients. To define the growth type, further regression was performed for all cases using log-transformed data. Values of the allometric constant (b) were examined by Student's t-test (Sokal & Rohlf, 1987), using H_0: b = 1 in the case of BL-CL relationship (b = 1: isometry; b < 1: negative allometry; b > 1: positive allometry) or H_0: b = 3 in the case of BW vs. CL relationship (b = 3: isometry; b < 3: negative allometry; b > 3: positive allometry) (Hartnoll, 1982). Analysis of covariance (ANCOVA) was used to analyze the differences of the slopes and intercepts between the regression lines (Zar, 1996). Microsoft Excel 2013, PAST 3 and SYSTAT V13.2 software packages were used to conduct the statistical analyses. All statistical analyses were considered significant at a confidence interval of 95%.

4.4.3. RESULTS

A total of 640 females of *M. provocatoria owstoni* with CL ranging from 6.0 to 22.6 mm and BW varied from 0.18 to 6.70 g, were used in the present study. The relative growth of BL to CL in female *M. provocatoria owstoni* using early and late phases, separated by a specific

Table 4.4.1. Estimated slope and intercept of the linear regression representing the relationship between carapace length (CL, mm) and body length (BL, mm) in female *Metapenaeopsis provocatoria owstoni* and residual sum of square (RSS) in different sets of two phases. *N*, number of individuals. The CL value at which the lowest total RSS was found is marked in bold format.

Relationship	Breakpoint (CL, mm)	Early phase				Late phase				Total RSS
		Slope	Intercept	*N*	RSS	Slope	Intercept	*N*	RSS	
BL vs. CL	8.0	2.16	16.82	13	12.23	3.68	7.87	627	2077.55	2089.78
	8.5	3.19	9.49	33	44.45	3.63	8.66	607	1937.43	1981.88
	9.0	3.79	4.96	54	72.41	3.57	9.50	586	1813.57	1885.98
	9.5	4.12	2.31	74	114.73	3.53	10.30	566	1694.46	1809.19
	10.0	4.08	2.57	95	205.48	3.45	11.57	545	1442.83	1648.31
	10.5	4.31	0.66	111	244.81	3.41	12.16	529	1373.87	1618.68
	10.7	4.22	1.44	116	257.89	3.39	12.56	524	1317.31	1575.2
	11.0	4.37	0.14	133	281.95	3.36	13.09	507	1234.12	1516.07
	11.2	4.33	0.52	140	302.05	3.34	13.41	500	1202.03	1504.08
	11.5	4.42	-0.30	153	325.31	3.32	13.69	487	1177.44	1502.75
	11.7	4.45	-0.52	162	332.26	3.31	13.91	478	1164.47	1496.73
	12.0	4.44	-0.46	171	340.32	3.30	14.15	469	1147.74	1488.06
	12.1	4.39	-0.05	178	379.68	3.27	14.58	462	1086.92	1466.6
	12.3	4.39	-0.01	187	394.94	3.25	14.90	453	1056.38	1451.32
	12.5	4.41	-0.21	198	421.86	3.26	14.84	442	1029.77	1451.63
	12.7	4.44	-0.44	208	443.39	3.25	14.94	432	1018.37	1461.76
	12.8	4.42	-0.31	214	455.23	3.24	15.11	426	1003.51	1458.74
	12.9	4.41	-0.17	219	480.12	3.22	15.39	421	957.41	1437.53
	13.0	4.36	0.31	228	529.83	3.19	15.85	412	914.37	1444.2
	13.3	4.29	0.93	244	586.05	3.14	16.82	396	835.55	1421.6
	13.4	4.28	1.06	248	593.45	3.13	16.96	392	829.18	1422.63
	13.5	4.28	1.04	259	599.35	3.12	17.16	381	820.83	1420.18
	13.7	4.26	1.21	268	608.89	3.10	17.58	372	803.88	1412.77
	13.8	4.25	1.37	279	620.87	3.08	17.97	361	785.40	1406.27
	13.9	4.25	1.32	281	622.70	3.05	18.00	359	783.55	1406.25
	14.0	4.24	1.39	285	626.24	3.07	18.18	355	778.44	1404.68
	14.1	4.24	1.44	288	627.73	3.06	18.22	352	778.06	1405.79
	14.2	4.24	1.46	292	639.24	3.04	18.49	348	742.27	1381.51
	14.3	4.21	1.69	301	683.16	3.03	18.72	339	715.56	1398.72
	14.4	4.21	1.76	306	703.98	3.03	18.85	334	693.59	1397.57
	14.5	4.20	1.78	309	713.80	3.02	19.00	331	684.28	1398.08
	14.6	4.18	2.00	317	729.08	2.99	19.48	323	666.42	1395.5
	14.7	4.17	2.10	327	755.65	2.99	19.49	313	638.01	1393.66
	14.8	4.17	2.08	331	769.98	2.98	19.61	309	627.92	1397.9
	14.9	4.16	2.19	341	782.85	2.98	19.77	299	616.76	1399.61
	15.0	4.14	2.40	347	808.81	2.95	20.28	293	593.18	1401.99
	15.1	4.14	2.47	354	815.55	2.94	20.43	286	585.67	1401.22
	15.2	4.13	2.50	364	839.12	2.94	20.43	276	561.59	1400.71
	15.3	4.14	2.40	374	854.67	2.97	19.86	266	540.20	1394.87
	15.4	4.13	2.51	386	867.63	2.97	19.86	254	530.45	1398.08
	15.5	4.14	2.45	392	871.74	2.98	19.61	248	514.02	1385.75
	15.6	**4.14**	**2.42**	**399**	**898.82**	**3.01**	**19.14**	**241**	**479.78**	**1378.60**
	15.7	4.12	2.65	417	934.68	3.02	18.98	223	454.78	1389.46
	15.8	4.11	2.71	425	949.36	3.03	18.68	215	441.16	1390.52
	15.9	4.10	2.80	435	979.55	3.06	18.28	205	413.03	1392.58
	16.0	4.10	2.86	443	991.93	3.09	17.70	197	397.35	1389.28
	16.3	4.07	3.14	460	1058.89	3.11	17.19	180	354.38	1413.27
	16.5	4.04	3.55	476	1170.40	3.07	18.06	164	303.58	1473.98
	17.0	3.98	4.15	510	1295.08	3.11	17.19	130	245.19	1540.27
Single line using all data		3.71	7.43	640	2157.40	—	—	—	—	—

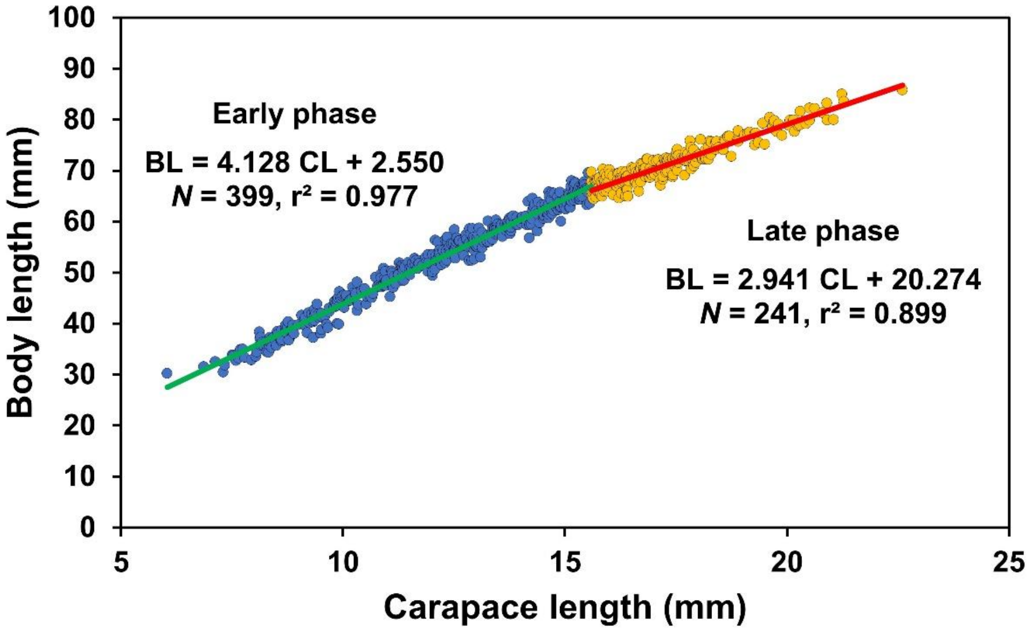


Figure 4.4.2. Relationship of body length (BL) with carapace length (CL), as estimated by the least squares regression with untransformed data of female *Metapenaeopsis provocatoria owstoni*.

CL value as a hypothesized transition point, and the RSS values of the regression analyses are shown in Table 4.4.1. Comparisons of RSS indicated that two separate linear BL vs. CL models, one for each life phase (Figure 4.4.2), fit the data better than a single linear model that was applied over the entire CL range for both sexes (Table 4.4.1). Based on the lowest value of the total RSS, clear transition points were noted. The transition point in the BL vs. CL relationship possibly reflecting morphological sexual maturity was estimated to be 15.6 mm CL. A significant decrease in the slope and a corresponding increase in the intercept (ANCOVA, $P < 0.001$) for late-phased females (CL ≥ 15.6 mm) compared with early-phased females (CL < 15.6 mm). BL showed negative allometric growth with CL in both early- phased (Student's t-test, $P < 0.01$) and late-phased females (Student's t-test, $P < 0.01$). However, in late-phased females, the growth rate significantly slowed down (ANCOVA, $P < 0.001$; Table 4.4.2).

The relative growth rate of BW to CL for early-phased females varied significantly versus that of late-phased females (ANCOVA, $P < 0.001$). In early-phased females, negative allometric growth was found between BW and CL (Student's t-test, $P < 0.01$). Negative allometric growth (Student's t-test, $P < 0.001$) was also reported for late-phased females but the rate declined drastically than that of the early-phased females ($P < 0.001$; Figure 4.4.3, Table 4.4.2).

4.4.4. DISCUSSION

The relationship between BL and CL revealed the existence of dimorphic growth in female *M. provocatoria owstoni* as was also reported for two other species of the genus, *M. kyushuensis* (see Chapter 4, section 4.3) and *M. sibogae* (Rahman & Ohtomi, 2018b). Clear transition points were detected in the analysis of RSS favoring two separate linear BL vs. CL models that fit the data better than a single linear model that was applied over the entire CL range, indicating two life phases (Somerton, 1980; Rahman & Ohtomi, 2018b). A change in allometry between early- and late-phased females was indicated by a 28.8% decrease in the slope of the BL vs. CL relationship (Figure 4.4.2). A change in allometry between early-and late-phased females was reflected by a 21.5% decrease in the slope of the BL vs. CL relationship in *M. sibogae* (Rahman & Ohtomi, 2018b) and by a 19.2% decrease in *M. kyushuensis* (see Chapter 4, Section 4.3).

The rapid reduction in the slope of the BL vs. CL relationship designates that the carapace grows faster corresponding to the abdomen in late-phased individuals than that in the early-phased individuals, because BL is essentially the sum of CL and the abdomen length (Diaz *et al.*, 2001; Rahman & Ohtomi, 2018b). As reported by Solis (1988), the cephalothorax in penaeid shrimps holds most internal organs, including major portions of the ovaries of fully mature females and the whole testes, whereas, the abdomen only contains a minor portion of the ovaries (Diaz *et al.*, 2001; Rahman & Ohtomi, 2018b). The change in the BL vs. CL relationship in female *M. provocatoria owstoni* thus reflects the changing biological requirements of individuals reaching sexual maturity, as described by Rahman & Ohtomi (2018b) for *M. sibogae* and by Diaz *et al.* (2001) for the penaeid shrimp *P. duorarum*. The transition points in the BL vs. CL relationships (15.6 mm CL) therefore possibly indicate sexual maturation (Hartnoll, 1982; Lovett & Felder, 1989; Ahamed & Ohtomi, 2014; Pescinelli *et al.*, 2014, 2015; Diaz *et al.*, 2001; Rahman & Ohtomi, 2018b, 2018c). The minimum size at sexual maturity of *M. provocatoria owstoni* as verified by histological observation was 12.8 mm CL (see Chapter 2, Section 2.4). The variation between the minimum size at maturity and the morphological sexual maturity is also found in *M. kyushuensis* (see Chapter 2, Section 2.3 and Chapter 4, Section 4.3) and *M. sibogae* (2017, 2018b). Rahman & Ohtomi (2018b) reported that the morphological sexual maturity size approximates the 50% size at sexual maturity as was also reported for *M. kyushuensis* (see Chapter 4, Section 4.3). There is no information available on the 50% size at sexual maturity of *M. provocatoria owstoni*. It is therefore

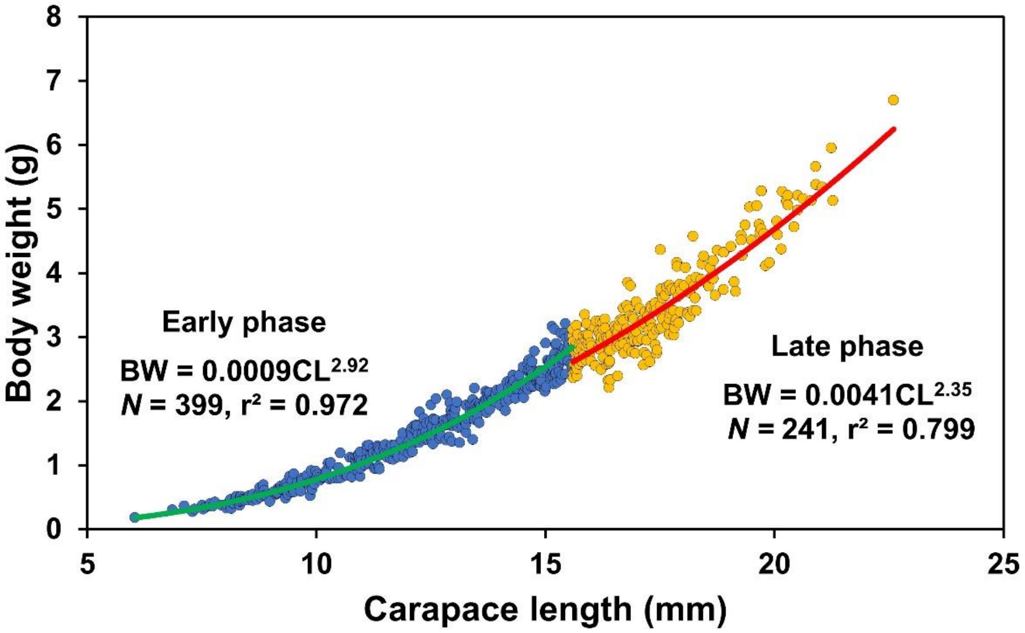


Figure 4.4.3. Relationship of body weight (BW) with carapace length (CL) of female *Metapenaeopsis provocatoria owstoni*, as estimated by the least squares regression with untransformed data.

Table 4.4.2. Regression coefficients for the estimation of body length (BL) and body weight (BW) from carapace length (CL) for early and late phases of female *Metapenaeopsis provocatoria owstoni* (by least square estimate, with log-transformed data). Results of Student's t-test, using H_0: slope = 1 in the case of BL-CL relationship or H_0: slope = 3 in the case of BW-CL relationship.

Relationship	Sex, phase	*N*	Intercept	Slope	r^2	Allometry	*P*
BL vs. CL	Female, early phase	399	4.87	0.95	0.98	–	<0.01
	Female, late phase	241	9.13	0.72	0.89	–	<0.001
BW vs. CL	Female, early phase	399	0.0009	2.92	0.97	–	<0.001
	Female, late phase	241	0.0041	2.35	0.799	–	<0.001

N, sample size; r^2, coefficient of determination; *P*, probability; 0, isometry; –, negative allometry; +, positive allometry.

hypothesized the morphological size at sexual maturity of female *M. kyushuensis* estimated in this study might approximate the 50% size at sexual maturity of the species. Defining mature individuals through histological observation of gonads and subsequent estimation of size at sexual maturity using logistic equation is the most accurate method but is expensive and time consuming (Rahman & Ohtomi, 2018b). It is therefore highly convenient for regular monitoring of size at sexual maturity of a species through observing critical morphological changes by analyzing relative growth once validated by histological observation.

The analysis of the BW vs. CL relationship provides further insights into the differential growth in female *M. provocatoria owstoni*. The growth rate of BW in relation to CL slowed significantly after morphological sexual maturity was reached, demonstrating the existence of dimorphic growth. The exact reason for this decrease in growth rate in the late-phased females is yet to be unraveled, but it is often hypothesized that it results from subsidized energy allocation for somatic growth, because aquatic animals must optimize the use of resources (energy) to grow or reproduce or perform some combination of these demands (Heino & Kaitala, 1999; Rahman & Ohtomi, 2018b, 2018c). While energy is supposed to be allocated entirely for growth in early-phased individuals, it has been hypothesized to be divided for reproduction and growth in late-phased individuals, causing reproduction to impede somatic growth (Paulraj *et al.*, 1982; Taylor & Gabriel, 1992; Pescinelli *et al.*, 2015; Rahman & Ohtomi, 2018b, 2018c).

The analysis of relative growth to figure out the critical morphological changes to estimate sexual maturity is widely used in many crustaceans including some caridean shrimps. In penaeid shrimps, however, this methodology is rarely used. The present study provided for the first time information on the allometry of female *M. provocatoria owstoni*, revealing the existence of dimorphic growth, which is possibly associated with sexual maturity. The findings of this study together with the findings of Rahman & Ohtomi (2018b, 2018c) clearly indicate that morphological sexual maturity could be detected through relative growth analysis in penaeid shrimps as well. Future research on the identification of male *M. provovatoria owstoni* through morphological analysis and supported by genetic markers, subsequent reproduction and growth studies are highly recommended.

CHAPTER 5: DISTRIBUTION PATTERNS AND POPULATION DYNAMICS OF *METAPENAEOPSIS* SPECIES IN KAGOSHIMA BAY, SOUTHERN JAPAN

5.1: BACKGROUND OF THE STUDY

Kagoshima Bay is a semi-enclosed deep-water bay with a maximum water depth of more than 230 m. The bay is uniquely characterized by the steep slopes on both sides and the bottom topography influenced by volcanic formation (Matumoto, 1943). Despite its unique structure, bottom topography, and influence of volcanic eruption from one of the most active volcanoes of the world, Sakurajima, Kagoshima Bay is rich in benthic biodiversity, inhabited by 124 species from 33 different families of decapod crustacean (Ohtomi *et al.*, 2008). Some of these species are rare and reported to inhabit only Kagoshima Bay among Japanese waters, such as *Alpheus kagoshimanus* Hayashi & Nagata, 2000, *A. longipalma* Komai & Ohtomi, 2018, *M. sibogae* and *Periclimenes thermohydrophilus* Hayashi & Ohtomi, 2001. *M. sibogae* was first recorded in Japanese waters from Kagoshima Bay in 2004 (Ohtomi & Nagata, 2004). This species was previously reported to inhabit only the deep waters of Indonesia, New Caledonia, and the Philippines (Crosnier, 1987, 2007; De Grave & Fransen, 2011). There are five more species of *Metapenaeopsis* inhabit the bay as well, of them two are morphologically similar with *M. sibogae*- *M. kyushuensis* and *M. provocatoria owstoni* (Ohtomi & Nagata, 2004). In just over a decade since being first recorded, *M. sibogae* has become one of the most dominant species in the benthic community of Kagoshima Bay and is gaining economic importance (Rahman & Ohtomi, unpublished). The adaptation and dominance of *M. sibogae* in the bay is remarkable, and its impact on other *Metapenaeopsis* species as well as on the other crustacean species needs to be evaluated.

Detail information on the population dynamics of a species is indispensable for supporting optimal exploitation and sustainable management regimes, which will illustrate the species recruitment patterns, nursery and spawning grounds, mortality, possible migratory behavior, and will aid to explain the seasonal distribution and abundance patterns (Ohtomi *et al.*, 2018). Such studies are essential for understanding the life histories of a species and would be helpful for comparative studies among species. There are, however, no studies available on the spatiotemporal distribution and population dynamics of any of the three mentioned *Metapenaeopsis* species, which are essential to support their optimal exploitation and sustainable management regimes.

5.2: DISTRIBUTION PATTERNS AND POPULATION DYNAMICS OF *METAPENAEOPSIS SIBOGAE*

5.2.1. INTRODUCTION

M. sibogae was first recorded in Japanese waters from Kagoshima Bay in 2004 (Ohtomi & Nagata, 2004). This species was previously reported to inhabit only the deep waters of Indonesia, New Caledonia, and the Philippines (Crosnier, 1987, 2007; De Grave & Fransen, 2011). There are two morphologically similar *Metapenaeopsis* species inhabit the bay as well- *M. kyushuensis* and *M. provocatoria owstoni* (Ohtomi & Nagata, 2004). In just over a decade since being first recorded, *M. sibogae* has become one of the most dominant species in the benthic community of Kagoshima Bay and is gaining economic importance (Rahman & Ohtomi, unpublished). The adaptation and dominance of *M. sibogae* in the bay is remarkable, and its impact on the two *Metapenaeopsis* species as well as on the other crustacean species needs to be evaluated for appropriate resource management. There is, however, no study available on the distribution patterns and population dynamics of this species, which is essential for comparing the life history among species. The present study therefore aimed to reveal the distribution patterns and population dynamics of *M. sibogae* for the first time using a large number of samples collected from Kagoshima Bay.

5.2.2. MATERIALS AND METHODS

5.2.2.1. *Sampling procedures and data analysis*

The present study was conducted in the semi-enclosed deep-water Kagoshima Bay, southern Japan (Figure 5.2.1). The bay was demarcated into four areas based on the oceanographic and bathymetric characteristics following Ohtomi *et al.* (2018): **(1) bay head**, a semi-circular enclosure running up to 140 m in depth; **(2) central basin**, with maximum

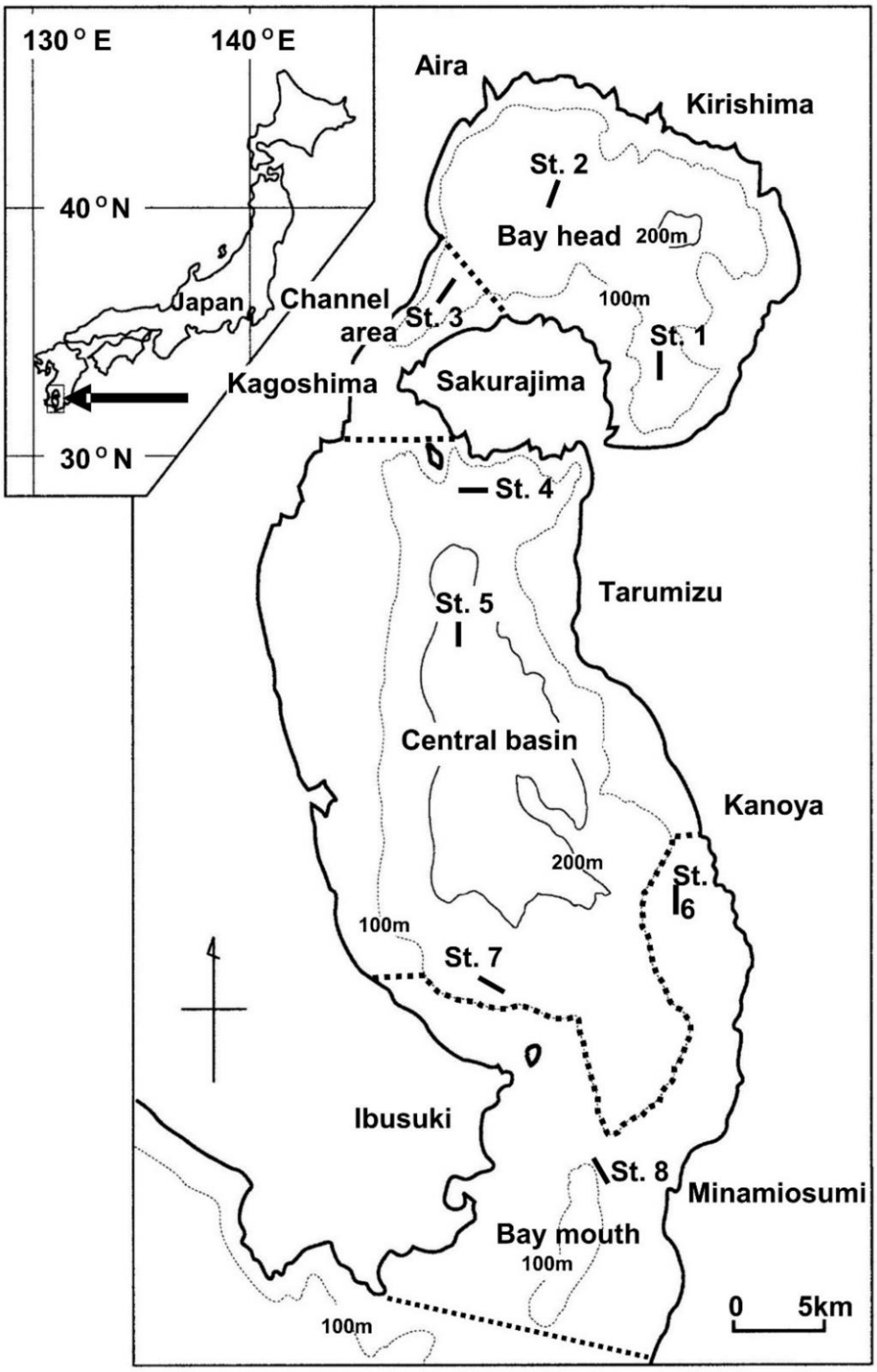


Figure 5.2.1. Map of Kagoshima Bay, southern Japan, showing the demarcation of the study area into the bay head, channel area, central basin, and bay mouth (shown by dotted lines) as well as the location of the eight sampling stations.

water depths reaching about 230 m; **(3) channel area**, connecting the bay head and central basin; and **(4) bay mouth**, opening to the Pacific and the East China Sea to the south and west, respectively. Eight sampling stations were established in these areas: Sts. 1 and 2 in the bay head, St. 3 in the channel area, Sts. 4, 5 and 7 in the central basin, and Sts. 6 and 8 in the bay mouth (Table 5.2.1). According to Ohtomi *et al.* (2018), the proportion of clay content was low and did not vary considerably among the stations. The majority of the stations, including Sts. 1, 2, 4, 5, and 7, were dominated by silty sediment. The proportion of silt and sand was almost equal at St. 3, while Sts. 6 and 8 were dominated by silty-sand sediment (Ohtomi *et al.*, 2018).

Samples of *M. sibogae* were collected by experimental trawl surveys conducted in the bay from February 2004 to October 2017. Monthly sampling was conducted in 3 or 4 stations while seasonal sampling (January, April, July, and October) was conducted from all 8 stations. In the present study, seasons were defined following Ohtomi *et al.* (2018) as: winter (December to February), spring (March to May), summer (June to August) and autumn (September to November). Sampling was conducted onboard the training vessel “Nansei-Maru” (175 t) of the Faculty of Fisheries, Kagoshima University. The vessel is equipped with an onboard split-beam quantitative echo-sounder (KFC-3000, KAIJO, Japan) and global positioning system (GPS) (JLR-7700 MK-II Navigator, WMJ Marine, USA) for navigation. A total of 576 trawls were conducted using a simple trawl net carrying canvas kites on the tip of the wings (LC-VI, Nichimo Co., Japan). The net was 23.5 m long and 8.0 m wide with mesh size of 37.9 mm and 20.2 mm at the net body and cod end, respectively (Ohtomi *et al.*, 2004). Towing was conducted during the daytime for a preset tow duration of 10 min at a speed of 2 kt. At each haul, net depth bottom water temperature and salinity were recorded at 1 min intervals using submersible data loggers (Compact-TD ATD- HR and Compact-CT ACT-HR, JFE Advantech, Japan) attached to the head rope of the net. The salinity data was not possible to collect after

Table 5.2.1. Latitude-longitude coordinate at start and end of towing, mean water depth, warp length, and number of hauls at each sampling station of Kagoshima Bay, southern Japan.

Area	Station	Location				Mean water depth (m)	Warp length (m)
		Start of towing		End of towing			
		Latitude	Longitude	Latitude	Longitude		
Bay head	1	31°35.9'	130°45.1'	31°36.2'	130°45.3'	136.8	620
	2	31°40.1'	130°41.1'	31°40.4'	130°41.1'	143.7	650
Channel area	3	31°37.7'	130°37.4'	31°37.9'	130°37.7'	128.9	600
Central basin	4	31°31.7'	130°39.0'	31°31.7'	130°38.6'	180.0	700
	5	31°26.9'	130°37.8'	31°26.6'	130°37.9'	228.5	900
	6	31°20.6'	130°45.1'	31°20.3'	130°45.1'	77.5	430
	7	31°18.5'	130°39.1'	31°18.6'	130°38.8'	133.4	600
Bay mouth	8	31°11.3'	130°42.4'	31°11.6'	130°42.8'	99.0	500
Total							

2015 due to data logger trouble. In addition, water depth was recorded at same intervals with the echo-sounder (KFC-3000, KAIJO, Japan).

M. sibogae individuals were sorted out of each haul, and then counted and chilled immediately in ice. The samples were then transferred to the laboratory on the same day and fixed in 10% formalin pending further analysis. All specimens were classified by sex according to the presence of a petasma in males or a thelycum in females. For the specimens collected during January 2013 to December 2014, individual carapace length (CL), the shortest distance between the posterior margin of the orbit and the mid-dorsal posterior edge of the carapace, was measured using a digital slide caliper (CD-15PS, Mitutoyo, Japan) to the nearest 0.01 mm. Females of *M. sibogae* collected during January 2014 to December 2015 were macroscopically classified as immature, maturing or mature based on the ratio of ovary width to body width taken at the middle of the first abdominal segment (Rahman & Ohtomi, 2017) for the estimation of spawning ground.

Effective tow durations were determined from initial and final times of net contact with the sea bottom using logger-echo-sounder depth plots according to Fulanda & Ohtomi (2011). The mouth opening height of the trawl net used in the present study was 2.5 ± 0.3 m (mean ± SD) (Fuwa *et al.*, 2010). Therefore, the trawl net contact with the sea bottom was confirmed as long as ≤ 2.8 m difference between the echo sounder and the compact TD-logger readings was maintained (Fulanda & Ohtomi, 2011). The catch per unit effort (CPUE) was standardized to a 10 min preset tow duration according to Fulanda & Ohtomi (2011):

CPUE = Number of individuals collected per haul × 10 / Effective tow duration (min).

The CPUE was regarded as a measure of relative abundance of *M. sibogae*.

5.2.2.2. *Population dynamics of M. sibogae*

The dynamics of the *M. sibogae* population in Kagoshima Bay was studied by examining the distribution patterns of a cohort with progression of age following Ohtomi *et al.* (2018). To serve this purpose, monthly length-frequency distributions for each sex were constructed with 1 mm intervals of CL using samples collected from January 2013 to December 2014 (except September 2014) (see Figures 3.2.2 & 3.2.3). A series of component normal distributions were fitted to the length-frequency of each sample by sex, using a computer analysis (Microsoft Excel-add-in-Solver) based on Hasselblad's maximum likelihood method (Hasselblad, 1966). Each component normal distribution was assumed to represent an age group in the population. Then, ages in months were assigned to the mean CLs belonging to each of the cohorts by assigning 1st October, the approximate midpoint of the main spawning season, September-October (Rahman & Ohtomi, 2017), as the birth date (Ohtomi & Ohtomi, 2018a). In cases where two normal distributions representing two different age groups overlapped, individuals were separated into two age groups using a discriminate function: $Z_i = (L_m \times \sigma_n + L_n \times \sigma_m) / (\sigma_m + \sigma_n) - L_i$, where L_m is the mean CL and σ_m is the standard deviation at age m; L_n is the mean CL and σ_n is the standard deviation at age n; and L_i is the CL of individual i. If $Z_i > 0$, i belonged to m age group; if $Z_i < 0$, i belonged to n age group. One particular cohort for each sex was traced, which first appeared in January 2013 and existed until December 2014 for males and January 2015 for females from the length-frequency distributions for investigating the distribution patterns with progressing age.

5.2.2.3. *Spawning grounds*

The spawning grounds were defined as the areas near the sampling stations where mature females occurred. The main spawning grounds were defined as the areas near the sampling stations where ≥ 25% of the total mature females occurred based on CPUE analysis.

5.2.3. RESULTS

5.2.3.1. *Spatiotemporal distribution*

A total of 39,398 individuals of *M. sibogae* were collected from Kagoshima Bay during the present study. High variations in CPUE values were observed among surveys at different stations ranging from 0 to 1,214, with highest mean CPUE at St. 4 (221 ± 55) followed by St. 7 (178 ± 89) in the central basin, while the lowest mean CPUE was recorded at Sts. 6 and 8 in the bay mouth (1 ± 1) (Figure 5.2.2).

Seasonal and temporal variations in mean CPUE of *M. sibogae* are shown in Figures 5.2.3 and 5.2.4. The mean CPUE was the highest during spring (71 ± 108) followed by autumn (64 ± 98), while the lowest mean CPUE was recorded in winter (48 ± 81). *M. sibogae* was abundant in Sts. 4 and 7 in the central basin in all the season, however, higher CPUE was recorded in St. 4 in autumn whereas it was higher in spring in St. 7. In the bay head, highest CPUE was recorded in spring followed by autumn and summer. Analysis of mean CPUE throughout the study period revealed that *M. sibogae* population in the bay started to increase drastically in 2007 and continued until 2010. From 2011 to 2014, a significant decrease in CPUE can be observed followed by another gradual increase in the subsequent years. CPUE analysis further revealed that *M. sibogae* was more abundant in the southern part of the central basin (St. 7) in the initial years of its first record in 2004, however, it becomes more abundant in the northern part of the central basin (St. 4) in the following years. In recent years, *M. sibogae* is getting abundant in the bay head and channel area as well.

5.2.3.2. *Population dynamics of M. sibogae*

The distribution patterns with progression of age revealing the population dynamics of *M. sibogae* showed similar patterns for both sexes (Figures 5.2.5 & 5.2.6). Juveniles at the age of 3.7 months were found to be widely distributed in the bay, although with a low mean CPUE

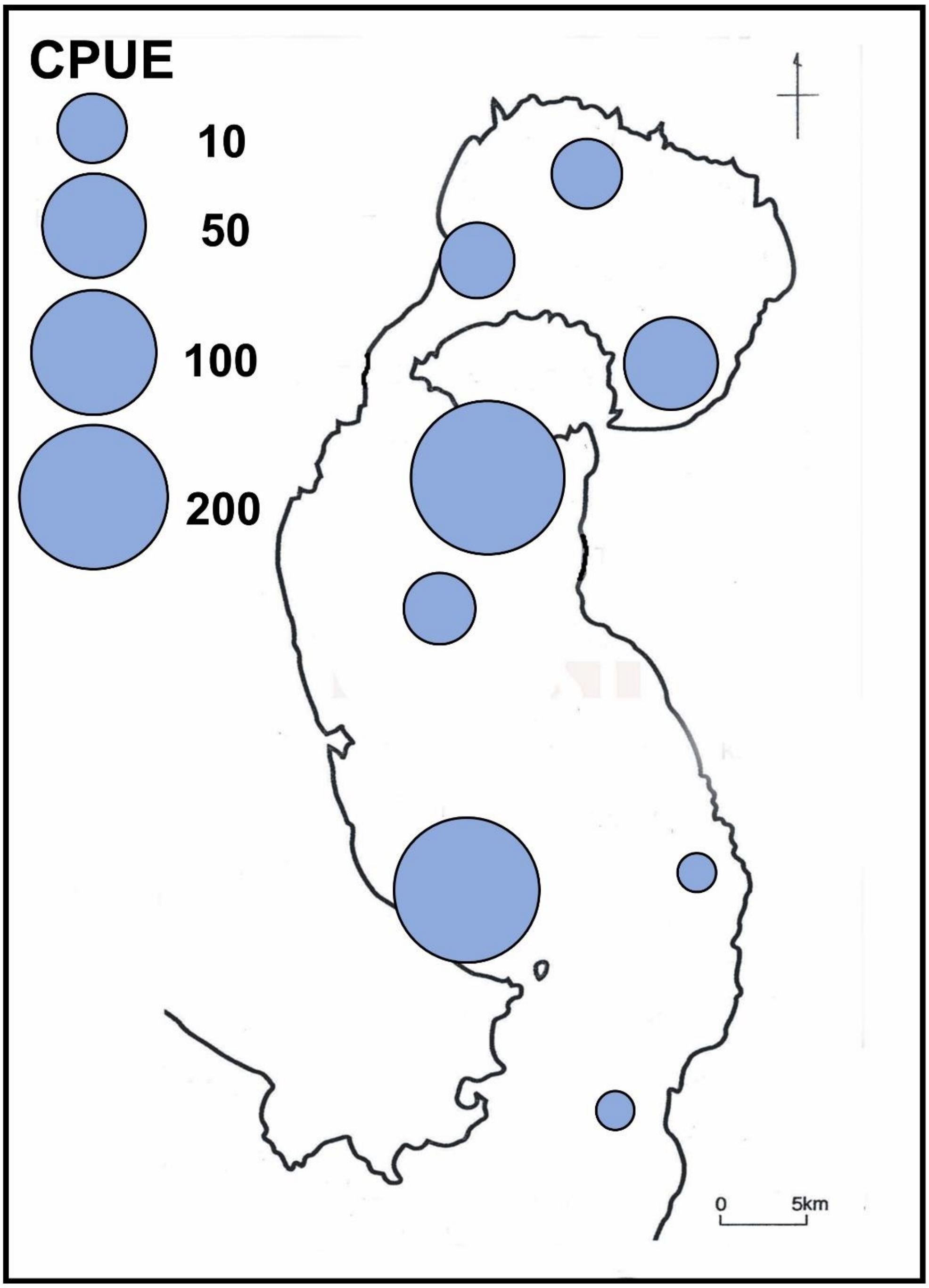


Figure 5.2.2. Mean catch per unit effort (CPUE) of *Metapenaeopsis sibogae* at each sampling station in Kagoshima Bay, southern Japan during February 2004 to October 2017.

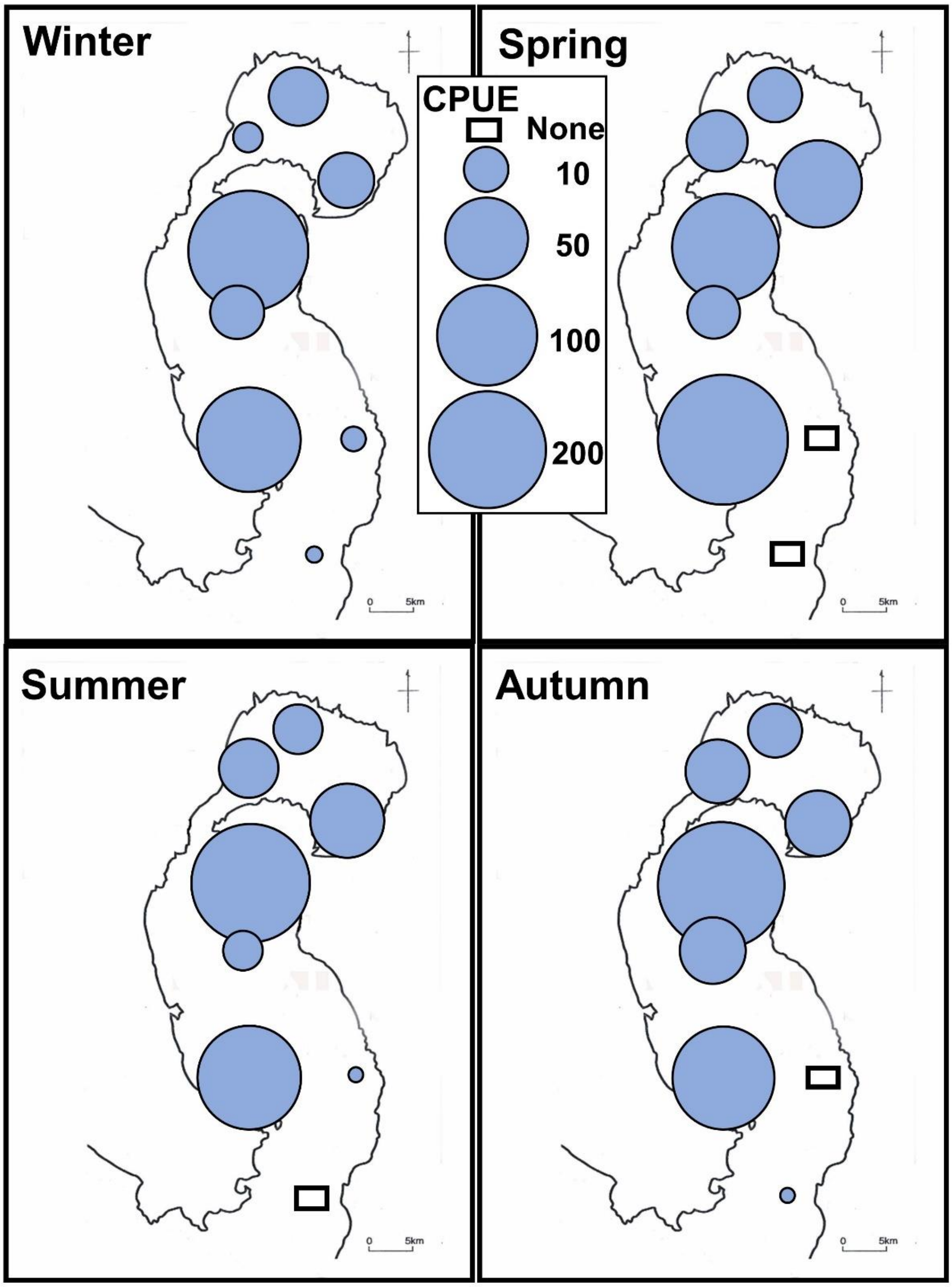


Figure 5.2.3. Seasonal changes in mean catch per unit effort (CPUE) of *Metapenaeopsis sibogae* at each sampling station in Kagoshima Bay, southern Japan during February 2004 to October 2017.

(7 ± 12). As age progressed (at the age of 6.5 months), their abundance increased drastically at all the stations with the highest CPUE (185) was recorded in the southern part of the central basin. At the ages of 9.7 months and 12.6 months, a rapid increase of CPUE was observed in the northern part of the central basin. At the age of 15. 5 months, the CPUE decreased drastically throughout the bay, and individuals became concentrated in the northern part of the central basin (158). Thereafter, the CPUE of these cohorts gradually decreased with an increased tendency of individuals inhabiting the deeper waters was observed before they disappeared from the time series of length-frequency distributions.

5.2.3.3. *Spawning grounds*

Mature females of *M. sibogae* occurred in the entire areas of its distribution except in the shallowest (Sts. 6 and 8) and deepest (St. 5) areas of the bay (Figure 5.2.7). Mean CPUE of mature females during the main spawning season were highest at St. 4 followed by St. 7 and more than 75% of the mature females were distributed in the central basin. We, therefore, concluded that *M. sibogae* spawned in areas with an average water depth ranging from ~130 m to ~180 m in Kagoshima Bay with the northern and southern part of the central basin being their main spawning grounds.

5.2.3.4. *Physical and chemical environmental factors*

The seasonal variations in bottom water temperature and salinity during the study period are shown in Figure 5.2.8. The lowest (14.9 ºC) and highest (18.7 ºC) water temperature were recorded in spring at St. 5 and in autumn at St. 6, respectively with a mean value of 16.0 ± 0.7 ºC. On the other hand, the lowest (32.5) and highest (33.6) salinities were recorded in winter at St. 2 and in autumn at St. 8, respectively with a mean value of 33.2 ± 0.3. A slight variation in seasonal bottom water temperature (except in autumn at St. 6, where the bottom water temperature was much higher than the other stations) and salinity was observed among the stations with no definite spatial or seasonal trends.

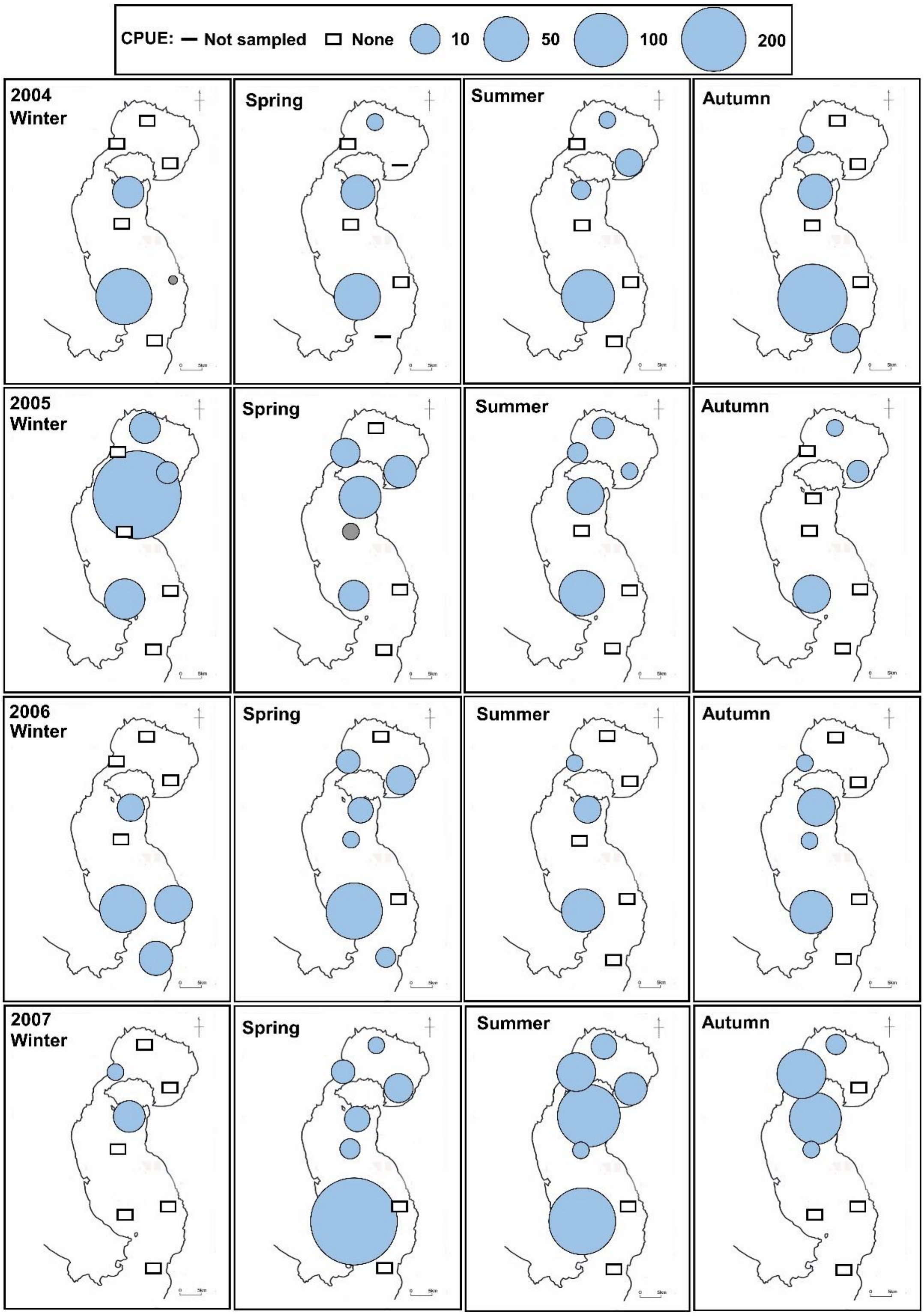


Figure 5.2.4. Seasonal and temporal changes in mean catch per unit effort (CPUE) of *Metapenaeopsis sibogae* at each sampling station in Kagoshima Bay, southern Japan during February 2004 to October 2017.

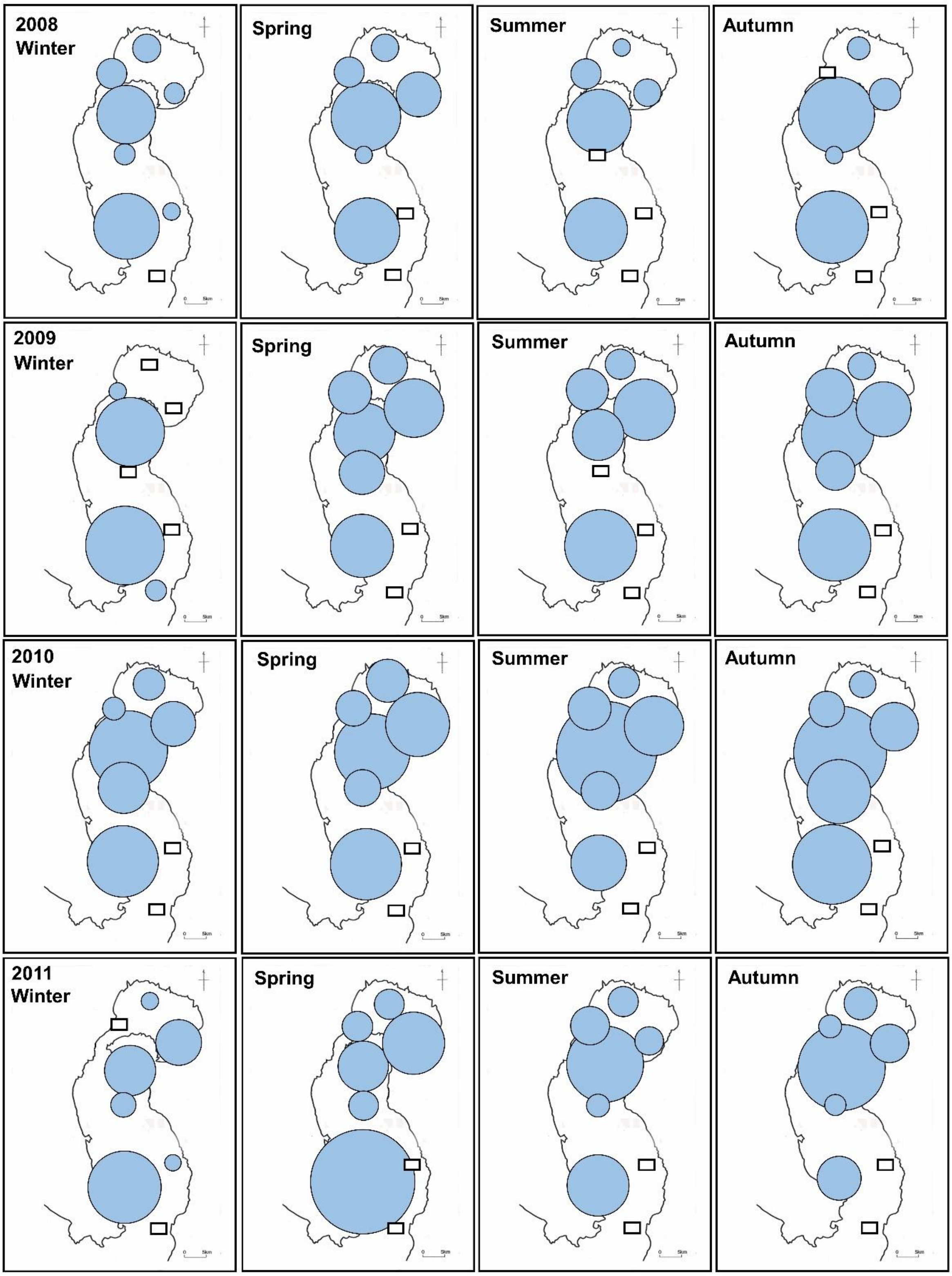
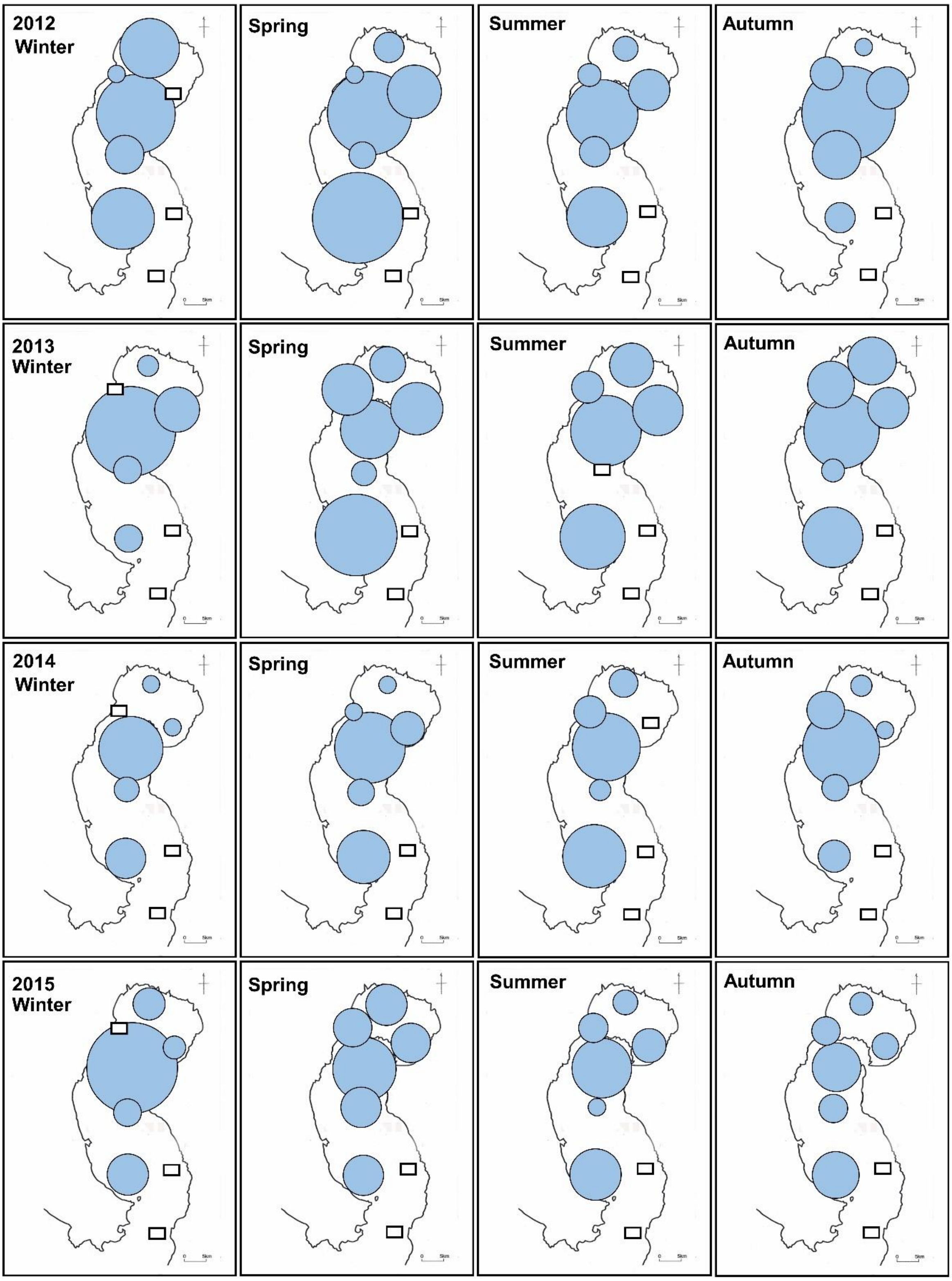


Figure 5.2.4. continued



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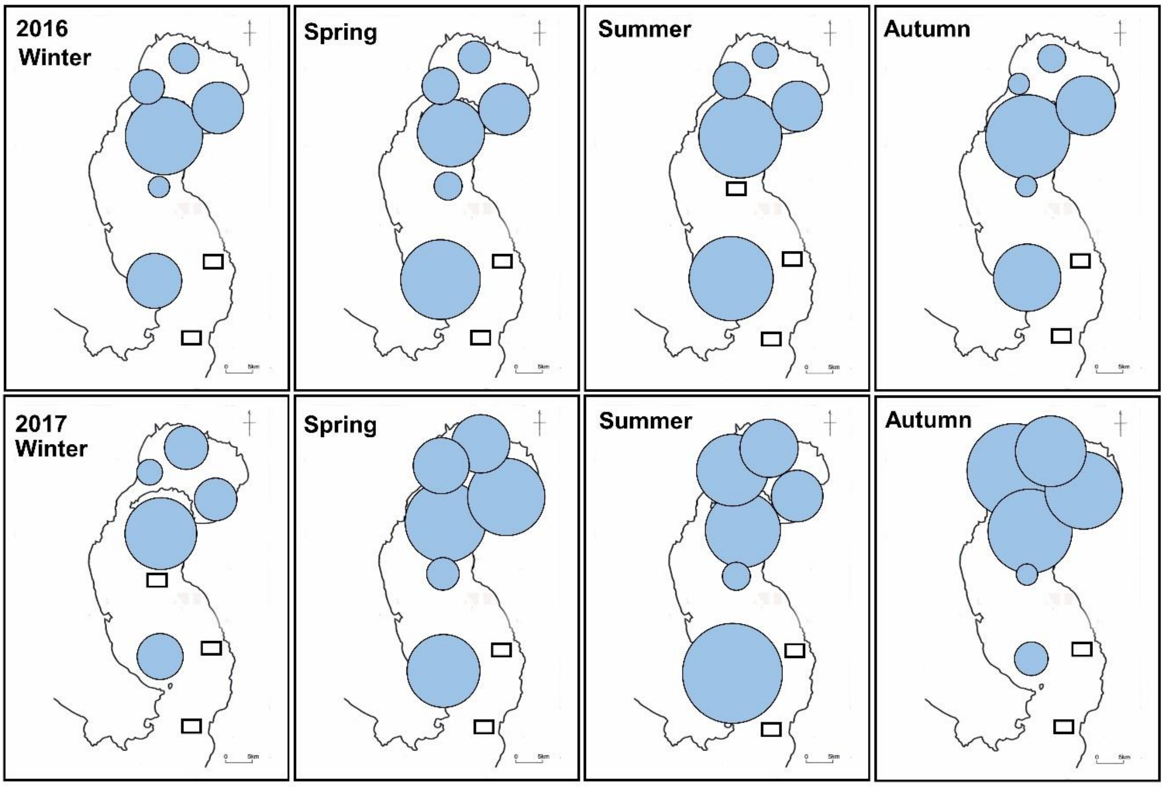


Figure 5.2.4. continued

5.2.4. DISCUSSION

M. sibogae was found to have a wide spatial and bathymetric distribution in Kagoshima Bay. Apart from Kagoshima Bay, the shallowest habitat record of this shrimp was 247 m in Saleh-Bay, Indonesia (Grave & Fransen, 2011). Ohtomi & Nagata (2004) reported *M. sibogae* from Kagoshima Bay at a depth of ~130 m, which was the shallowest record of this species. The present study confirms the presence of *M. sibogae* at a water depth of 77 m, which is the new shallowest habitat record of this species. It is important to note that during the present study, no sampling could be conducted at depths < 77 m due to steep slopes on both sides of the bay (Matumoto, 1943) and a heavy congestion of fixed gear (set-nets, traps, etc.) set by commercial fisheries (Ohtomi *et al.*, 2018). The comparatively shallower bathymetric distribution of *M. sibogae* in the bay, could be an adaptation of the local population to cope with the steep slopes of the bay. *P. izumiae* in Kagoshima Bay also showed adaptive behavior as they adapted to inhabit the comparatively deeper waters in the bay (Ohtomi *et al.*, 2018).

M. sibogae was found to be predominant in the northern and southern part of the central basin. The distribution of shrimps and other crustaceans is known to depend on several environmental factors, including water temperature, salinity, bottom sediment, and food availability, which influence population densities and set geographical limits to their distribution (Abelló *et al.*, 1988; Cartes *et al.*, 2002; Fanelli & Cartes, 2004; Ohtomi *et al.*, 2018). The abundance and biomass of *T. curvirostris* was found to be significantly correlated with water temperature (Cha *et al.*, 2004a). However, during the present study, the physical and chemical environmental parameters (bottom-water temperature and salinity) showed only small variations, as reported for deeper-waters (>100 m) of Kagoshima Bay by several researchers (e.g., Oki, 1989; Ahamed & Ohtomi, 2011; Rahman & Ohtomi, 2017, 2018a), and was thus not considered as a crucial factor for determining the distribution and relative abundance of *M. sibogae* within the bay.

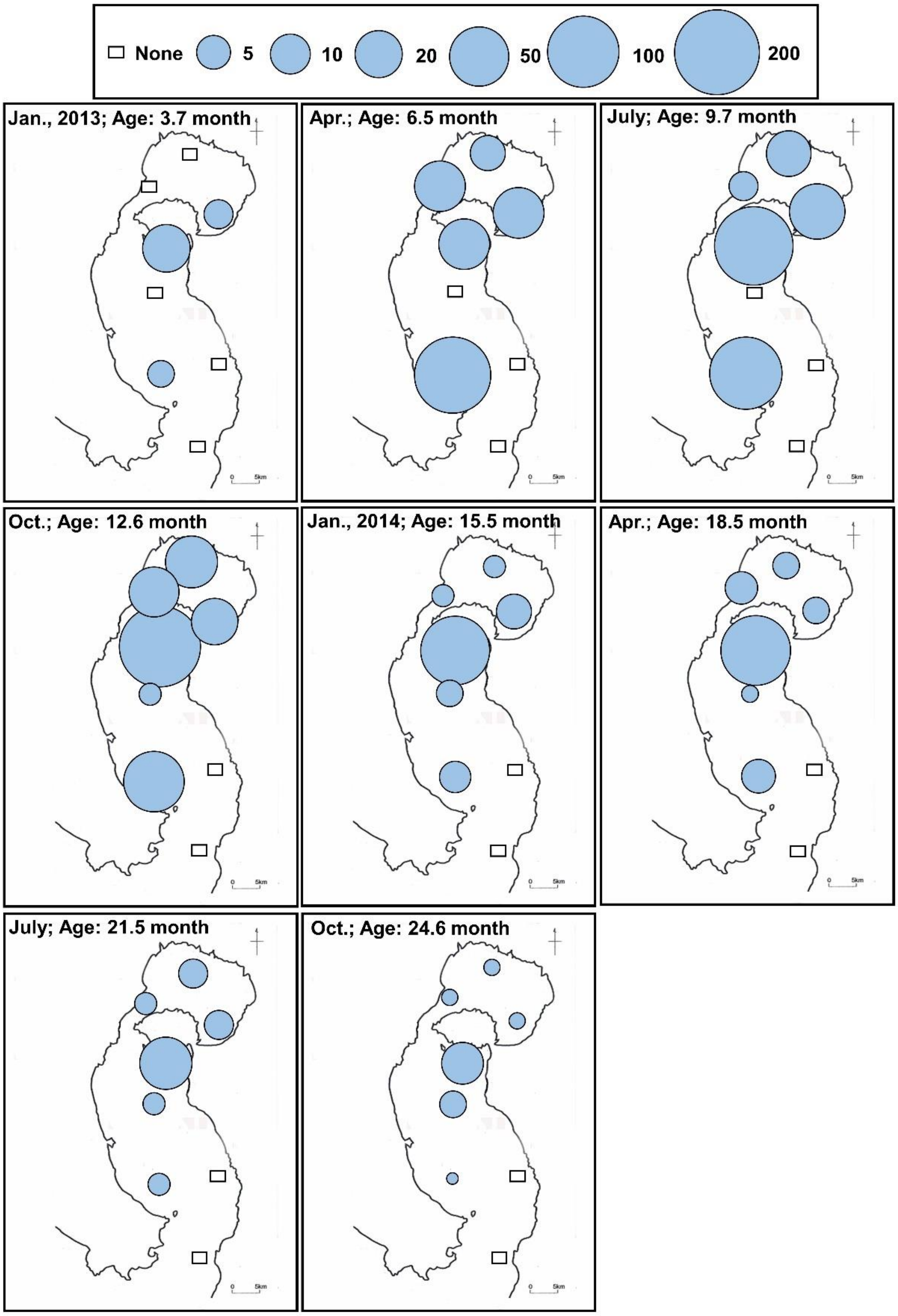


Figure 5.2.5. Distribution patterns with progression of age of a single cohort of male *Metapenaeopsis sibogae* at each sampling station in Kagoshima Bay, southern Japan.

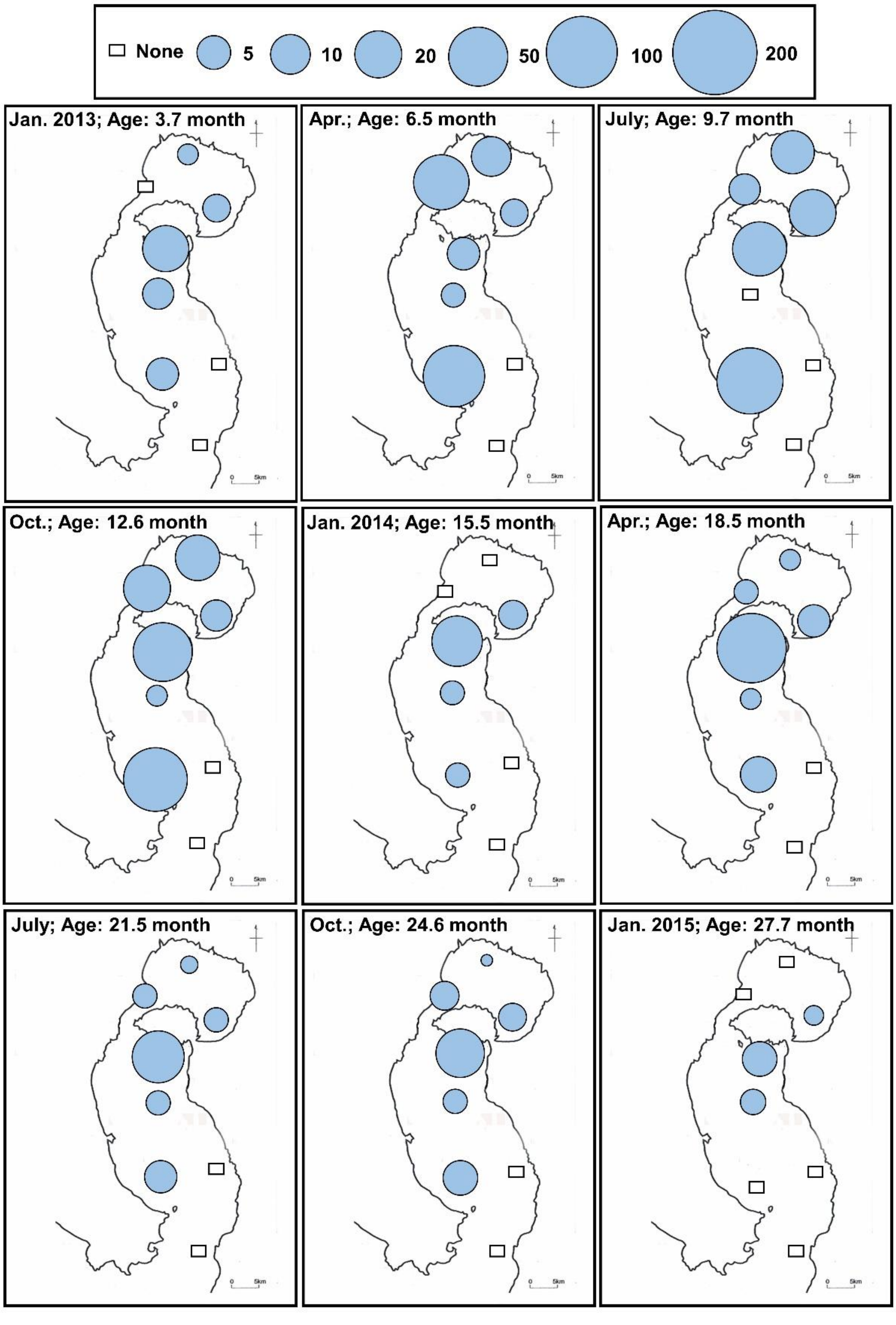


Figure 5.2.6. Distribution patterns with progression of age of a single cohort of female *Metapenaeopsis sibogae* at each sampling station in Kagoshima Bay, southern Japan.

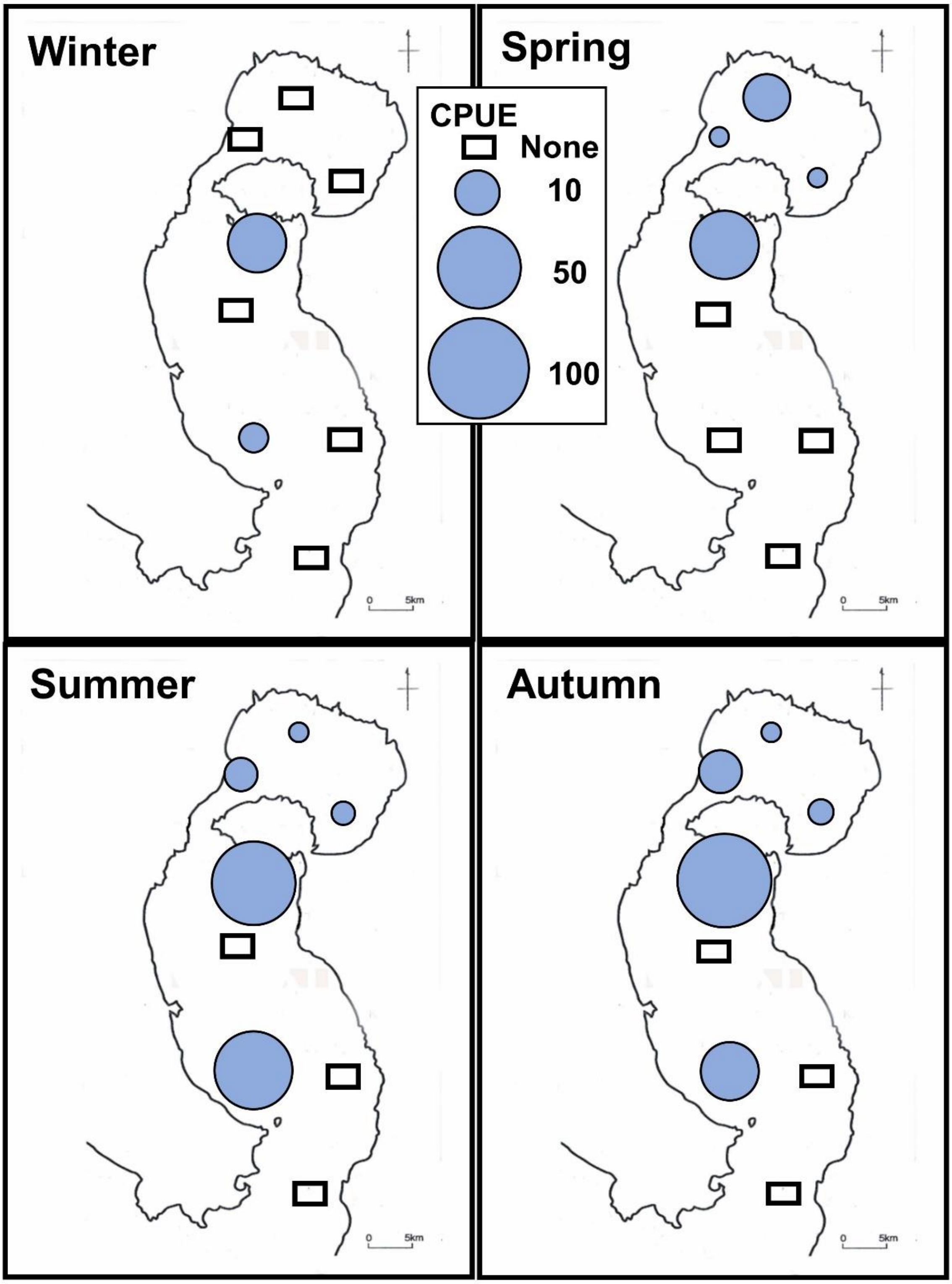


Figure 5.2.7. Mean catch per unit effort (CPUE) of mature females of *Metapenaeopsis sibogae* at each sampling station in Kagoshima Bay, southern Japan.

In contrast, the bottom sediments of Kagoshima Bay differed among distinct areas (Ohtomi *et al.*, 2018) and seemed to have an impact on the distribution and relative abundance of *M. sibogae.* Several authors reported that bottom sediment characteristics are one of the key factors influencing spatial distribution of crustaceans (Wenner *et al.*, 1983; Abelló *et al.*, 1988). The influence of bottom sediment characteristics on distribution was reported for several penaeid shrimps, such as *T. curvirostris* in the eastern Mediterranean Sea (Galil, 2000) and *P. longirostris* in the Mediterranean Sea and eastern northern Atlantic Ocean (Heldt, 1954; Holthuis, 1980). *M. sibogae* was distributed throughout the bay when the bottom sediment varied from silty to silty-sand, however, they were most abundant in areas where the bottom is made of silty sediment. Interestingly, *M. sibogae* was more abundant in the central basin than in the bay head, though the sediment characteristics of these two areas were similar. Even within the central basin, *M. sibogae* was more abundant in the northern and southern parts (comparatively shallower) than the deeper central area. Water depth of the sampling stations among these areas were different. It is, therefore, hypothesized that water depth might be one of the factors behind the higher relative abundance of *M. sibogae* in the northern and southern areas than that in the central area of the central basin. The average water depth of the sampling stations in the bay head approximates that of the southern part of the central basin, but *M. sibogae* was more abundant in the southern part of the central basin. No specific factor could be identified in this study, however, water current among these two areas might have an impact among other possibly influencing factors such as primary food resources (Guijarro *et al.*, 2012) and light intensity (Cartes *et al.*, 1993; Aguzzi *et al.*, 2007).

The distribution patterns with progression of age that reveal the dynamics of the *M. sibogae* population, were studied by selecting one cohort from each sex and tracing them throughout their life cycle. Mature females of *M. sibogae* occurred in areas with water depths ranging from ~130 m to ~180 m, however, most of the mature females occurred in the northern

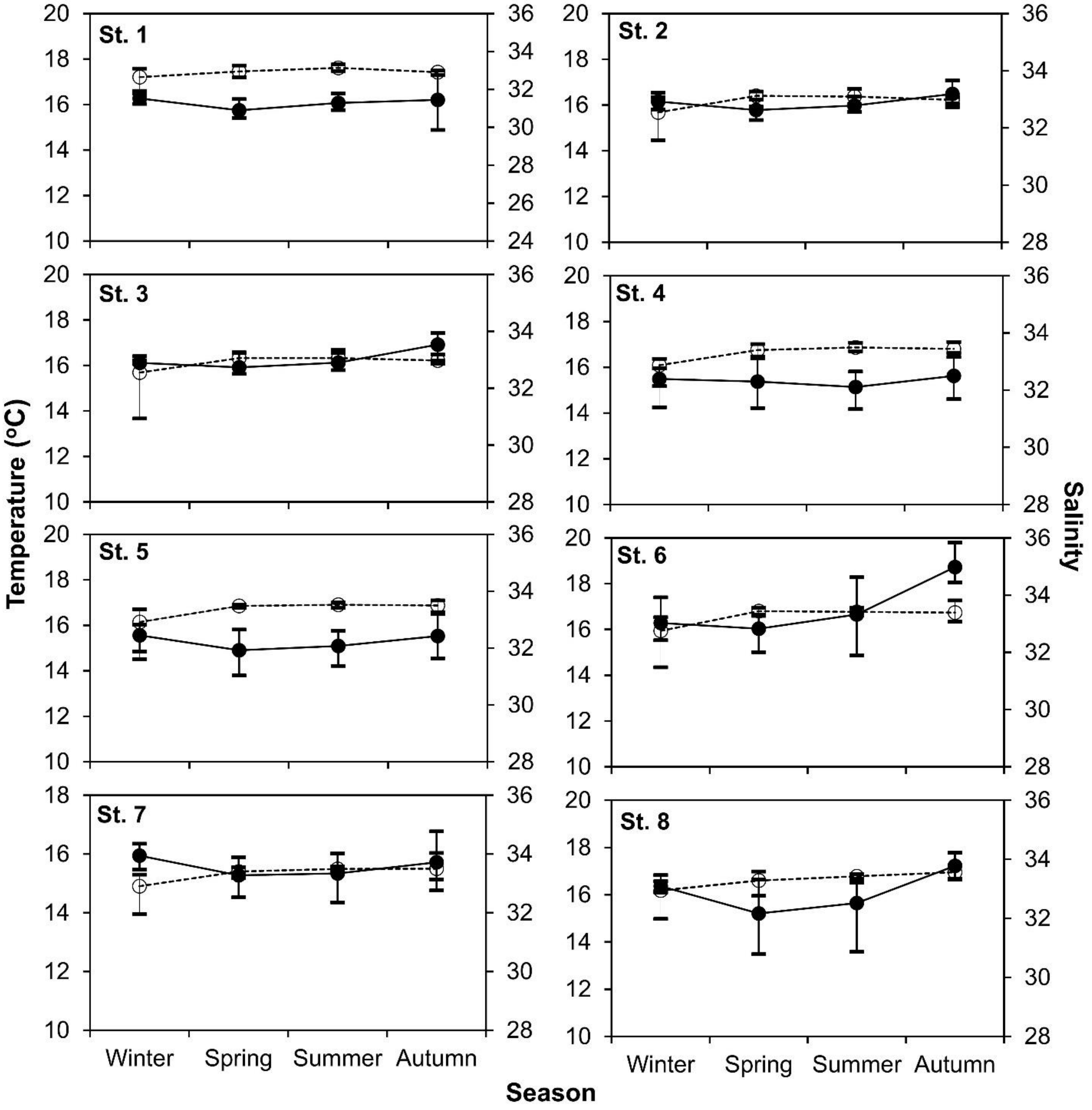


Figure 5.2.8. Seasonal changes in bottom water temperature (solid circles) and salinity (open circles) at each station in Kagoshima Bay, southern Japan.

and the southern part of the central basin and are thus considered as the main spawning grounds of this species. However, the juveniles of *M. sibogae* occurred throughout its distribution areas at the age of 3.7 months: though with a low mean CPUE, even in the main spawning grounds. According to the study by Rahman & Ohtomi (2018a), both male and female *M. sibogae* were first recruited in winter (January) and the recruitment continued until spring (April). It was therefore hypothesized that the lower abundance of juveniles at the age of 3.7 months could have resulted from the ongoing recruitment process. The drastic increase in relative abundance at the age of 6.5 months throughout the distribution areas, especially in the northern and southern parts of the central basin, might be indicating the completion of recruitment. However, several studies reported the influence of hydrological factors in the recruitment dynamics of shrimp, and smaller individuals appeared to be distributed in regions shallower than their main distribution areas (Cartes *et al.*, 2008; Massuti *et al.*, 2008; Palmas *et al.*, 2015). The comparatively deeper recruitment areas of *M. sibogae* in Kagoshima Bay might be an adaptation of this shrimp to the unique topography of the bay, as was reported for another shrimp, *P. izumiae* in the bay (Ohtomi *et al.*, 2018). Future studies to reveal the details of larval settlement and of the recruitment patterns of this shrimp are recommended.

As age progressed, at the ages of 9.7 months and 12.6 months, the CPUE increased rapidly in the northern part of the central basin, however, without any noticeable shift in CPUE being marked in other areas. By the age of 12.6 months, most of the shrimps become sexually mature (Rahman & Ohtomi, 2017, 2018a). It is very likely that no noticeable spawning migration occurs in this species. A drastic decrease in the CPUE was observed at the age of 15.5 months. Thereafter, the CPUE of these cohorts gradually decreased with an increased tendency of individuals to inhabit the deeper waters being observed before disappearing from the time series of length-frequency distributions. The tendency of larger-sized individuals to inhabit deeper waters of their distribution areas is a well described feature in shrimps including some penaeids such as *T. curvirostris* in Sendai Bay (Kosaka, 1979), *P. lanceolatus* in Kagoshima Bay (Farhana & Ohtomi, unpublished).

Seasonal differences in CPUE of *M. sibogae* were also observed in the bay, with higher values recorded in spring versus lower values in winter. These differences might be related to the life history of the shrimp. The new cohorts of *M. sibogae* first appeared in winter; however, these cohorts were fully recruited in the following spring and continued to thrive and grow through the summer and autumn, which was reflected in the higher CPUE of the shrimp in these seasons. On the other hand, in winter, usually two age groups were present in males and three in females. However, the CPUE of both age groups in males were significantly low as the older one drastically reduced after spawning, whereas the new one just started to be recruited in the fishery. Similarly, in females, the oldest age group declined as they reached their maximum life expectancy, the middle age group rapidly declined after spawning, the other new one just started to be recruited in the fishery. These phenomena resulted in overall lower CPUE values in winter. The present study recorded a drastic increase in the relative abundance of *M. sibogae* during 2007-2010 followed by a significant decrease during 2011-2014 and a subsequent steady increase, although the factors driving the phenomena were not identified in the present study. *M. sibogae* showed strong adaptability and flexibility in their habitat suitability, which together with the stable physical and chemical environmental parameters of the bay might be some of the reasons for this species' rapid dominance in just over a decade since being first recorded in the bay in 2004 by Ohtomi & Nagata (2004).

The present study has revealed the distribution patterns and population dynamics of *M. sibogae*, which would clarify the life history of this shrimp and thereby be applied in formulating effective strategies for its sustainable management. Studies on the influence of primary food resources and light intensity on the distribution patterns of this shrimp are recommended in order to make the picture of its life cycle in Kagoshima Bay complete. In addition, the comparative distribution and abundance pattern analysis should be conducted for the major species to enlighten the possible impact of the newly recorded species on the benthic community of the bay. The adaptation of *M. sibogae* in Kagoshima Bay, its rapid abundance and dominance signify the importance of identification of its origin and source of introduction in Japanese waters as a priority research.

5.3: DISTRIBUTION PATTERNS AND POPULATION DYNAMICS OF *METAPENAEOPSIS KYUSHUENSIS*

5.3.1. INTRODUCTION

M. kyushuensis is one of the dominant species in the benthic community of Kagoshima Bay and is getting economic importance. There is, however, no study available on the distribution patterns and population dynamics of this species, which are essential for knowing recruitment patterns, nursery and spawning grounds, possible migratory behavior in order to formulate sustainable management regime. The present study therefore aimed to reveal the distribution patterns and population dynamics of *M. kyushuensis* for the first time using a large number of samples collected from Kagoshima Bay.

5.3.2. MATERIALS AND METHODS

5.3.2.1. *Sampling procedures and data analysis*

The present study was conducted in the semi-enclosed deep-water Kagoshima Bay, southern Japan (see Figure 5.2.1). The bay was demarcated into four areas based on the oceanographic and bathymetric characteristics following Ohtomi *et al.* (2018): **(1) bay head**, a semi-circular enclosure running up to 140 m in depth; **(2) central basin**, with maximum water depths reaching about 230 m; **(3) channel area**, connecting the bay head and central basin; and **(4) bay mouth**, opening to the Pacific and the East China Sea to the south and west, respectively. Eight sampling stations were established in these areas: Sts. 1 and 2 in the bay head, St. 3 in the channel area, Sts. 4, 5 and 7 in the central basin, and Sts. 6 and 8 in the bay mouth (see Table 5.2.1).

Samples of *M. kyushuensis* were collected by experimental trawl surveys conducted in the bay from February 2004 to December 2017. Monthly sampling was conducted in 3 or 4 stations while seasonal sampling (January, April, July, and October) was conducted from all 8 stations. In the present study, seasons were defined following Ohtomi *et al.* (2018) as: winter (December to February), spring (March to May), summer (June to August) and autumn (September to November). Sampling was conducted onboard the training vessel "Nansei-Maru" (175 t) of the Faculty of Fisheries, Kagoshima University. The vessel is equipped with an onboard split-beam quantitative echo-sounder (KFC-3000, KAIJO, Japan) and global positioning system (GPS) (JLR-7700 MK-II Navigator, WMJ Marine, USA) for navigation. A total of 576 trawls were conducted using a simple trawl net carrying canvas kites on the tip of the wings (LC-VI, Nichimo Co., Japan). The net was 23.5 m long and 8.0 m wide with mesh size of 37.9 mm and 20.2 mm at the net body and cod end, respectively (Ohtomi *et al.*, 2004). Towing was conducted during the daytime for a preset tow duration of 10 min at a speed of 2 kt. At each haul, net depth was recorded at 1 min intervals using submersible data loggers (Compact-TD ATD-HR, JFE Advantech, Japan) attached to the head rope of the net. In addition, water depth was recorded at same intervals with the echo-sounder (KFC-3000, KAIJO, Japan).

Individuals of *M. kyushuensis* were sorted out of each haul based on the shape of a petasma in males or a thelycum in females. *M. kyushuensis* is morphologically similar with *M. provocatoria owstoni*. The females of these two species can be identified based on the differences in thelycum (see Figure 3.2.2A, B). However, the shape and size of petasma in males were similar, and it was impossible to distinguish the males of these two species (see Figure 3.2.2C). Therefore, during data analysis, the number of males were divided into two portions based on the ratio of females of *M. kyushuensis* and *M. provocatoria owstoni* in the sample. Individuals were counted onboard and chilled immediately in ice. The samples were then transferred to the laboratory on the same day and fixed in 10% formalin pending further analysis. For the females collected during January 2016 to December 2017, individual carapace length (CL), the shortest distance between the posterior margin of the orbit and the mid-dorsal posterior edge of the carapace, was measured using a digital slide caliper (CD-15PS, Mitutoyo, Japan) to the nearest 0.01 mm. Females collected during the same period were macroscopically classified as immature, maturing or mature based on the ratio of ovary width to body width taken at the middle of the first abdominal segment (Rahman & Ohtomi, 2017) for the estimation of spawning ground.

Effective tow durations were determined from initial and final times of net contact with the sea bottom using logger-echo-sounder depth plots according to Fulanda & Ohtomi (2011). The mouth opening height of the trawl net used in the present study was 2.5 ± 0.3 m (mean ± SD) (Fuwa *et al.*, 2010). Therefore, the trawl net contact with the sea bottom was confirmed as long as ≤ 2.8 m difference between the echo sounder and the compact TD-logger readings was maintained (Fulanda & Ohtomi, 2011). The catch per unit effort (CPUE) was standardized to a 10 min preset tow duration according to Fulanda & Ohtomi (2011):

CPUE = Number of individuals collected per haul × 10 / Effective tow duration (min).

The CPUE was regarded as a measure of relative abundance of *M. kyushuensis*.

5.3.2.2. *Population dynamics of M. kyushuensis*

The dynamics of *M. kyushuensis* population in Kagoshima Bay was studied following Ohtomi *et al.* (2018) by examining the distribution patterns of a cohort with progression of age. To serve this purpose, monthly length-frequency distributions of females were constructed with 1 mm intervals of CL using samples collected from January 2016 to December 2017 (see Figure 3.3.3). A series of component normal distributions were fitted to the length-frequency of each sample, using a computer analysis (Microsoft Excel-add-in-Solver) based on Hasselblad's maximum likelihood method (Hasselblad, 1966). Each component normal distribution was assumed to represent an age group in the population. Then, ages in months were assigned to the mean CLs belonging to each of the cohorts by assigning 1st August, the approximate midpoint of the main spawning season, June-September (see Chapter 2, section 2.3), as the birth date (see Chapter 3, Section 3.3). In cases, where two normal distributions representing two different age groups overlapped, individuals were separated into two age groups using a discriminate function: $Z_i = (L_m \times \sigma_n + L_n \times \sigma_m) / (\sigma_m + \sigma_n) - L_i$, where L_m is the mean CL and σ_m is the standard deviation at age m; L_n is the mean CL and σ_n is the standard deviation at age n; and L_i is the CL of individual i. If $Z_i > 0$, i belonged to m age group; if $Z_i < 0$, i belonged to n age group. A particular cohort of females was traced, which first appeared in January 2016 and existed until July 2017 from the length-frequency distributions for investigating the distribution patterns with progressing age.

5.3.2.3. *Spawning grounds*

The spawning grounds were defined as the areas near the sampling stations where mature females occurred. The main spawning grounds were defined as the areas near the sampling stations where ≥ 25% of the total mature females occurred based on CPUE analysis.

5.3.3. RESULTS

5.3.3.1. *Spatiotemporal distribution*

A total of 7510 individuals of *M. kyushuensis* were collected from Kagoshima Bay during the present study. CPUE values varied significantly among surveys at different stations ranging from 0 to 510, with the highest mean CPUE at St. 7 (50 ± 45) followed by St. 4 (18 ± 4) in the central basin, while the lowest mean CPUE was recorded at Sts. 6 and 8 in the bay mouth (1 ± 1) (Figure 5.3.1). Seasonal and temporal variations in mean CPUE of *M. kyushuensis* are shown in Figures 5.3.2 and 5.3.3. The mean CPUE was the highest during spring (17 ± 39) followed by summer (8 ± 16), while the lowest mean CPUE was recorded in winter (6 ± 11). *M. kyushuensis* was abundant in Sts. 7 and 4 in the central basin in all the season, however, the highest CPUE was recorded in St. 7 in spring whereas it was higher in winter in St. 4. In the

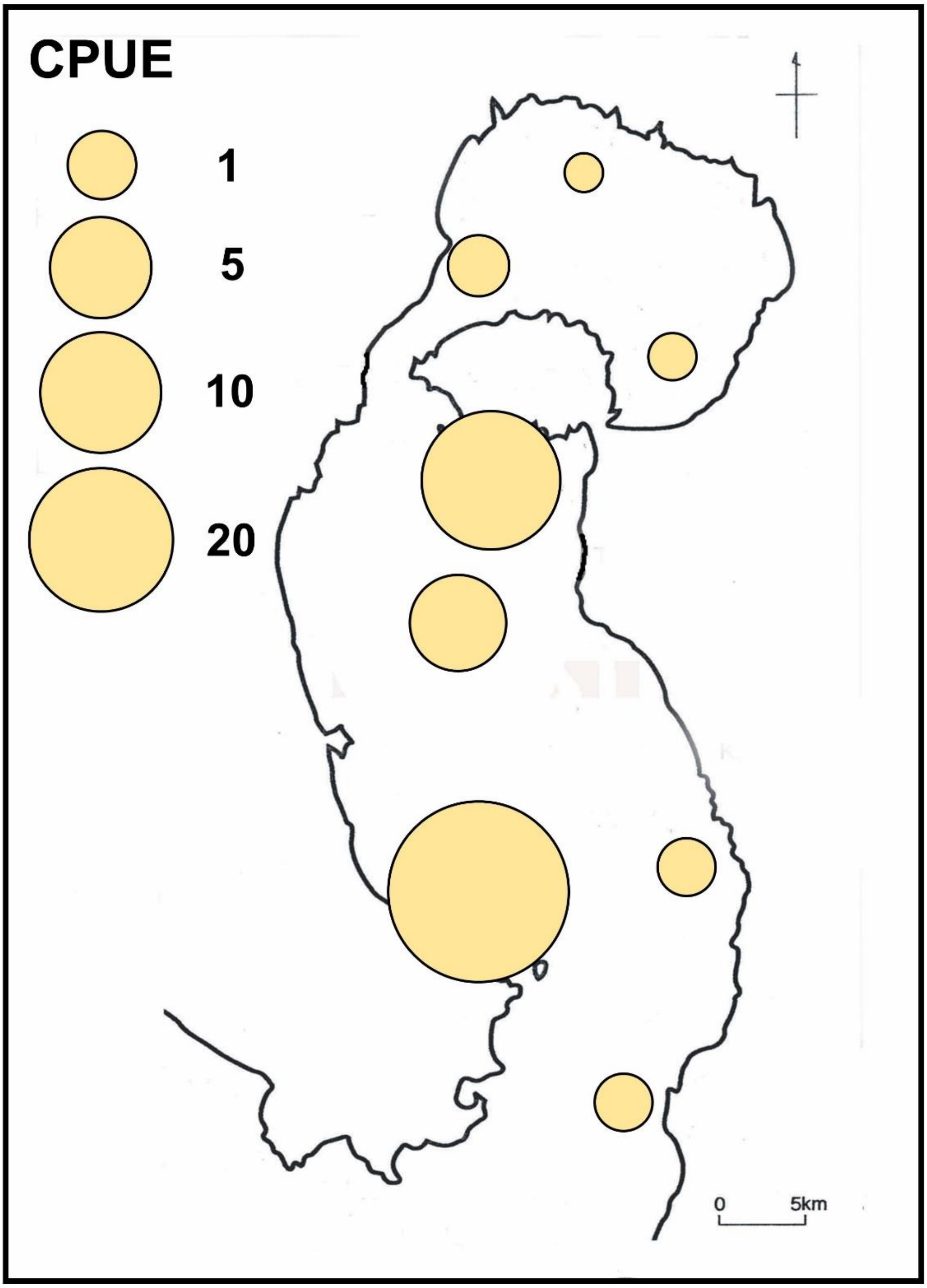


Figure 5.3.1. Mean catch per unit effort (CPUE) of *Metapenaeopsis kyushuensis* at each sampling station in Kagoshima Bay, southern Japan during February 2004 to October 2017.

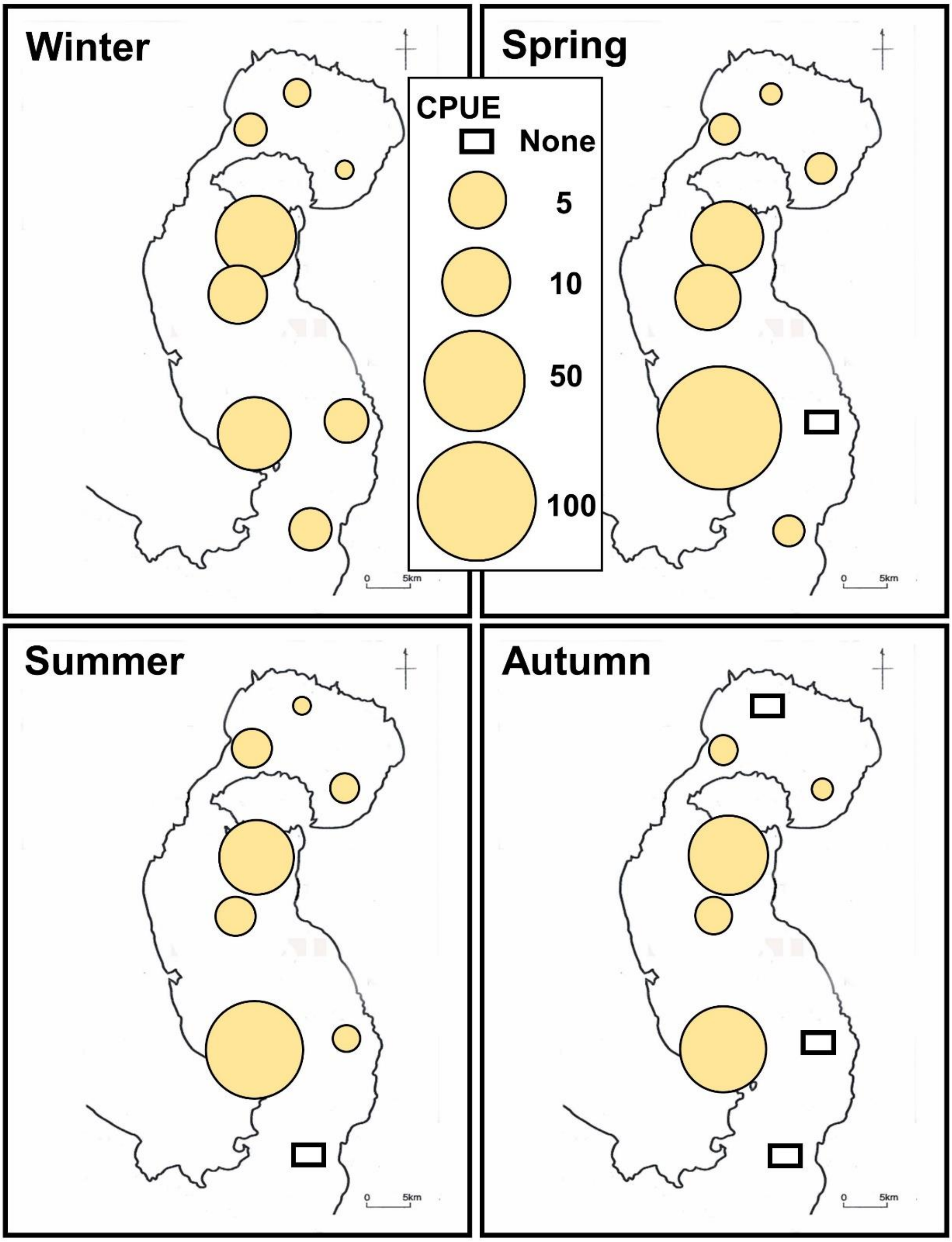


Figure 5.3.2. Seasonal changes in mean catch per unit effort (CPUE) of *Metapenaeopsis kyushuensis* at each sampling station in Kagoshima Bay, southern Japan during February 2004 to October 2017.

bay head, channel area and in the bay mouth, the CPUEs were low throughout the year. Analysis of mean CPUE during the study period revealed that *M. kyushuensis* population declined rapidly during 2009 to 2012. A gradual increase in the population has been recorded in the subsequent years.

5.3.3.2. ***Population dynamics of M. kyushuensis***

The distribution patterns with progression of age revealing the population dynamics of female *M. kyushuensis* showed that juveniles at the age of 6.6 month were mainly distributed in the northern and southern area of the central basin with a low mean CPUE (3 ± 2). As age progressed (at the age of 9.6 months), their abundance increased drastically in both the areas with the highest CPUE was recorded in the southern part of the central basin. At the age of 12.5 months, a rapid decline in CPUE was observed in the southern area of the central basin. Thereafter, individuals started to show the tendency to inhabit the deeper areas of the bay as increased CPUEs were recorded at northern and central part of the central basin (at the ages of 15.6 months and 18.4 months). Subsequently, the CPUE of these cohorts drastically decreased before disappearing from the time series of length-frequency distributions.

5.3.3.3. ***Spawning grounds***

Mature females of *M. kyushuensis* occurred in the entire areas of its distribution except in the shallowest (Sts. 6 and 8) areas of the bay (Figure 5.3.5). Mean CPUE of mature females during the main spawning season were highest at St. 7 followed by St. 4 and more than 75% of the mature females were distributed in the central basin. It was therefore concluded that *M. kyushuensis* spawned in areas with an average water depth ranging from ~130 m to ~230 m in Kagoshima Bay with the northern and southern part of the central basin being the main spawning grounds.

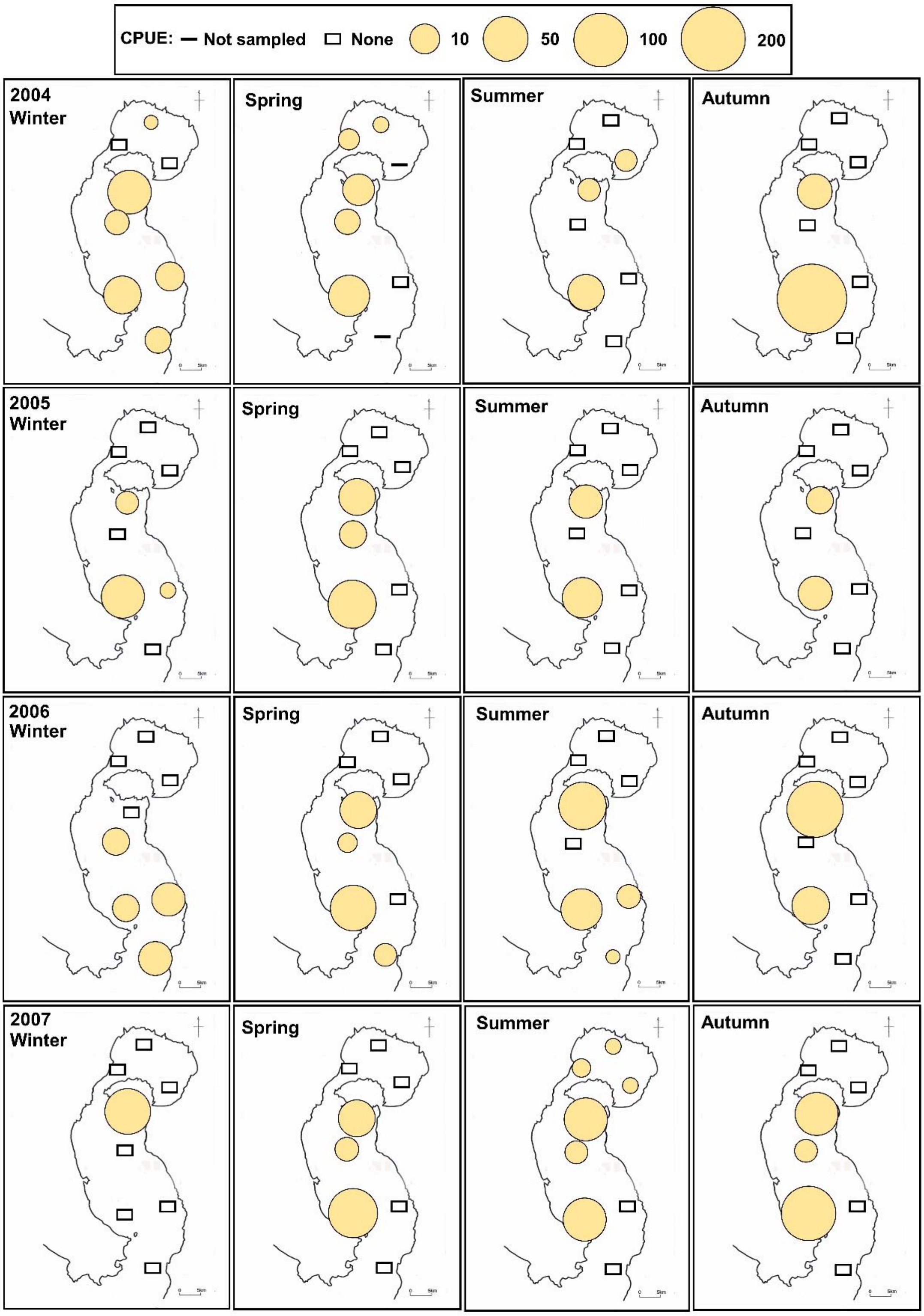
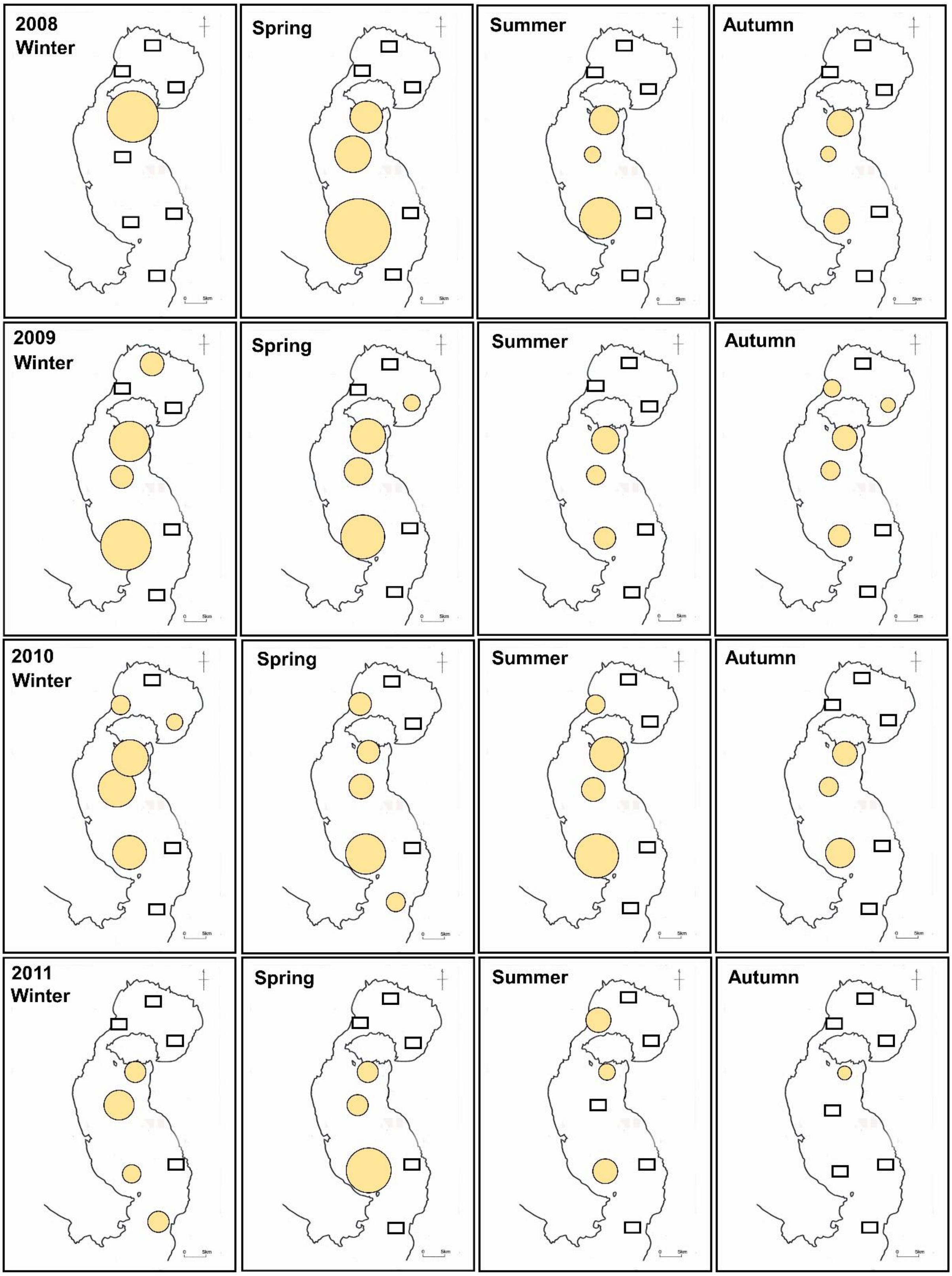


Figure 5.3.3. Seasonal and temporal changes in mean catch per unit effort (CPUE) of *Metapenaeopsis kyushuensis* at each sampling station in Kagoshima Bay, southern Japan during February 2004 to October 2017.



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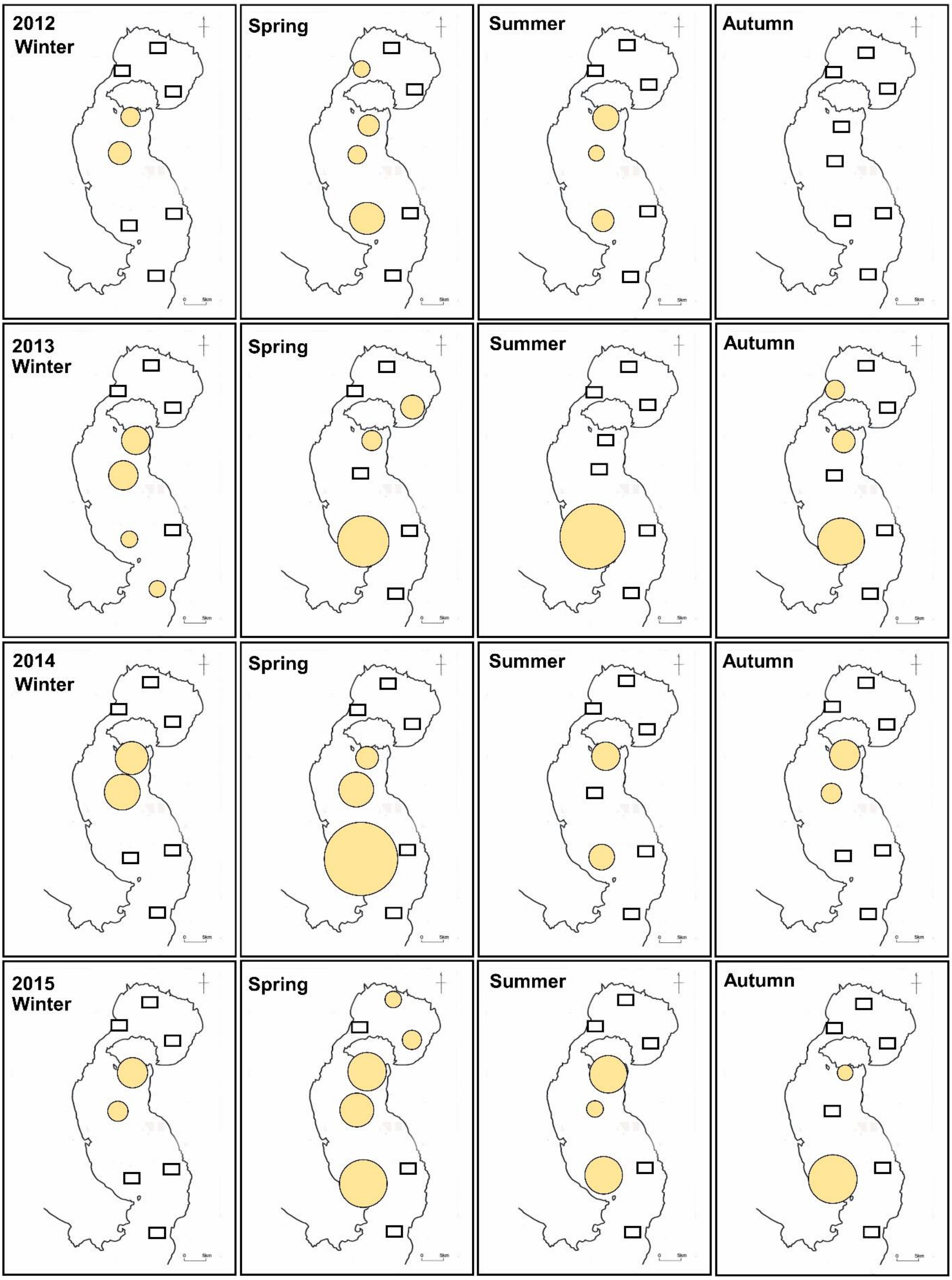

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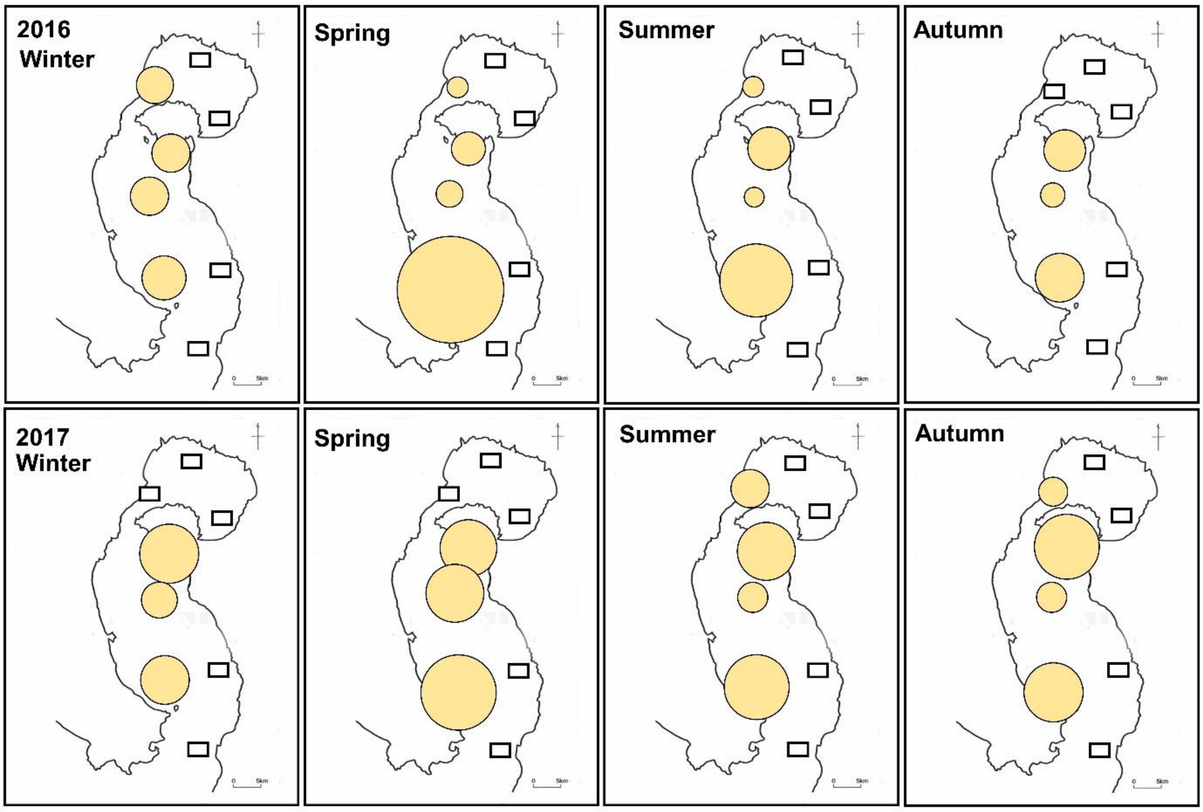


Figure 5.3.3. continued

5.3.4. DISCUSSION

Studies on deep-water shrimp populations are quite difficult to conduct because large samples are hard to collect (Ohtomi & Irieda, 1997). In the present study, it was possible to collect long time series data that enabled the study of spatiotemporal and bathymetric distribution patterns together with the population dynamics of *M. kyushuensis* in Kagoshima Bay. The present study confirms the bathymetric distribution range of *M. kyushuensis* from around 80 m to around 230 m. It is important to note that, during the present study, no sampling could be conducted at depths < 77 m due to steep slopes on both sides of the bay (Matumoto, 1943) and a heavy congestion of fixed gear (set-nets, traps, etc.) set by commercial fisheries (Ohtomi *et al.*, 2018). Wide bathymetric distribution has also been reported for another *Metapenaeopsis* species, *M. sibogae* in the bay (see Chapter 5, Section 5.2). Even though *M. kyushuensis* showed wide spatial distribution in the bay, the shrimp was mostly dominant in the northern and southern areas of the central basin. The distribution of shrimps and other crustaceans is known to depend on several environmental factors, including water temperature, salinity, bottom sediment, and food availability, which influence population densities and set geographical limits to their distribution (Abelló *et al.*, 1988; Cartes *et al.*, 2002; Fanelli & Cartes, 2004; Ohtomi *et al.*, 2018). The abundance and biomass of *T. curvirostris* was found to be significantly correlated with water temperature (Cha *et al.*, 2004a). However, the bottom-water temperature and salinity remained stable in Kagoshima Bay (see Figure 5.2.8), as also reported by several studies that in the deeper-waters (>100 m) of the bay, temperature and salinity tend to be stable throughout the year (e.g., Oki, 1989; Ahamed & Ohtomi, 2011; Rahman& Ohtomi, 2017, 2018a), and was thus not considered as a crucial factor for determining the distribution and relative abundance of *M. kyushuensis* in the bay.

Bottom sediment is one of the key factors known to influence the spatial distribution of crustaceans (Wenner *et al.*, 1983; Abelló *et al.*, 1988). The bottom sediment of Kagoshima Bay

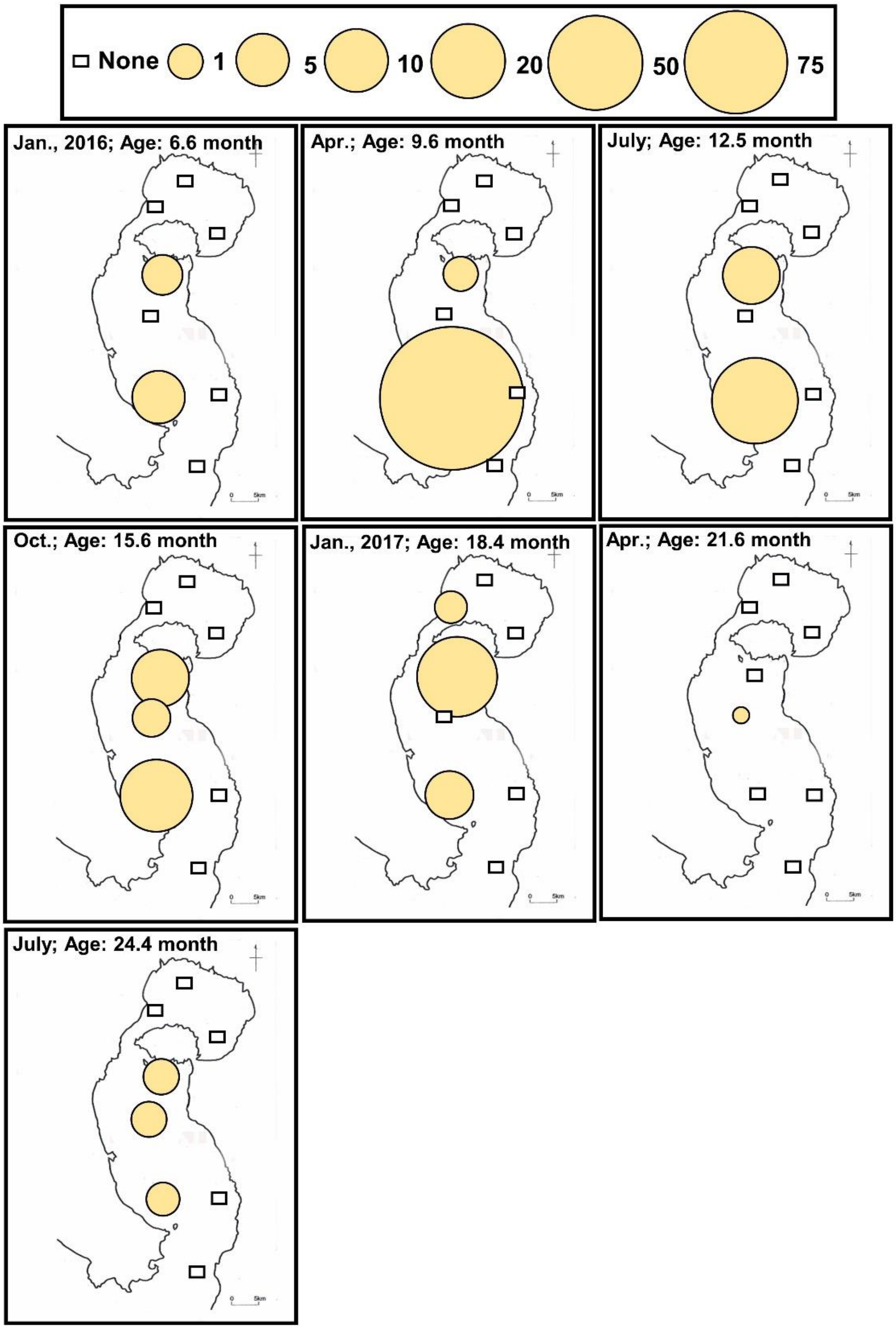


Figure 5.3.4. Distribution patterns with progression of age of a single cohort of female *Metapenaeopsis kyushuensis* at each sampling station in Kagoshima Bay, southern Japan.

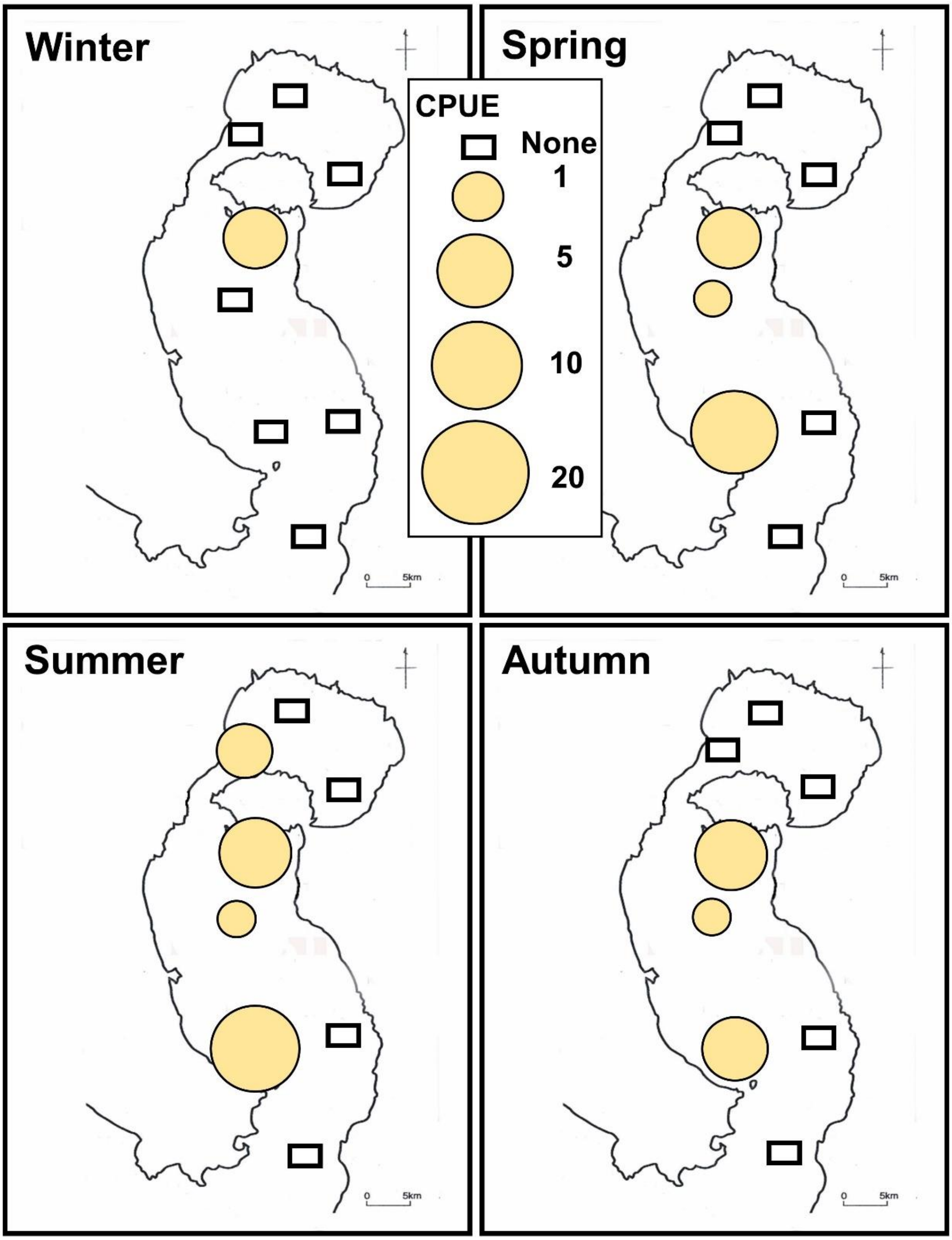


Figure 5.3.5. Mean catch per unit effort (CPUE) of mature females of *Metapenaeopsis kyushuensis* at each sampling station in Kagoshima Bay, southern Japan.

has been reported to vary among distinct areas (Ohtomi *et al.*, 2018), and it might have an impact on determining the distribution and relative abundance of *M. kyushuensis*. The shrimp was distributed throughout the bay where the bottom sediment varied from silty to silty-sand, however, they were most abundant in areas where the bottom is made of silty sediment. The influence of bottom sediment characteristics on distribution was also reported for several penaeid shrimps, such as *T. curvirostris* in the eastern Mediterranean Sea (Galil, 2000), *P. longirostris* in the Mediterranean Sea and eastern northern Atlantic Ocean (Heldt, 1954; Holthuis, 1980), and *M. sibogae* in Kagoshima Bay (see Chapter 5, Section 5.2). Interestingly, *M. kyushuensis* was significantly more abundant in the central basin than in the bay head, though the sediment characteristics of these two areas were similar. Even within central basin, *M. kyushuensis* was more abundant in the southern part (comparatively shallower) than the deeper northern and central area. It is, therefore, hypothesized that water depth might also be one of the factors behind the higher relative abundance of *M. kyushuensis* in the southern areas than that in the northern and central areas of the central basin and in the bay head. There is, however, no specific factor could be identified in this study. Water current might have an impact on determining the distribution patterns of crustaceans among other possible influencing factors such as primary food resources (Guijarro *et al.*, 2012) and light intensity (Cartes *et al.*, 1993; Aguzzi *et al.*, 2007), and are recommended to clarify in the future studies.

Recruitment of *M. kyushuensis* was found to occur in the northern and southern areas of the central basin, which seemed to be synchronized with the main spawning grounds of the species, however, with a very low CPUE. Females of *M. kyushuensis* were first recruited in winter (December) and the recruitment continued until mid-spring (April). It was therefore hypothesized that the lower abundance of juveniles at the age of 6.6 months could have resulted from the ongoing recruitment process. The drastic increase in relative abundance at the age of 9.6 months might be indicating the completion of recruitment. Similar findings have been reported for another two shrimp in the bay, *M. sibogae* (see Chapter 5, Section 5.2) and *P. izumiae* (Ohtomi *et al.*, 2018). Several studies reported the influence of hydrological factors in the recruitment dynamics of shrimp, and smaller individuals appeared to be distributed in regions shallower than their main distribution areas (Cartes *et al.*, 2008; Massuti *et al.*, 2008; Palmas *et al.*, 2015). The comparatively deeper recruitment areas of *M. kyushuensis* in Kagoshima Bay might be an adaptation of this shrimp to the unique topography of the bay, as was also reported for *M. sibogae* (see Chapter 5, Section 5.2) and *P. izumiae* (Ohtomi *et al.*, 2018). Future studies to reveal the details of larval settlement and the recruitment patterns of this shrimp are recommended.

A rapid decrease in the CPUE was observed at the age of 12.5 month. By the age of 12.5 months, most of the shrimp become sexually mature (see Chapter 2, Section 2.3). It is very likely that mass mortality might have occurred after the first spawning event. Thereafter, the CPUE of the selected cohort gradually decreased with an increased tendency for individuals of inhabiting the deeper waters being observed before disappearing from the time series of length-frequency distributions. The tendency of larger-sized individuals to inhabit deeper waters of their distribution areas is a well described feature in shrimps including some penaeids such as- *T. curvirostris* in Sendai Bay (Kosaka, 1979), *P. lanceolatus* (Farhana & Ohtomi, unpublished) and *M. sibogae* (see Chapter 5, Section 5.2) in Kagoshima Bay.

Seasonal differences in CPUE of *M. kyushuensis* were also observed in the bay, with higher values recorded in spring versus lower values in winter. These differences might be related to the life history of the shrimp. The new cohort of *M. kyushuensis* first appeared in winter; however, this cohort was fully recruited in the following spring and continued to thrive and grow in size through the summer and autumn, which was reflected in comparatively higher CPUE of the shrimp in these seasons. On the other hand, in winter, two age groups were present. However, the CPUE of both age groups were significantly low as the new one just started to be recruited in the fishery, whereas the older one was drastically reduced after spawning. These phenomena resulted in the overall lower CPUE values in winter. The present study recorded a significant decrease in the relative abundance of *M. kyushuensis* during 2009-2012 followed by a slight increase in the subsequent years. The decrease in *M. kyushuensis* population in the bay coincided with the rapid increase of *M. sibogae* population in the bay. The main distribution areas of *M. kyushuensis* in the bay are similar with those of *M. sibogae*. Therefore, habitat competition might have been a cause for the decrease of the *M. kyushuensis* population in the bay, however, more detail studies are needed.

The present study revealed the distribution patterns and population dynamics of *M. kyushuensis* for the first time, which would clarify the life history of this shrimp and thereby will aid in formulating effective strategies for its sustainable management. Studies on the influence of primary food resources and light intensity on the distribution patterns of this shrimp are recommended in order to make the picture of its life cycle in Kagoshima Bay complete. It was not possible to separate the males of *M. kyushuensis* from the males of *M. provocatoria owstoni*, and therefore, the population dynamics of female individuals were studied. No significant differences have been reported between the trends of population dynamics between males and females in *P. izumiae* (Ohtomi *et al.*, 2018) and *M. sibogae* (see Chapter 5, Section 5.2) in the bay. Therefore, the population dynamics of female *M. kyushuensis* can be considered as a whole picture for the species. It is however crucial to distinguish the males of these species through genetic analysis, which would also lead to the development of morphological identification key, and is recommended for future studies.

5.4: DISTRIBUTION PATTERNS AND POPULATION DYNAMICS OF *METAPENAEOPSIS PROVOCATORIA OWSTONI*

5.4.1. INTRODUCTION

Information on the distribution patterns of a species including the spatiotemporal distributions and spawning ground is crucial for the inference of stock structure, and ultimately for fisheries management (Frank & Brickman, 2001; Smedbol & Stephenson, 2001). In addition, research on nursery and spawning areas is essential for effective management of vulnerable stages of the life cycle of a species (FAO, 2003). *M. provocatoria owstoni* is a small penaeid shrimp distributed in the waters of Japan, Korea and Taiwan (Shinomiya & Sakai, 2000). It is one of the dominant *Metapenaeopsis* species in Kagoshima Bay and is gaining in commercially importance. There is, however, no study available on the distribution patterns and population dynamics of this species that are essential for exploring the recruitment patterns, nursery and spawning grounds, possible migratory behavior in order to manage the fishery successfully. The present study therefore aimed at revealing the distribution patterns and population dynamics of *M. provocatoria owstoni* for the first time using samples collected from Kagoshima Bay.

5.4.2. MATERIALS AND METHODS

5.4.2.1. *Sampling procedures and data analysis*

The present study was conducted in the semi-enclosed deep-water Kagoshima Bay, southern Japan (see Figure 5.2.1). The bay was demarcated into four areas based on the oceanographic and bathymetric characteristics following Ohtomi *et al.* (2018): **(1) bay head**, a semi-circular enclosure running up to 140 m in depth; **(2) central basin**, with maximum water depths reaching about 230 m; **(3) channel area**, connecting the bay head and central basin; and **(4) bay mouth**, opening to the Pacific and the East China Sea to the south and west, respectively. Eight sampling stations were established in these areas: Sts. 1 and 2 in the bay head, St. 3 in the channel area, Sts. 4, 5 and 7 in the central basin, and Sts. 6 and 8 in the bay mouth (see Table 5.2.1).

Samples of *M. provocatoria owstoni* were collected by experimental trawl surveys conducted in the bay from February 2004 to December 2017. Monthly sampling was conducted in 3 or 4 stations while seasonal sampling (January, April, July, and October) was conducted from all 8 stations. In the present study, seasons were defined following Ohtomi *et al.* (2018) as: winter (December to February), spring (March to May), summer (June to August) and autumn (September to November). Sampling was conducted onboard the training vessel "Nansei-Maru" (175 t) of the Faculty of Fisheries, Kagoshima University. The vessel is equipped with an onboard split-beam quantitative echo-sounder (KFC-3000, KAIJO, Japan) and global positioning system (GPS) (JLR-7700 MK-II Navigator, WMJ Marine, USA) for navigation. A total of 697 trawls were conducted using a simple trawl net carrying canvas kites on the tip of the wings (LC-VI, Nichimo Co., Japan). The net was 23.5 m long and 8.0 m wide with mesh size of 37.9 mm and 20.2 mm at the net body and cod end, respectively (Ohtomi *et al.*, 2004). Towing was conducted during the daytime for a preset tow duration of 10 min at a speed of 2 kt. At each haul, net depth was recorded at 1 min intervals using submersible data loggers (Compact-TD ATD-HR, JFE Advantech, Japan) attached to the head rope of the net. In addition, water depth was recorded at same intervals with the echo-sounder (KFC-3000, KAIJO, Japan).

Individuals of *M. provocatoria owstoni* were sorted out of each haul based on the shape of a petasma in males or a thelycum in females. *M. provocatoria owstoni* is morphologically similar with *M. kyushuensis*. The females of these two species can be identified based on the differences in thelycum (see Figure 3.3.2A, B). However, the shape and size of petasma in males were similar, and it was impossible to distinguish the males of these two species (see Figure 3.2.2C). Therefore, during data analysis, the number of males were divided into two portions based on the ratio of the females of *M. provocatoria owstoni* and *M. kyushuensis* in the sample. Individuals were counted onboard and chilled immediately in ice. The samples were then transferred to the laboratory on the same day and fixed in 10% formalin pending further analysis. For the females collected during January 2013 to December 2017, individual carapace length (CL), the shortest distance between the posterior margin of the orbit and the mid-dorsal posterior edge of the carapace, was measured using a digital slide caliper (CD-15PS, Mitutoyo, Japan) to the nearest 0.01 mm. Females collected during the same period were macroscopically classified as immature, maturing or mature based on the ratio of ovary width to body width taken at the middle of the first abdominal segment (Rahman & Ohtomi, 2017) for the estimation of spawning ground.

Effective tow durations were determined from initial and final times of net contact with the sea bottom using logger-echo-sounder depth plots according to Fulanda & Ohtomi (2011). The mouth opening height of the trawl net used in the present study was 2.5 ± 0.3 m (mean ± SD) (Fuwa *et al.*, 2010). Therefore, the trawl net contact with the sea bottom was confirmed as long as ≤ 2.8 m difference between the echo sounder and the compact TD-logger readings was maintained (Fulanda & Ohtomi, 2011). The catch per unit effort (CPUE) was standardized to a 10 min preset tow duration according to Fulanda & Ohtomi (2011):

CPUE = Number of individuals collected per haul × 10 / Effective tow duration (min).

The CPUE was regarded as a measure of relative abundance of *M. provocatoria owstoni*.

5.4.2.2. *Population dynamics of M. provocatoria owstoni*

Due to the limited number of individuals that could be collected per month throughout the study period, the data collected from January 2013 to December 2017 were merged and considered as a one-year dataset. The dynamics of *M. provocatoria owstoni* population in

Kagoshima Bay was then studied by identifying different cohorts in the seasonal samples, assigning age and tracing their distribution patterns. To serve this purpose, monthly length-frequency distributions of females were constructed with 1 mm intervals of CL (see Figure 3.4.3). A series of component normal distributions were fitted to the length-frequency of each sample, using a computer analysis (Microsoft Excel-add-in-Solver) based on Hasselblad's maximum likelihood method (Hasselblad, 1966). Each component normal distribution was assumed to represent an age group in the population. Then, ages in months were assigned to the mean CLs belonging to each of the cohorts by assigning 1st June, the approximate midpoint of the main spawning season, May-June (see Chapter 2, section 2.4), as the birth date (see Chapter 3, Section 3.4). In cases, where two normal distributions representing two different age groups overlapped, individuals were separated into two age groups using a discriminate function: $Z_i = (L_m \times \sigma_n + L_n \times \sigma_m) / (\sigma_m + \sigma_n) - L_i$, where L_m is the mean CL and σ_m is the standard deviation at age m; L_n is the mean CL and σ_n is the standard deviation at age n; and L_i is the CL of individual i. If $Z_i > 0$, i belonged to m age group; if $Z_i < 0$, i belonged to n age group.

5.4.2.3. *Spawning grounds*

The spawning grounds were defined as the areas near the sampling stations where mature females occurred. The main spawning grounds were defined as the areas near the sampling stations where ≥ 25% of the total mature females occurred based on CPUE analysis.

5.4.3. RESULTS

5.4.3.1. *Spatiotemporal distribution*

A total of 2978 individuals of *M. provocatoria owstoni* were collected from Kagoshima Bay during the present study. CPUE values varied significantly among surveys at different stations ranging from 0 to 111, with highest mean CPUE at St. 7 (22 ± 7) followed by St. 4 (8

± 4) in the central basin, while the lowest mean CPUE was recorded at Sts. 6 in the bay mouth (1 ± 1) (Figure 5.4.1).

Seasonal and temporal variations in mean CPUE of *M. provocatoria owstoni* are shown in Figures 5.4.2 and 5.4.3. The mean CPUE was the highest in winter (6 ± 8) followed by spring (5 ± 9), while the lowest mean CPUE was recorded in autumn (3 ± 5) followed by summer (4 ± 9). *M. provocatoria owstoni* was most abundant in Sts. 7 (23 ± 7) and 4 (7 ± 4) in the central basin in all the seasons, however, the highest CPUE was recorded in St. 7 in spring whereas it was higher in winter in St. 4. In the bay head, channel area and in the bay mouth, the CPUEs were low throughout the year. Analysis of mean CPUE during the study period revealed that *M. provocatoria owstoni* population gradually declined from 2004 to 2008. A rapidly decline can be noticed during 2009 to 2014. Thereafter, a slight increase in the population has been recorded.

5.4.3.2. *Population dynamics of M. provocatoria owstoni*

The distribution patterns with progression of age revealing the population dynamics of female *M. provocatoria owstoni* showed that juveniles at the age of 7.5 months were distributed throughout the distribution areas with a low mean CPUE (1 ± 2). The CPUE of juveniles was, however, significantly higher in St. 7. As age progressed (at the age of 10.5 months), their abundance increased significantly throughout the bay with the highest CPUE was recorded in the southern area of the central basin. At the age of 13.6 months, a rapid decline in CPUE was observed and the individuals were mostly distributed in the central basin. Thereafter, individuals started to show the tendency to inhabit the deeper areas of the bay as increased CPUEs were recorded at northern and central part of the central basin (at the ages of 19.5 months and 22.5 months). The CPUEs of this cohort afterwards were minimal before finally disappearing from the time series of the length-frequency distributions.

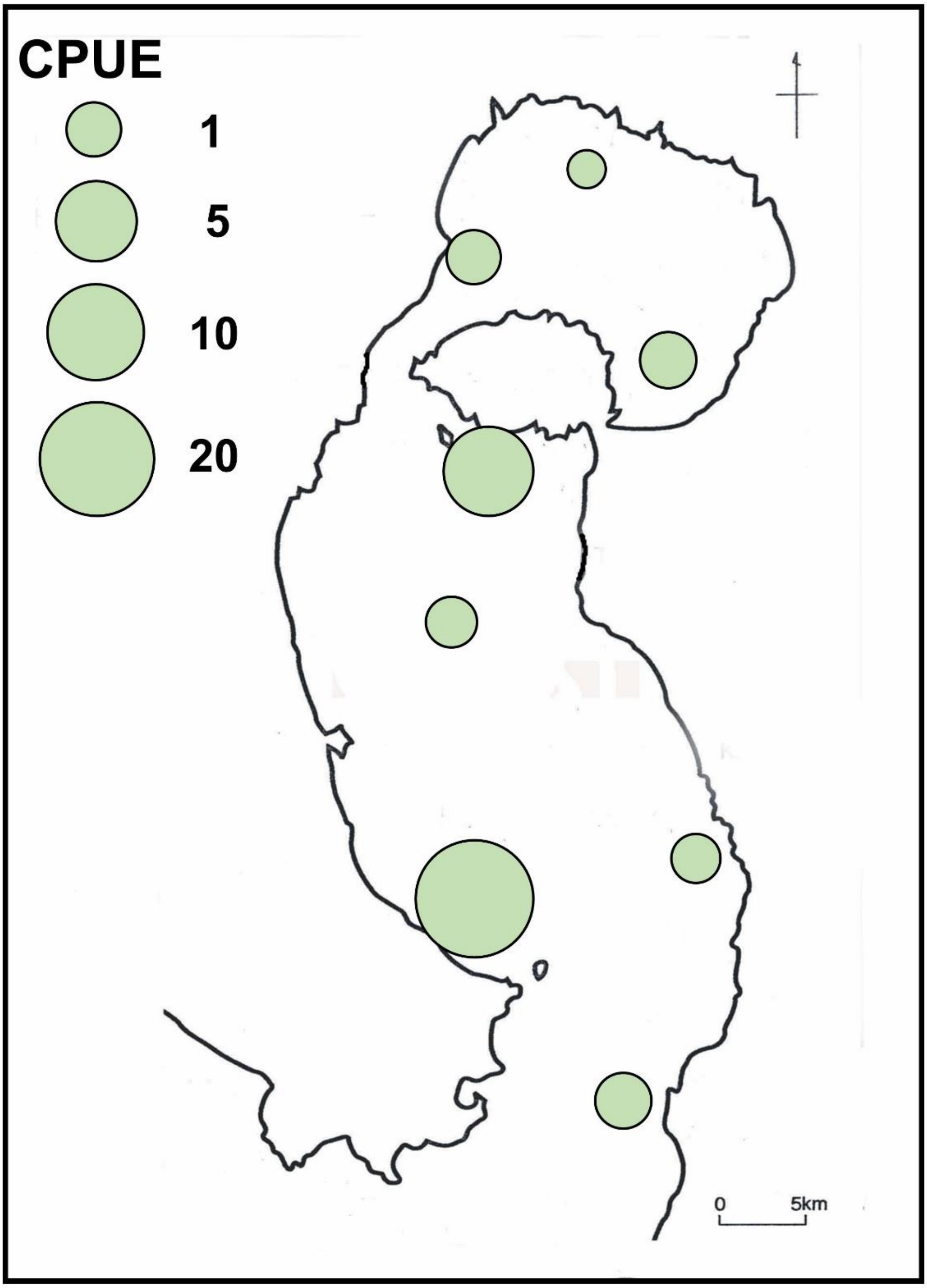


Figure 5.4.1. Mean catch per unit effort (CPUE) of *Metapenaeopsis provocatoria owstoni* at each sampling station in Kagoshima Bay, southern Japan during February 2004 to October 2017.

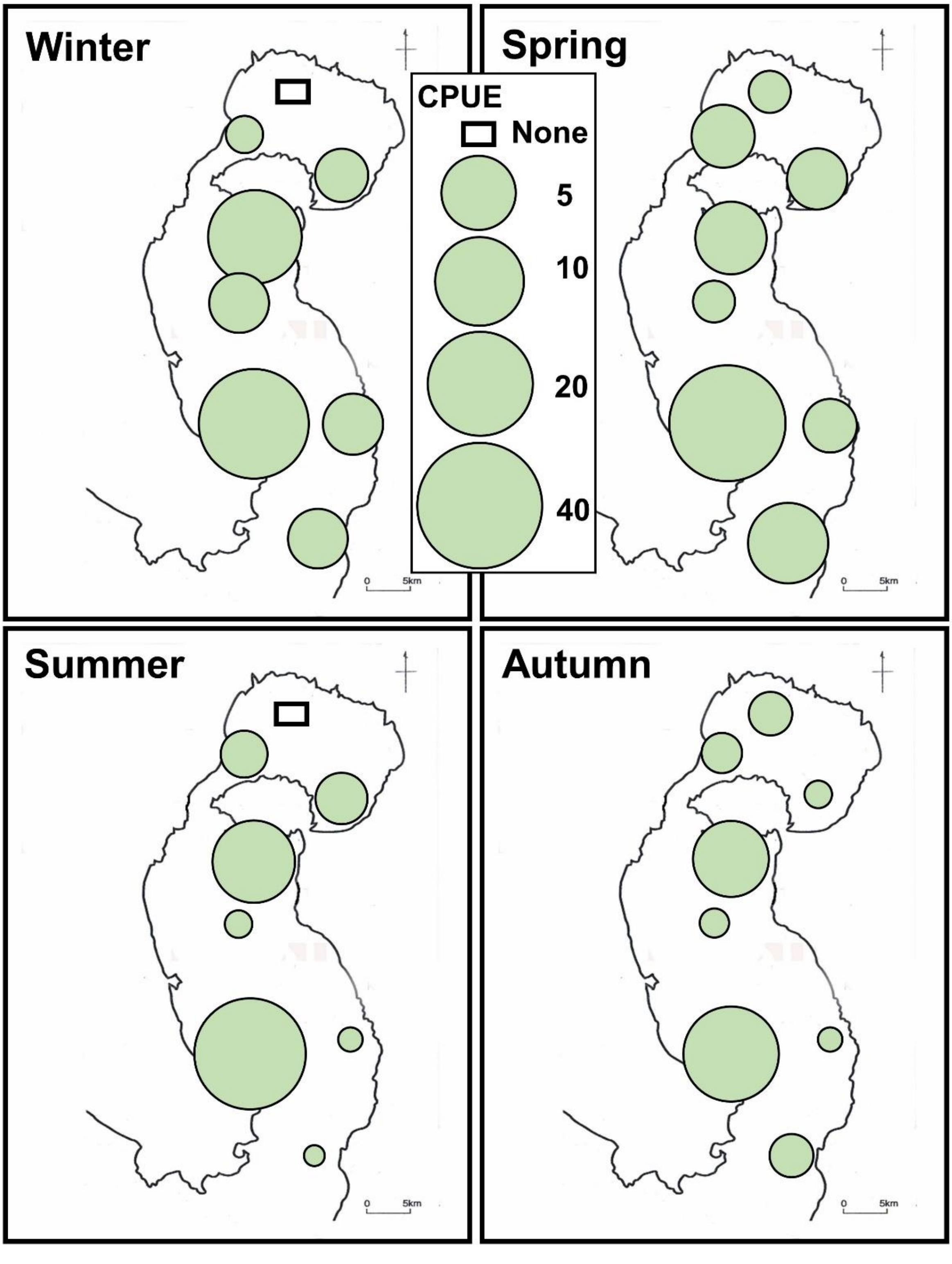
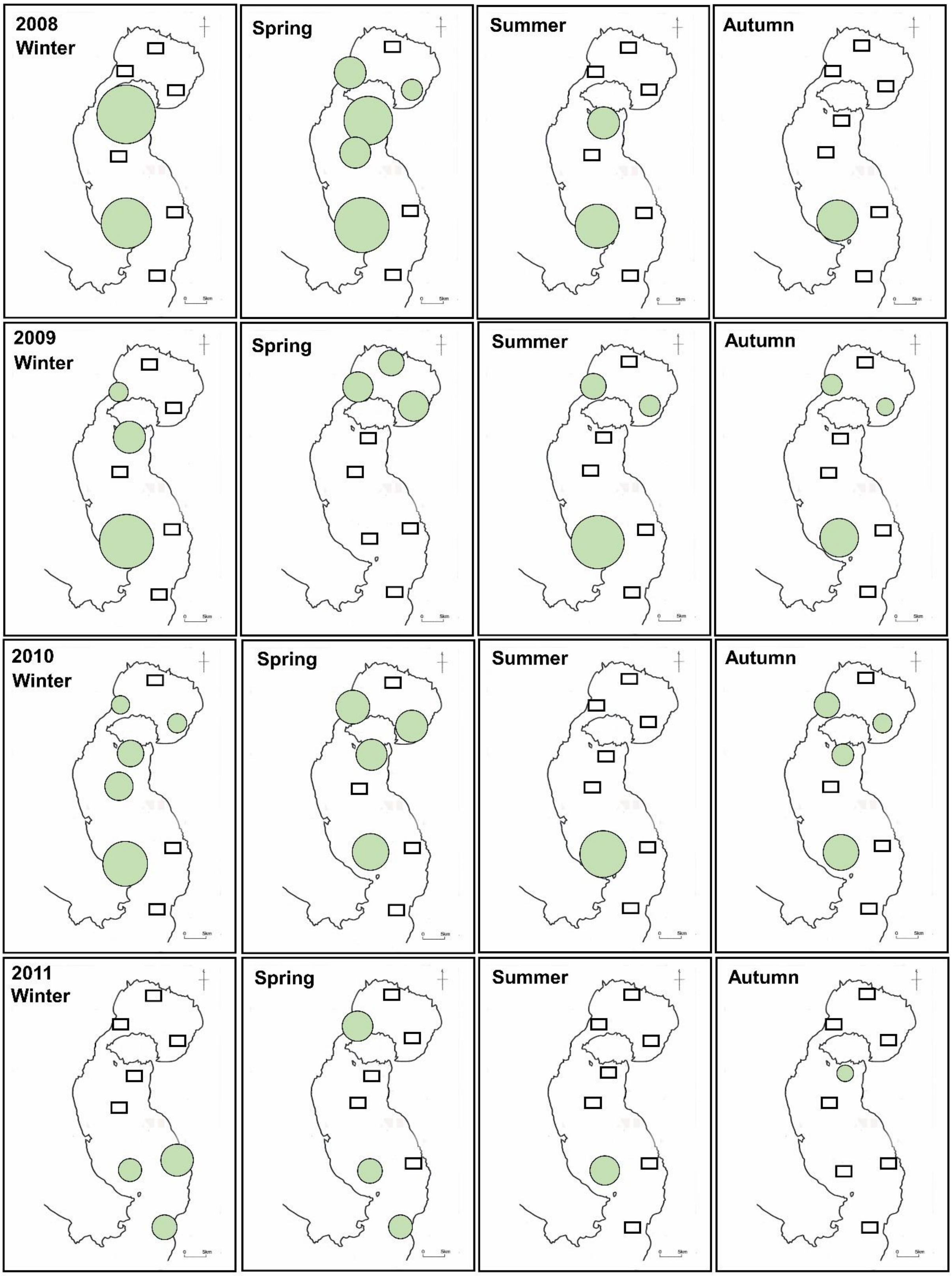


Figure 5.4.2. Seasonal changes in mean catch per unit effort (CPUE) of *Metapenaeopsis provocatoria owstoni* at each sampling station in Kagoshima Bay, southern Japan during February 2004 to October 2017.

Figure 5.4.3. Seasonal and temporal changes in mean catch per unit effort (CPUE) of *Metapenaeopsis provocatoria owstoni* at each sampling station in Kagoshima Bay, southern Japan during February 2004 to October 2017.



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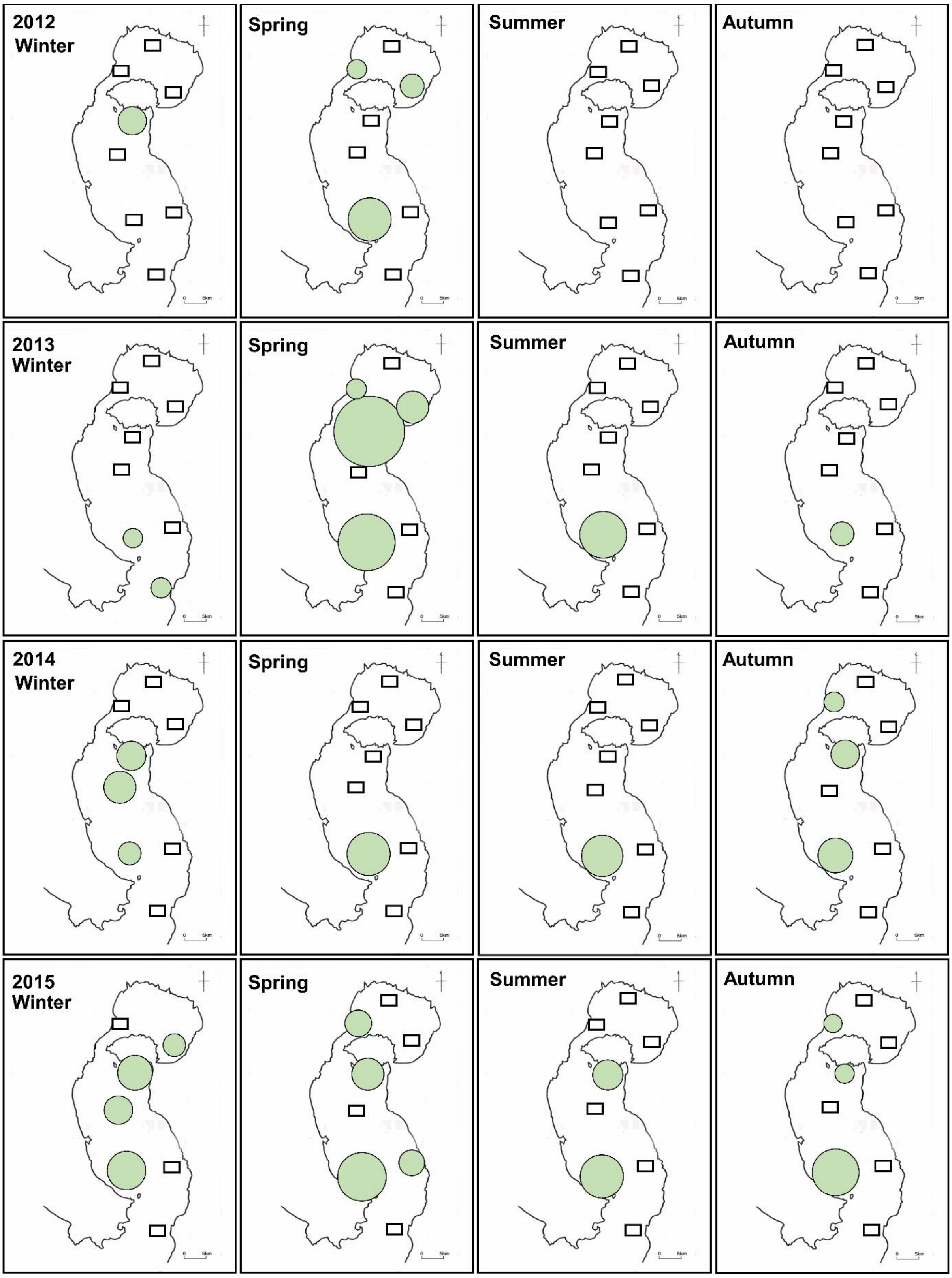

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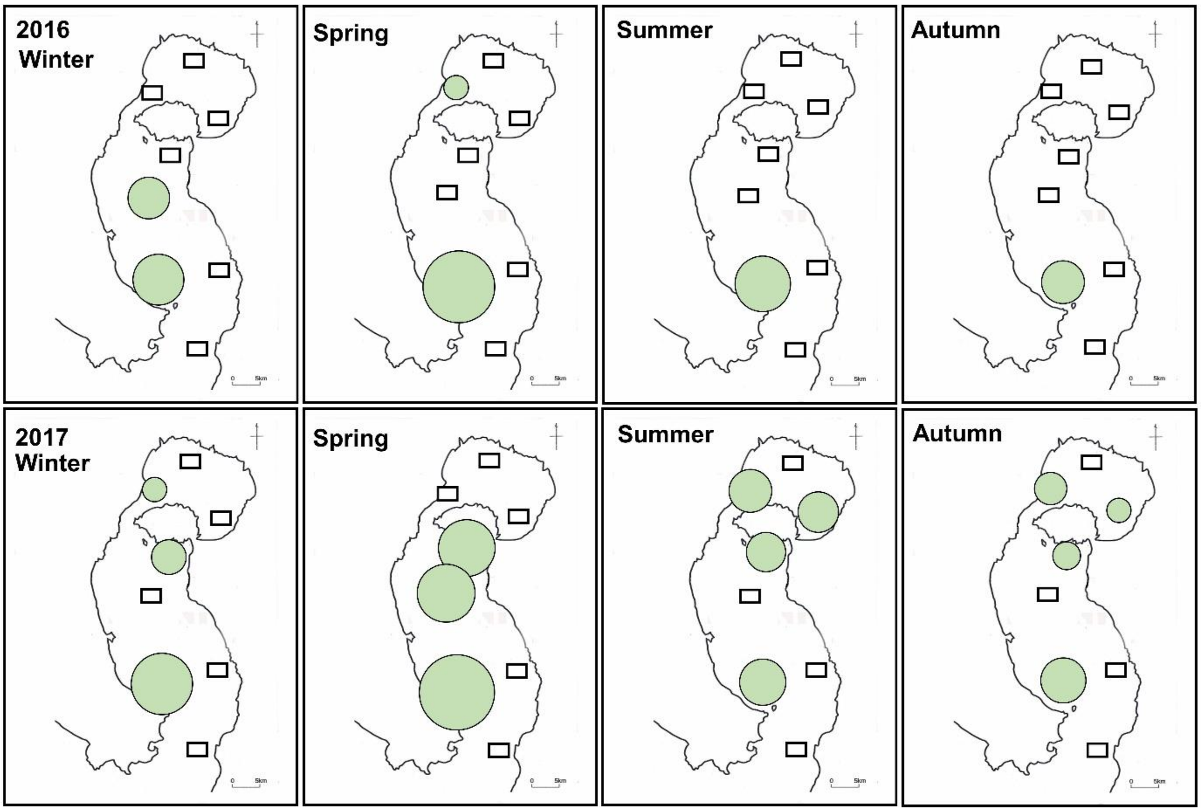


Figure 5.4.3. continued

5.4.3.3. *Spawning grounds*

Mature females of *M. provocatoria owstoni* occurred in the southern area of the central basin and in the bay head (Figure 5.4.5). Mean CPUE of mature females during the main spawning season were highest at St. 7 followed by St. 1 and more than 75% of the mature females were distributed in the southern area of the central basin. It was therefore concluded that *M. provocatoria owstoni* spawned in areas with an average water depth ranging from ~130 m to ~140 m in Kagoshima Bay with the southern area of the central basin being the main spawning grounds.

5.4.4. DISCUSSION

The population dynamics of a species is indispensable for supporting its optimal exploitation and sustainable management regimes, which will illustrate the species recruitment patterns, nursery and spawning grounds, mortality, possible migratory behavior, and will aid to explain the seasonal distribution and abundance patterns (Ohtomi *et al.*, 2018). Such studies on deep-water shrimp populations are, however, quite difficult to conduct because large samples over a long period of time are hard to collect (Ohtomi & Irieda, 1997). In the present study, it was possible to collect long time series data that enabled the study of spatial, temporal and bathymetric distribution patterns together with the population dynamics of *M. provocatoria owstoni* in Kagoshima Bay.

The present study confirms the bathymetric distribution range of *M. provocatoria owstoni* from 77 m to around 230 m. It is important to note that, during the present study, no sampling could be conducted at depths < 77 m due to steep slopes on both sides of the bay (Matumoto, 1943) and a heavy congestion of fixed gear (set-nets, traps, etc.) set by commercial fisheries (Ohtomi *et al.*, 2018). Wide bathymetric distribution has also been reported for another two *Metapenaeopsis* species in the bay, *M. sibogae* and *M. provocatoria owstoni* (see

Chapter 5, Sections 5.2 and 5.3). *M. provocatoria owstoni* showed wide spatial distribution in the bay, however, they were predominant in the northern and southern areas of the central basin, more specifically the southern area. Similar distribution patterns were also recorded for *M. kyushuensis* in the bay. The distribution of shrimps and other crustaceans is known to depend on several environmental factors, including water temperature, salinity, bottom sediment, and food availability, which influence population densities and set geographical limits to their distribution (Abelló *et al.*, 1988; Cartes *et al.*, 2002; Fanelli & Cartes, 2004; Ohtomi *et al.*, 2018). The abundance and biomass of *T. curvirostris* was found to be significantly correlated with water temperature (Cha *et al.*, 2004a). However, the bottom-water temperature and salinity remained stable in the bay (see Figure 5.2.8), as several studies have also reported that the temperature and salinity in the deeper-waters (>100 m) of Kagoshima Bay tend to be constant throughout the year (e.g., Oki, 1989; Ahamed & Ohtomi, 2011; Rahman & Ohtomi, 2017, 2018a), and was thus not considered as a crucial factor for determining the distribution and relative abundance of *M. provocatoria owstoni* in the bay.

Bottom sediment characteristic is one of the key factors influencing spatial distribution of crustaceans (Wenner *et al.*, 1983; Abelló *et al.*, 1988). According to Ohtomi *et al.* (2018), the bottom sediment of Kagoshima Bay varied among distinct areas and might have an impact on the distribution and relative abundance of *M. provocatoria owstoni*. The influence of bottom sediment characteristics on distribution was reported for several penaeid shrimps, such as *T. curvirostris* in the eastern Mediterranean Sea (Galil, 2000), *P. longirostris* in the Mediterranean Sea and eastern northern Atlantic Ocean (Heldt, 1954; Holthuis, 1980), and *M. sibogae* and *M. kyushuensis* in Kagoshima Bay (see Chapter 5, Sections 5.2 and 5.3). *M. provocatoria owstoni* was distributed throughout the bay with bottom sediment varied from silty to silty-sand, however, they were most abundant in areas where the bottom is made of silty sediment. Remarkably, *M. provocatoria owstoni* was significantly more abundant in the central basin

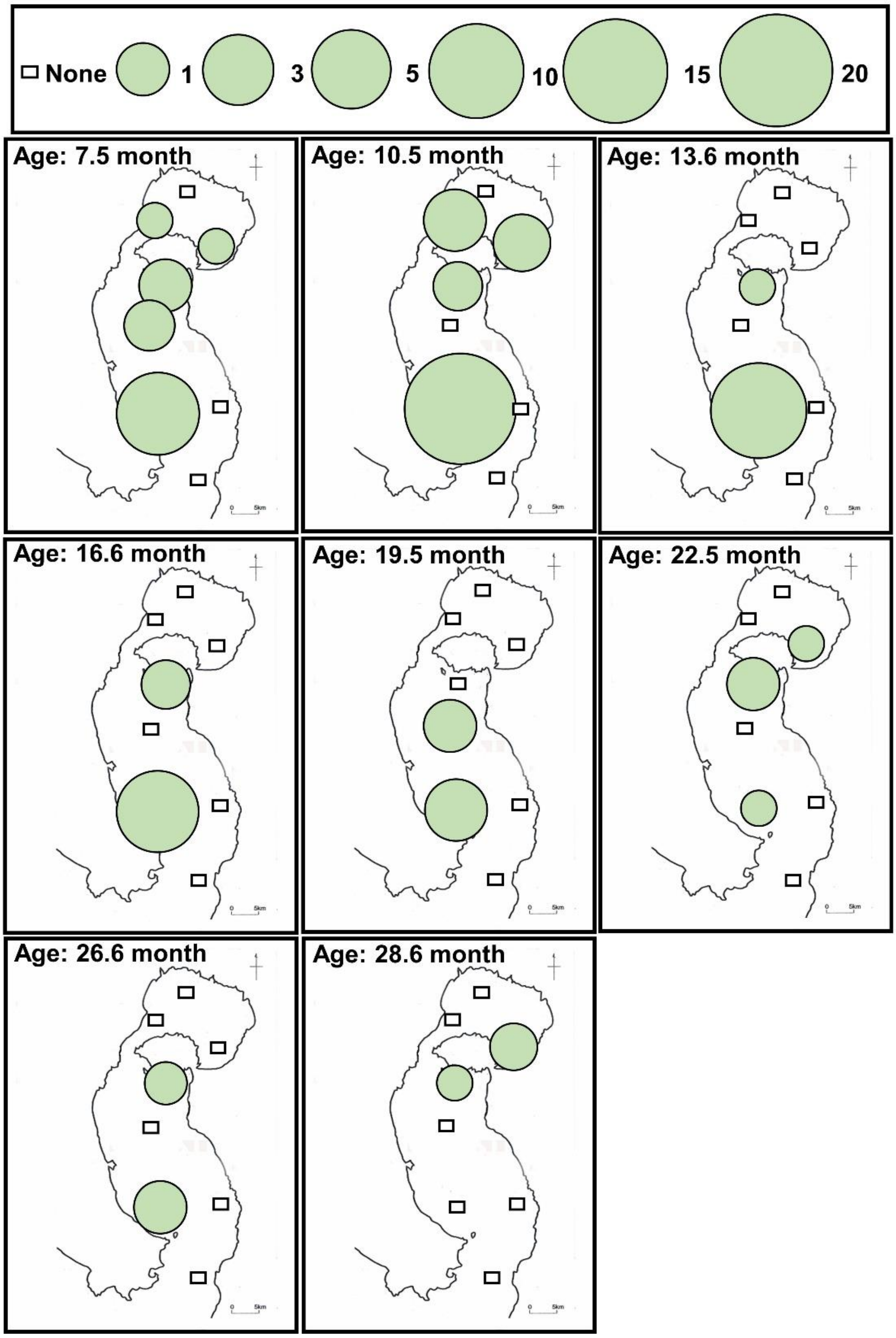


Figure 5.4.4. Distribution patterns with progression of age of a single cohort of female *Metapenaeopsis provocatoria owstoni* at each sampling station in Kagoshima Bay, southern Japan.

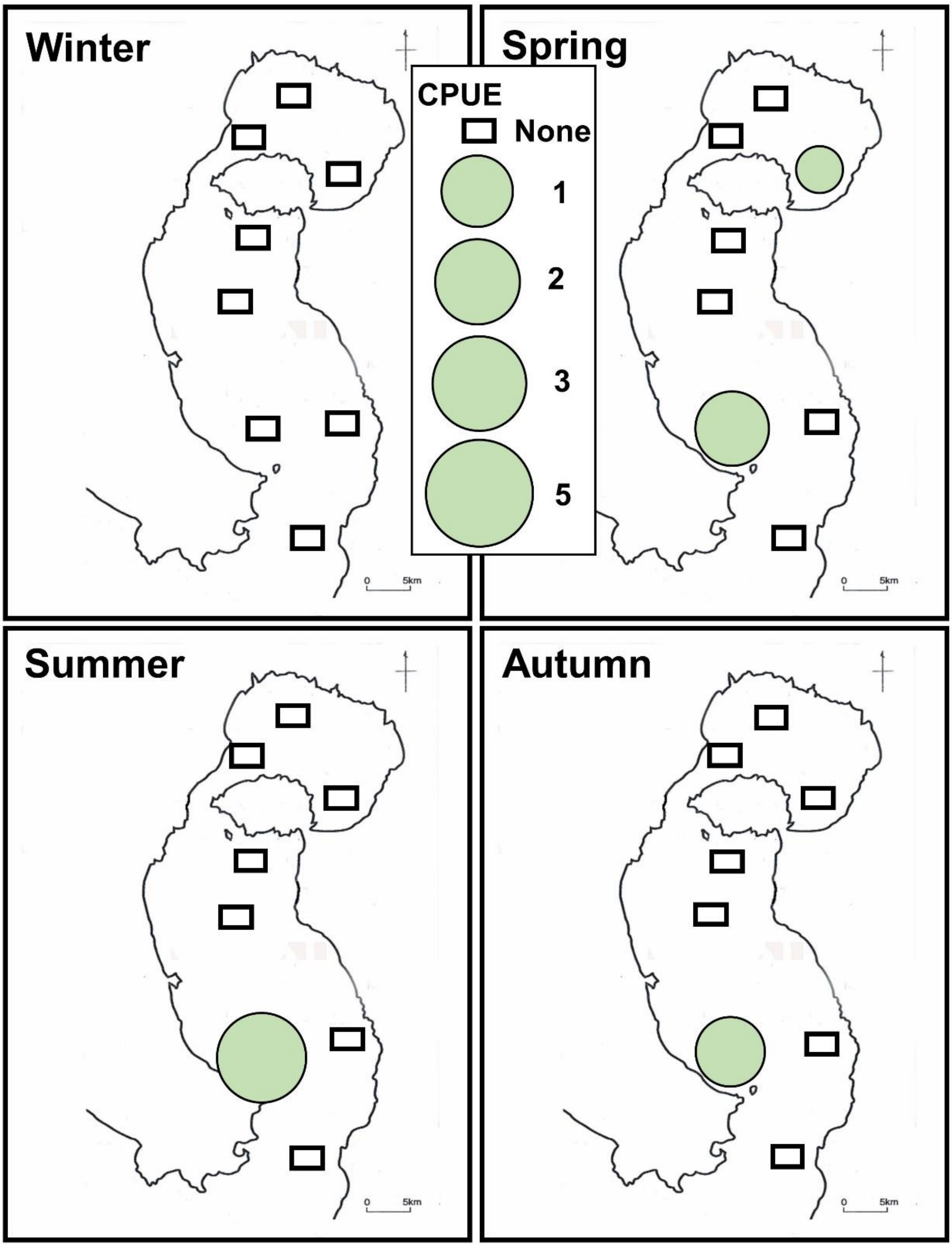


Figure 5.4.5. Mean catch per unit effort (CPUE) of mature females of *Metapenaeopsis provocatoria owstoni* at each sampling station in Kagoshima Bay, southern Japan.

than in the bay head, though the sediment characteristics of these two areas were similar. Even within the central basin, *M. provocatoria owstoni* was abundant in the southern area (comparatively shallower) than the deeper northern and central area. It is, therefore, hypothesized that water depth might be one of the factors behind the higher relative abundance of *M. provocatoria owstoni* in the southern area than that in the northern and central areas of the central basin and in the bay head. Depth is considered to be one of the major factors influencing the spatial distribution of penaeid shrimps (Dall *et al.*, 1990; Somers, 1994). Similar hypothesis has also been made for *M. sibogae* and *M. kyushuensis* in the bay. The distribution patterns of *M. sibogae, M. kyushuensis* and *M. provocatoria owstoni* in bay were similar, however, whereas *M. sibogae* was more abundant in the northern area of the central basin, *M. kyushuensis* and *M. provocatoria owstoni* were more abundant in the southern and comparatively shallower area of the central basin. Water current might have an impact on determining the distribution patterns of crustaceans among other possible influencing factors such as primary food resources (Guijarro *et al.*, 2012) and light intensity (Cartes *et al.*, 1993; Aguzzi *et al.*, 2007), and are recommended to clarify in the future studies.

Recruitment of *M. provocatoria owstoni* occurred throughout its distribution areas, with higher CPUE recorded at the southern area of the central basin, which seemed to be synchronized with the main spawning grounds of the species. Females of *M. provocatoria owstoni* were first recruited in autumn (November) and the recruitment continued until mid-spring (April). The significant increase in relative abundance at the age of 10.5 months might be indicating the completion of recruitment. Similar findings have been reported for another three shrimps in the bay, *M. sibogae* (see Chapter 5, Section 5.2), *M. kyushuensis* (see Chapter 5, Section 5.3) and *P. izumiae* (Ohtomi *et al.*, 2018). Several studies reported the influence of hydrological factors in the recruitment dynamics of shrimp, and smaller individuals appeared to be distributed in regions shallower than their main distribution areas (Cartes *et al.*, 2008;

Massuti *et al.*, 2008; Palmas *et al.*, 2015). The comparatively deeper recruitment areas of *M. provocatoria owstoni* in Kagoshima Bay might be an adaptation of this shrimp to the unique topography of the bay, as was also reported for *M. sibogae* (see Chapter 5, Section 5.2), *M. kyushuensis* (see Chapter 5, Section 5.2) and *P. izumiae* (Ohtomi *et al.*, 2018). Future studies to reveal the details of larval settlement and the recruitment patterns of this shrimp are recommended.

A rapid decrease in the CPUE was observed at the ages of 13.6 months and 16.6 months. By the age of 13.6 months, most of the females become sexually mature (see Chapter 2, Section 2.3), and by 16.6 months most of them gone through their first spawning event. It is therefore very likely that mass mortality might have occurred after the first spawning event. Thereafter, the CPUE of the selected cohort gradually decreased with an increased tendency of individuals of inhabiting the deeper waters being observed before disappearing from the time series of length-frequency distributions. The tendency of larger-sized individuals to inhabit deeper waters of their distribution areas is a well described feature in shrimps including some penaeids such as *T. curvirostris* in Sendai Bay (Kosaka, 1979), *P. lanceolatus* (Farhana & Ohtomi, unpublished), *M. sibogae* (see Chapter 5, Section 5.2) and *M. kyushuensis* (see Chapter 5, Section 5.3) in Kagoshima Bay. Specific factors relating to this phenomenon, however, remains unexplored.

Seasonal differences in CPUE of *M. provocatoria owstoni* were also observed in the bay, with higher values recorded in winter and spring versus lower values in summer and autumn. These differences might be related to the life history of the shrimp. The new cohort of *M. provocatoria owstoni* first appeared in late autumn (November); however, this cohort continued to be recruited in winter before being fully recruited in the following spring before being significantly reduced in summer. On the other hand, the second cohort was also present in winter, but the CPUE of this cohort was minimal in spring, summer and autumn as it approaches maximum life expectancy. These phenomena resulted in the overall higher CPUE values in winter and spring and lower values in summer and autumn. The present study recorded a significant decrease in the relative abundance of *M. provocatoria owstoni* during 2009-2014 followed by a slight increase in the subsequent years. A similar decreasing trend was also observed in the *M. kyushuensis* population in the bay, and this decreasing trend coincided with the rapid increase of *M. sibogae* population in the bay. The main distribution areas of *M. provocatoria owstoni* and *M. kyushuensis* in the bay are similar with that of *M. sibogae*. Therefore, habitat competition might have been a cause for the decrease of *M. kyushuensis* population in the bay, however, more detail studies are recommended.

The present study revealed the distribution patterns and population dynamics of *M. provocatoria owstoni* for the first time, which would clarify the life history of this shrimp and thereby will aid in formulating effective strategies for its sustainable management. Further studies on the influence of primary food resources, light intensity and diel pattern on the distribution of this shrimp are recommended in order to make the picture of its life cycle in Kagoshima Bay complete. In addition, the comparative distribution and abundance pattern analysis should be conducted for the major species of the bay to enlighten the possible impact of the newly recorded species, *M. sibogae* on the benthic community of the bay. It was not possible to separate the males of *M. provocatoria owstoni* from the males of *M. kyushuensis,* and therefore, only the population dynamics of female individuals were studied. No significant differences have been reported between the trends of population dynamics between males and females in *P. izumiae* (Ohtomi *et al.*, 2018) and *M. sibogae* (see Chapter 5, Section 5.2) in Kagoshima Bay. Therefore, the population dynamics of female *M. provocatoria owstoni* can be considered as a whole picture for the species. It is, however, vital to distinguish the males of these species through genetic analysis, which would also lead to the development of morphological identification key and is recommended for future studies.

CHAPTER 6: IDENTIFICATION OF PARASITES INFESTING *METAPENAEOPSIS* SPECIES IN KAGOSHIMA BAY AND ASSESSMENT OF PARASITIC IMPACT ON THE HOST

6.1. INTRODUTION

Kagoshima Bay is a semi-enclosed deep-water bay with a maximum water depth of more than 230 m. The bay is uniquely characterized by the steep slopes on both sides and the bottom topography influenced by volcanic formation (Matumoto, 1943). Despite its unique structure, bottom topography, and influence of volcanic eruption from one of the most active volcanoes of the world, Sakurajima, Kagoshima Bay is rich in benthic biodiversity, inhabited by 124 species from 33 different families of decapod crustaceans (Ohtomi *et al.*, 2008). There are 4 species and 2 subspecies of *Metapenaeopsis* inhabit the bay: *M. acclivis, M. palmensis, M. mogiensis mogiensis*, *M. kyushuensis, M. provocatoria owstoni*, *M. sibogae*, of which the later three species are dominant in the benthic community of the bay and are commercially important (Rahman & Ohtomi, unpublished).

Bopyrid isopods live on shrimp, brachyurans, and anomurans and cause a conspicuous bulge of the branchiostegite (Dall *et al.*, 1990). Bopyrids infesting penaeid shrimps grow with their hosts and have a similar longevity, although there is some loss of parasites as the shrimp approach asymptotic length (Owens & Glazebrook, 1985). Both growth and reproduction of the host can be affected by bopyrids (Abu-Hakima 1984; Chu & Leong, 1996; Choi *et al.*, 2004). There is no information available on parasite infestation of *M. sibogae, M. kyushuensis* and *M. provocatoria owstoni* from Kagoshima Bay. The present study therefore aimed at elucidating information on the occurrence and identification of parasites infesting these shrimp in addition to assess their vitiations in prevalence and impact on hosts' reproduction and growth.

6.2. MATERIALS AND METHODS

6.2.1. *Sampling*

Monthly samples were obtained from experimental trawl surveys conducted in Kagoshima Bay, southern Japan (31°25' N, 130°38' E) at depths of ~77 m to ~230 m with the training vessel *Nansei-Maru* (175 t) of the Faculty of Fisheries, Kagoshima University from January 2013 to December 2017. A simple trawl net (LC-VI; Nichimo Corporation, Tokyo), 23.5 m long, 37.9 mm mesh size in the body, 20.2 mm in the cod-end, carrying canvas kites on the tip of the wings (Ohtomi *et al.*, 2004) was used for sampling. The net was towed for a preset tow duration of 10 min at a speed of 2 knots. At each haul, target species were identified based on the shape of petasma in males or thelycum in females. It was not possible to distinguish between the males of *M. kyushuensis* and *M. provocatoria owstoni* due to the lack of identification key (see Chapter 3, Section 3.3). For the analysis of prevalence of parasite and assessing the parasitic impact on growth and reproduction of the host, samples collected during January 2014 to December 2015 were used for *M. sibogae*. For the same purpose, samples collected during January 2016 to December 2017 were used for *M. kyushuensis* while for *M. provocatoria owstoni*, samples collected during January 2013 to December 2017 were used. Seasons were defined following Ohtomi *et al.* (2018) as: winter (December to February), spring (March to May), summer (June to August) and autumn (September to November).

6.2.2. *Measurements*

For each individual, the occurrence of parasites was checked by observing the branchial chamber. In case of parasitized individuals, parasites were carefully removed and preserved in 70% alcohol. Parasite identification was done in collaboration with specialists from Kochi University and Ehime Prefecture Fisheries Research Center. Individual carapace length (CL) was measured to the nearest 0.01 mm from the posterior margin of the eyestalk to the dorsal midline of the posterior margin of the carapace with slide calipers (CD-15PS; Mitutoyo Corp., Kawasaki, Japan), and body weight (BW) was measured to the nearest 0.01 g by electronic balance (EB-430DW; Shimadzu Corp., Kyoto, Japan). The exoskeleton and muscles above the ovary of each female were removed, and the ovary and body widths were measured to the nearest 0.01 mm at the middle of the first abdominal segment. Ovaries were classified as one of three initial sexual maturity stages based on the ratio of ovary width to body width following Rahman & Ohtomi (2017): immature, ovary width < 1/5 body width; maturing, 1/5 body width ≤ ovary width < 1/3 body width; and mature, ovary width ≥ 1/3 body width. The whole gonad was then removed and weighed to the nearest 0.001 g using the electronic balance. The gonadosomatic index was calculated as:

$$\text{GSI (\%)} = 100 \times [\text{ovary weight} / \text{body weight}]$$

The GSI between parasitized females and non-parasitized females were compared using Mann-Whitney U test.

6.2.3. *Histological observation of the ovary*

The ovarian maturation of parasitized females was histologically observed. To server this purpose, ovaries of two parasitized females from each of *M. sibogae* (CL, 15.9-16.6 mm; GSI, 0.04-0.39%), *M. kyushuensis* (CL, 15.6-15.8 mm; GSI, 0.01-0.03%) and *M. provocatoria owstoni* (CL, 16.4-17.7 mm; GSI, 0.30-0.40%) samples were used. A small portion from the anterior regions of the abdominal lobes of each ovary was dehydrated in an ethanol series, infiltrated with paraffin, and sliced into 6 µm sections. The sections were stained with Mayer's hematoxylin and eosin, cover-slipped with a mounting medium, and examined under a microscope (DMLB; Leica, Wetzlar, Germany). The terminology and criteria for describing oocyte development and ovarian maturation followed Rahman & Ohtomi (2017).

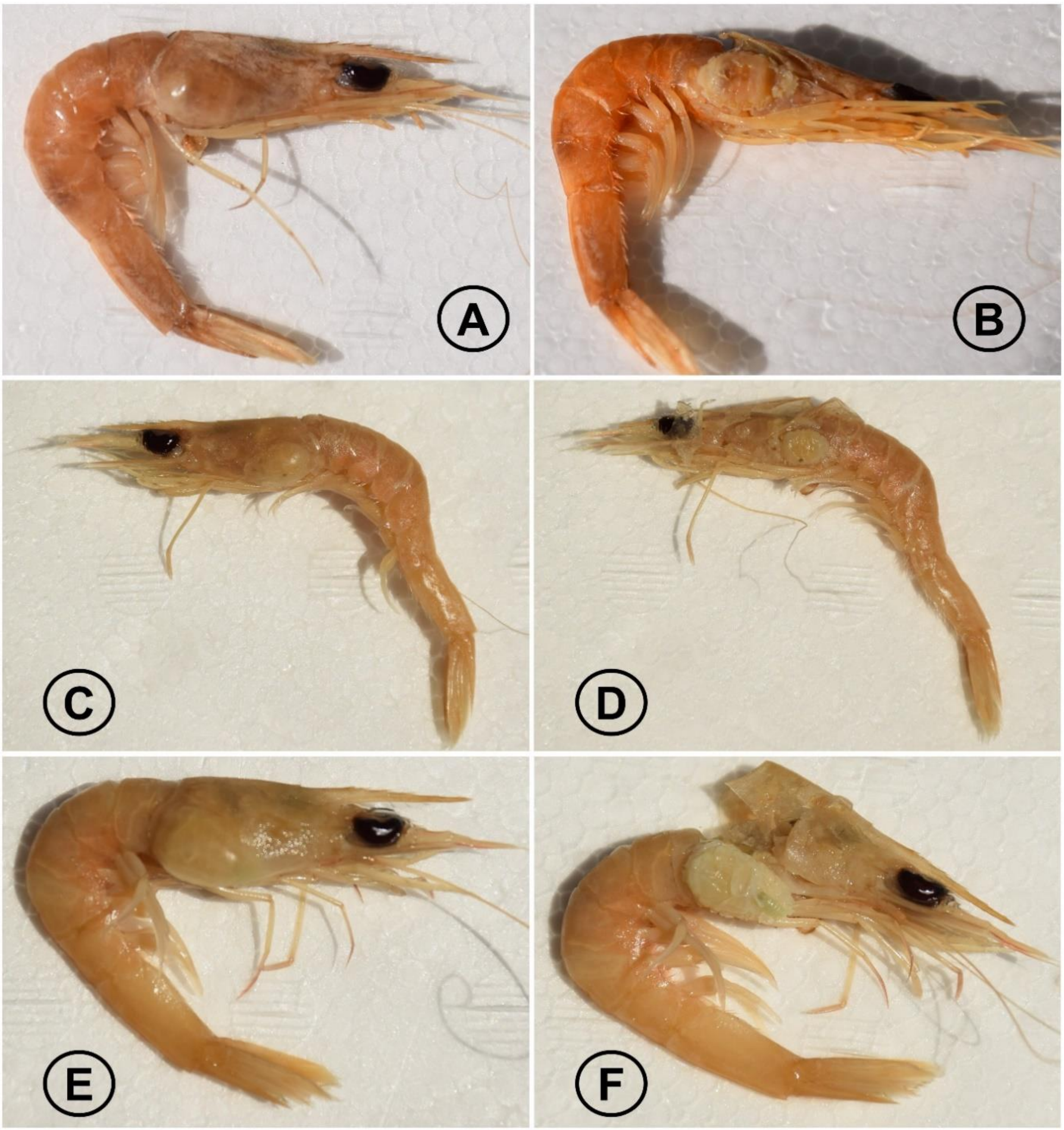


Figure 6.1. Occurrence of parasites in *Metapenaeopsis sibogae* (A, B), *M. kyushuensis* (C, D) and *M. provocatoria owstoni* (E, F) in Kagoshima Bay, southern Japan.

6.2.4. *Length-weight relationship*

The relationship between BW and CL was calculated based on the nonlinear power function using untransformed data as: BW = aCL^b, where, a and b are the regression coefficients. To define the growth type, further regression was performed using log-transformed data and the values of the allometric constant (b) were examined by Student's t-test (Sokal & Rohlf, 1987), using H_0: b = 3 (b = 3: isometry; b < 3: negative allometry; b > 3: positive allometry) (Hartnoll, 1982). Analysis of covariance (ANCOVA) was used to analyze the differences of the slopes and intercepts between the regression lines (Zar, 1996).

6.3. RESULTS

6.3.1. *Occurrence of parasites*

Parasites were found to occur in all the three studied *Metapenaeopsis* species (Figure 6.1). Female parasites filled most of and were tightly lodged within the host's branchial chamber, producing a distinguishing bulge in the host's branchiostegite. In *M. kyushuensis* and *M. sibogae,* male parasites oriented parallelely to the female parasite's body axis on the dorsal side of the female parasite's abdomen. On the other hand, male parasites oriented perpendicularly to the female parasite's body axis on the dorsal side of the female's abdomen in *M. provocatoria owstoni*. One male parasite was found to attach with one female parasite. In some cases, no male parasite could be traced.

6.3.2. *Identification of parasite*

Three different species of parasites were identified to infest the three species of *Metapenaeopsis* shrimps in the bay (Kume *et al.*, unpublished). *Minicopenaeon intermedium* Bourdon, 1981 was identified in a male specimen of *M. sibogae* (CL, 10.5 mm; BW, 0.87 g) (Figure 6.2). *Minicopenaeon liuruiyui* An, Boyko & Li 2013 was identified from a female specimen of *M. kyushuensis* (CL, 17.5 mm; BW, 3.41 g) (Figure 6.3). *Parapenaeon tertium*

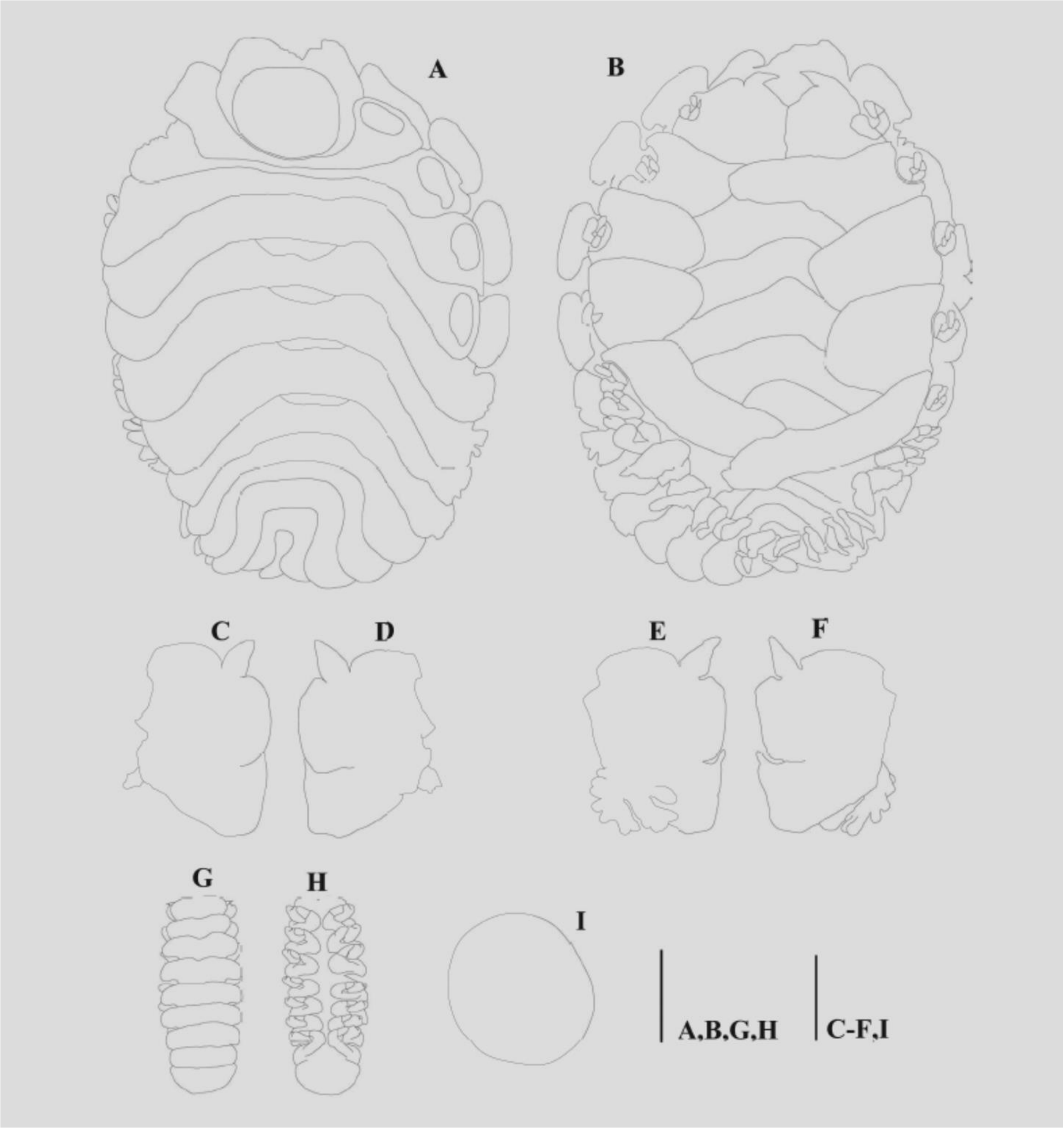


Figure 6.2. *Minicopenaeon intermedium* Bourdon, 1981, female (A-F, I) and male (G, H). A, dorsal view; B, ventral view; C, right maxilliped, external view; D, right maxilliped, internal view; E, left maxilliped and barbula, internal view; F, left maxilliped and barbula, external view; G, dorsal view; H, ventral view; I, egg. Scale bars, A, B, G, H, 1 mm; C-F, 0.5 mm; I, 0.1 mm (Kume *et al.*, unpublished).

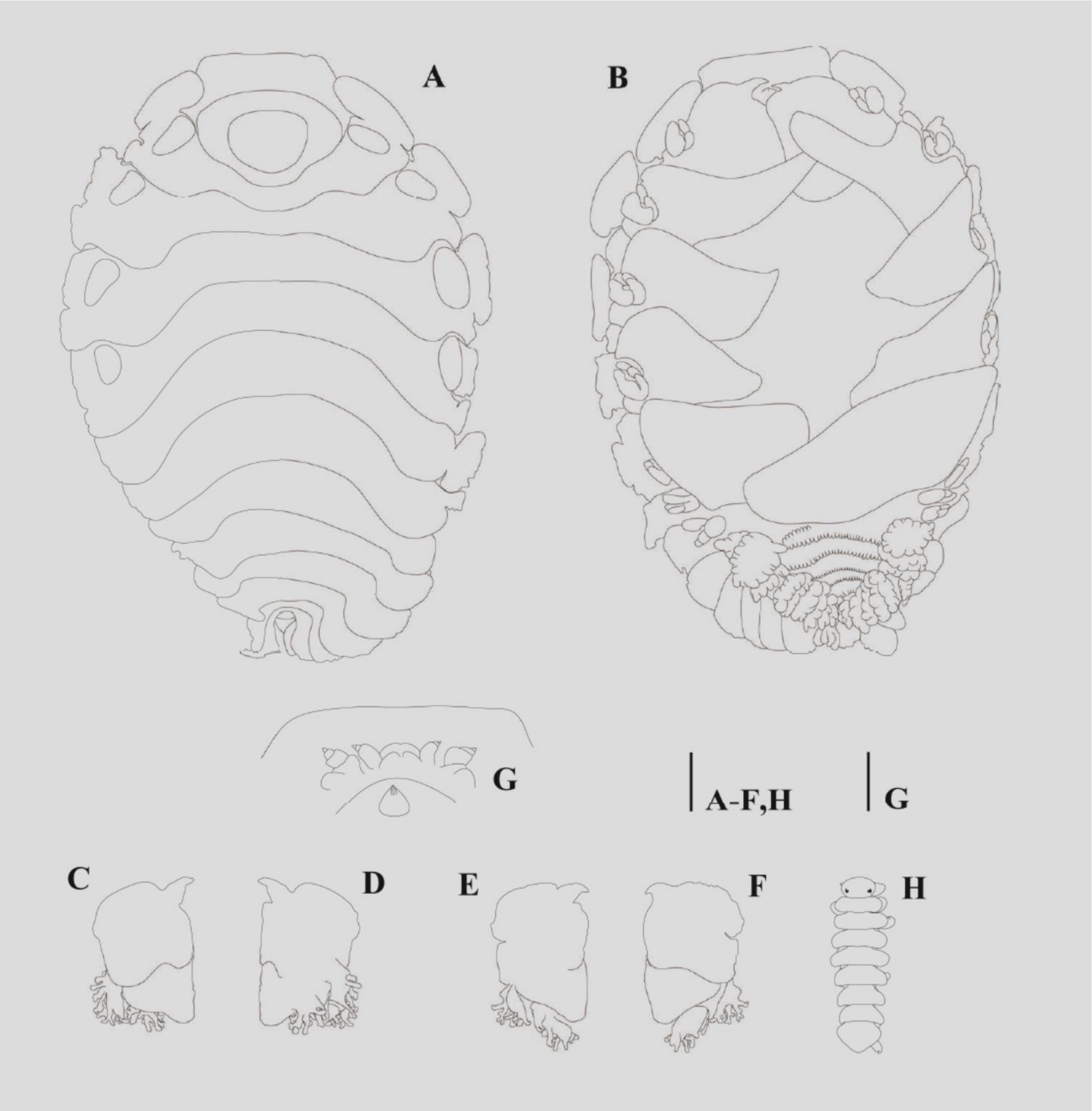


Figure 6.3. *Minicopenaeon liuruiyui* An, Boyko and Li, 2013, female (A-G) and male (H). A, dorsal view; B, ventral view; C, right maxilliped and barbula, external view; D, right maxilliped and barbula, internal view; E, left maxilliped and barbula, internal view; F, left maxilliped and barbula, external view; G, antennules and antenna; H, dorsal view. Scale bars, A-F, H, 1 mm; G, 0.5 mm (Kume *et al.*, unpublished).

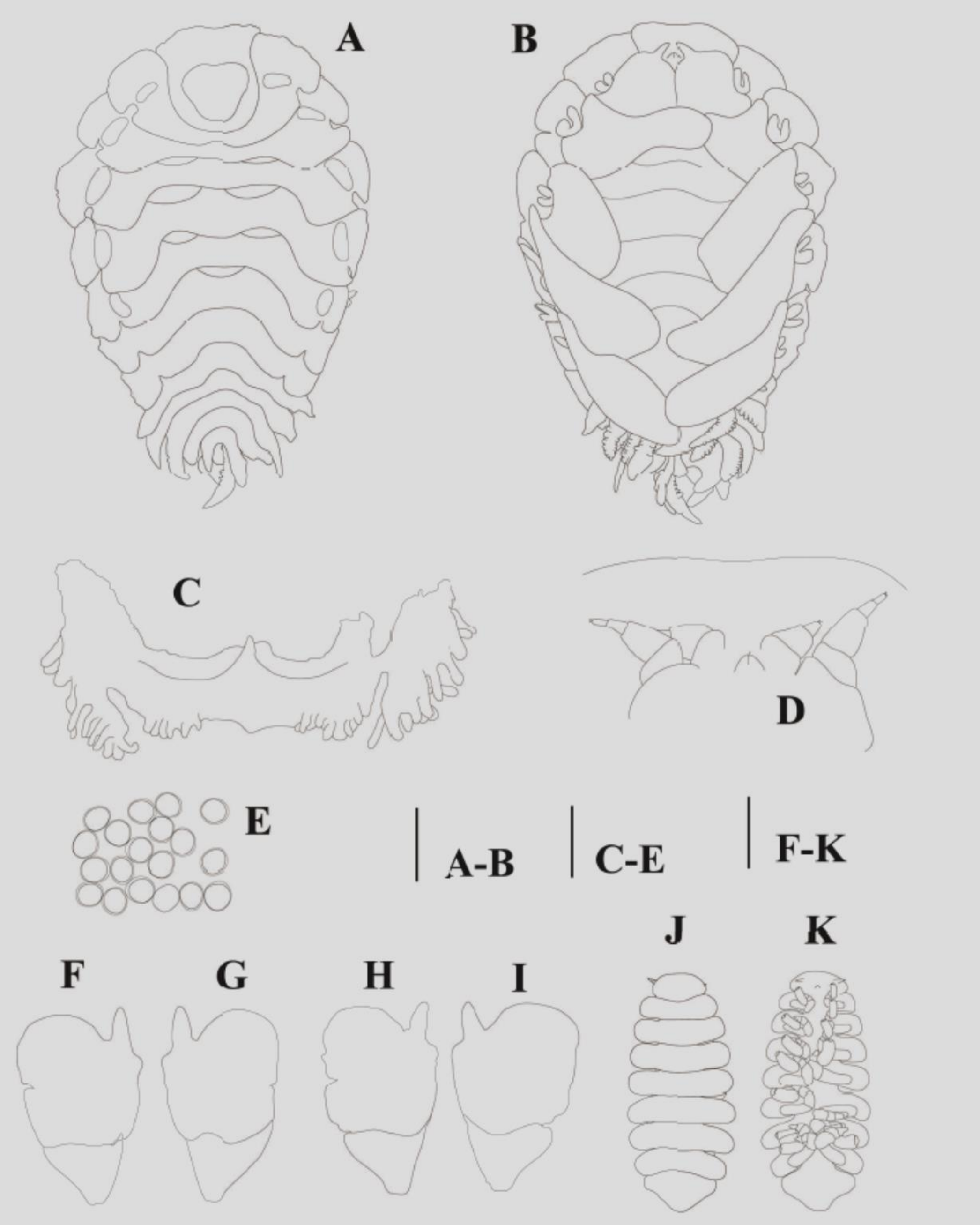


Figure 6.4. *Parapenaeon tertium* Nierstrasz and Brender à Brandis, 1932, female (A-I) and male (J, K). A, dorsal view; B, ventral view; C, barbula; D, antennules and antenna; E, egg; F, right maxilliped, external view; G, right maxilliped, internal view; H, left maxilliped, internal view; I, left maxilliped, external view; J, dorsal view; K, ventral view of male. Scale bars, A-B, 2 mm; C-E, 0.5 mm; J, K, 1 mm (Kume *et al.*, unpublished).

Nierstrasz and Brender à Brandi, 1932 was identified from a female of *M. provocatoria owstoni* (CL, 19.1 mm; BW, 4.49 g) (Figure 6.4).

6.3.3. *Variations in prevalence of parasite*

A total of 5433 males and 4342 females of *M. sibogae* were investigated, of which 0.07% were parasitized (0.07% each for both male and female) (Table 6.1). The highest and lowest infestation rates were recorded in autumn (0.15%) and winter (0.00%), respectively. A total of 2006 females of *M. kyushuensis* were investigated, of which 1.60% were found to be parasitized (Table 6.2). The highest and lowest infestation rates were recorded in autumn (3.65%) and winter (0.62%), respectively in female *M. kyushuensis*. Furthermore, a total of 640 females of *M. provocatoria owstoni* were investigated, of which 8.91% were parasitized (Table 6.3). The parasitic rate was highest in autumn (11.01%) and lowest in spring (7.34%) in female *M. provocatoria owstoni*. On the other hand, a total of 759 males of *M. kyushuensis* and *M. provocatoria owstoni* were investigated, of which 1.30% were parasitized (Table 6.4). The parasitic rate was highest in autumn (2.44%) and lowest in spring (0.57%).

6.3.4. *Impact of parasite infestation on the reproduction of host*

All the parasitized females of the studied three *Metapenaeopsis* species were found to be immature. Histological observation of the ovaries of the parasitized females showed that only oogonium-staged oocytes were present in the ovary (Figure 6.5). Oocytes of the parasitized females of *M. sibogae*, *M. kyushuensis* and *M. provocatoria* owstoni can not develop from the oogonium stage to further developmental stages. All the parasitized females were immature, whereas in case of non-parasitized females, all the three maturity stages were found.

The GSIs of parasitized females of *M. sibogae* ranged from 0.04-0.39%, whereas the GSI of the non-parasitized females ranged from 0.07-9.80%. The GSIs of the non-parasitized

Table 6.1. Variations in prevalence of bopyrid isopod infesting *Metapenaeopsis sibogae* in Kagoshima Bay, southern Japan during January 2014 to December 2015.

Season	**Number of non-parasitized individuals (Rate, %)**	**Number of parasitized individuals (Rate, %)**
Winter	2997 (100%)	0 (0.00%)
Spring	2339 (99.87%)	3 (0.13%)
Summer	2378 (99.96%)	1 (0.04%)
Autumn	2054 (99.85%)	3 (0.15%)
Overall	9768 (990.93%)	7 (0.07%)

Table 6.2. Variations in prevalence of bopyrid isopod infesting female *Metapenaeopsis kyushuensis* in Kagoshima Bay, southern Japan during January 2016 to December 2017.

Season	**Number of non-parasitized female (Rate, %)**	**Number of parasitized female (Rate, %)**
Winter	322 (99.38)	2 (0.62)
Spring	864 (99.08)	8 (0.92)
Summer	445 (98.02)	9 (1.98)
Autumn	343 (96.35)	13 (3.65)
Overall	1974 (98.40)	32 (1.60)

Table 6.3. Variations in prevalence of bopyrid isopod infesting female *Metapenaeopsis provocatoria owstoni* in Kagoshima Bay, southern Japan during January 2013 to December 2017.

Season	**Number of non-parasitized female (Rate, %)**	**Number of parasitized female (Rate, %)**
Winter	138 (89.03)	17 (10.97)
Spring	202 (92.66)	16 (7.34)
Summer	146 (92.41)	12 (7.59)
Autumn	97 (88.99)	12 (11.01)
Overall	583 (91.09)	57 (8.91)

Table 6.4. Variations in prevalence of bopyrid isopod infesting males of *Metapenaeopsis kyushuensis and M. provocatoria owstoni* in Kagoshima Bay, southern Japan during January to December 2016.

Season	**Number of non-parasitized individuals (Rate, %)**	**Number of parasitized individuals (Rate, %)**
Winter	174 (99.43%)	1 (0.57%)
Spring	396 (98.75%)	5 (1.25%)
Summer	109 (98.20%)	2 (1.80%)
Autumn	80 (97.56%)	2 (2.44%)
Overall	759 (98.70%)	10 (1.30%)

females were significantly higher than those of the parasitized females (*P* < 0.001). The GSI of parasitized females of *M. kyushuensis* ranged from 0.03-0.79%, whereas those of the non-parasitized females ranged from 0.07-9.43% (Table 6.5). The GSIs of the non-parasitized females were significantly higher than those of the parasitized females (*P* < 0.001). In case of parasitized females of *M. provocatoria owstoni*, the GSIs ranged from 0.02-0.95%, whereas the GSI of the non-parasitized females ranged from 0.05-9.29% (Table 6.6). The GSIs of the non-parasitized females were significantly higher than those of the parasitized females (*P* < 0.001). There was, however, no visible deformity observed in the structure of thelycum of the parasitized females of any of the studied *Metapenaeopsis* species. On the other hand, the petasma of the parasitized males did not grow in size in comparison to their non-parasitized counterparts.

6.3.5. *Effect of parasite infestation on the relative growth of the host*

The CL of the infested individuals of *M. sibogae* ranged from 10.4 to 15.8 mm. The CL of the infested females ranged from 12.2 to 18.6 mm in *M. kyushuensis*, whereas the CL of the infested females of *M. provocatoria owstoni* ranged from 8.4 to 22.6 mm. In case of males of *M. kyushuensis* and *M. provocatoria owstoni*, CL ranged from 12.8 to 17.9 mm. In all the cases, the relative growth of BW to CL was found to be negative allometric. The relative growth rate of BW in relation to CL was significantly lower in parasitized females than that of the non-parasitized females of *M. kyushuensis* (*P* < 0.01) (Figure 6.6). Similarly, the relative growth rate of BW to CL of parasitized females of *M. provocatoria owstoni* were significantly lower than that of the non-parasitized females (*P* < 0.05) (Figure 6.7).

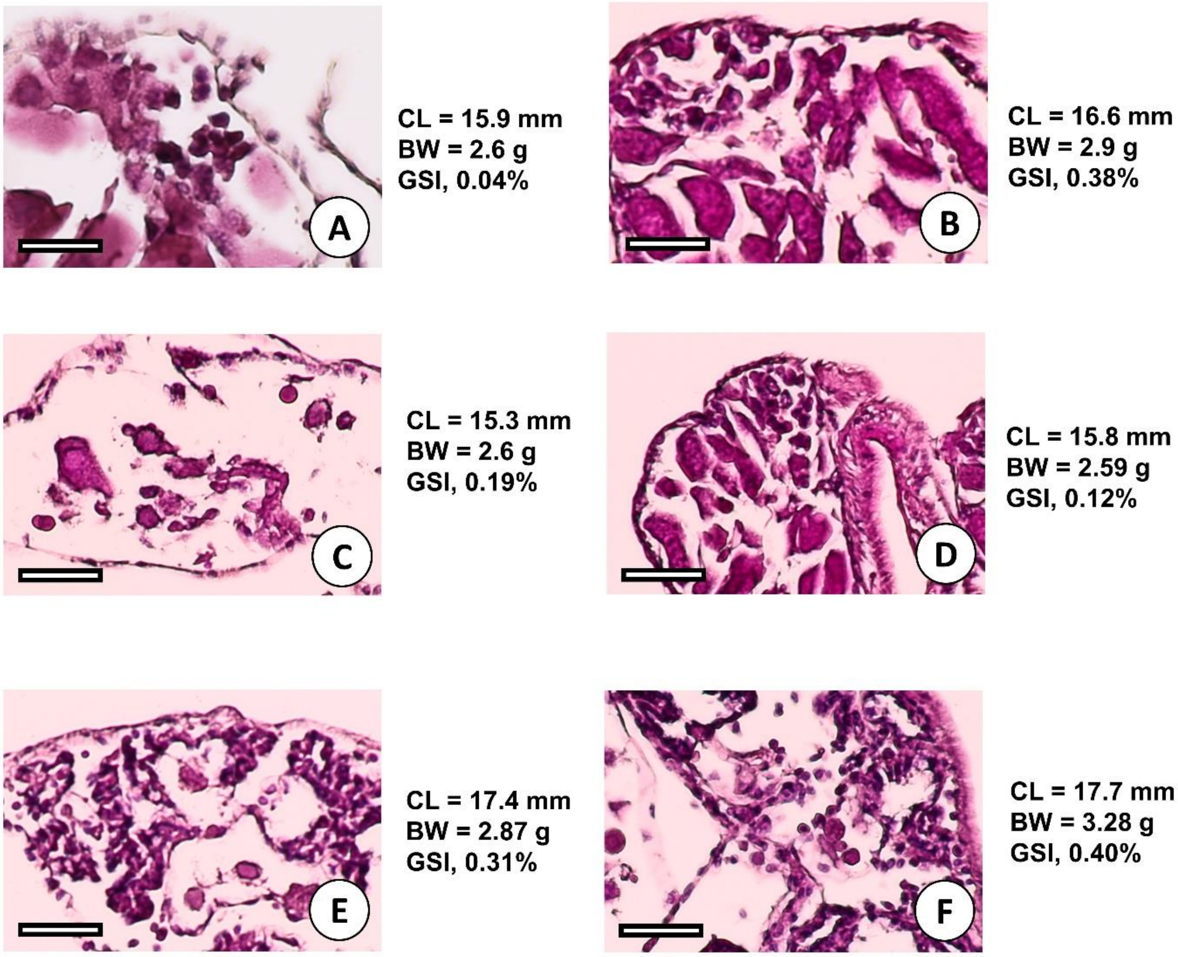


Figure 6.5. Histological sections of oocyte development of parasitized *Metapenaeopsis sibogae* (A, B), *M. kyushuensis* (C, D) and *M. provocatoria owstoni* (E, F). CL, carapace length; BW, body weight; GSI, gonadosomatic index. Scale bar = 20 µm.

Table 6.5. Size range, gonadosomatic index (GSI) and ovarian maturity status of the non-parasitized and parasitized females of *Metapenaeopsis kyushuensis* in Kagoshima Bay, southern Japan collected during January 2016 to December 2017.

	Non-parasitized female	**Parasitized female**
CL (mm)	7.2 to 19.7	12.2 to 18.6
BW (g)	0.29 to 4.62	1.43 to 3.60
GSI (%)	0.07 to 9.43	0.03 to 0.87
Immature	672	32
Maturing	1021	0
Mature	281	0

Table 6.6. Size range, gonadosomatic index (GSI) and ovarian maturity status of the non-parasitized and parasitized females of *Metapenaeopsis provocatoria owstoni* in Kagoshima Bay, southern Japan collected during January 2013 to December 2017.

	Non-parasitized female	**Parasitized female**
CL (mm)	6.0 to 21.2	8.4 to 22.6
BW (g)	0.18 to 5.95	0.51 to 6.70
GSI (%)	0.05 to 9.29	0.02 to 0.95
Immature	289	57
Maturing	231	0
Mature	63	0

6.4. DISCUSSION

Bopyrid isopods were found to infest all the three studied *Metapenaeopsis* species, however, the parasite species were found to be different in each species of shrimp (Kume *et al.*, unpublished). *M. intermedium* was identified from *M. sibogae*. Previously, *M. intermedium* had been reported from some other species of *Metapenaeopsis* (An *et al.*, 2013), however, in case of *M. sibogae,* this is the first record of infestation by a bopyrid isopod. *M. liuruiyui* was identified from *M. kyushuensis*. This is the first record of *M. liuruiyui* from Japanese waters and the first record from *M. kyushuensis*. The bopyrid isopod parasitizing *M. provocatoria owstoni* was identified as *P. tertium*. Previously, *P. tertium* was reported from Java, Indonesia to infest *Parapenaeus longipes* Alcock, 1905 (Nierstrasz & Brender à Brandis, 1932), and from Taiwan, where it infested *M. provocatoria owstoni* (Boyko, 2004). This is the first record of *P. tertium* and the first record from *M. provocatoria owstoni*.

The parasitic rate varied significantly among species, 0.07% in *M. sibogae*, 1.60% in female *M. kyushuensis,* 8.91% in female *M. provocatoria owstoni*, and 1.30% in male of *M. kyushuensis* and *M. provocatoria owstoni*. The difference in parasitic rates among these species could have resulted from their different habitat preferences, food habits and burrowing behaviors (Sakaji, 1995) apart from other possible influencing factors. The parasitic rate was reported to be 48% in case of *M. dalei* by the bopyrid *P. consolidatum* in waters of South Korea (Choi *et al.*, 2004), which was much higher than those reported for any of the studied *Metapenaeopsis* species. However, the parasitic rate was reported to be 0.24% in *Penaues longistylus* parasitized by *P. japonicum* and *Parapenaeon* prox. e*xpansus* in Queensland's east coast (Courtney, 1991). Prevalence of parasites fluctuated during the sampling years, as was observed for other bopyrid-host pairs (Beck, 1980; Somers & Kirkwood, 1991; Choi *et al.*, 2004). Seasonal fluctuations have also been observeds with the highest parasitic rates occurring in autumn for all the species. Such fluctuations are ascribed to the differential migration

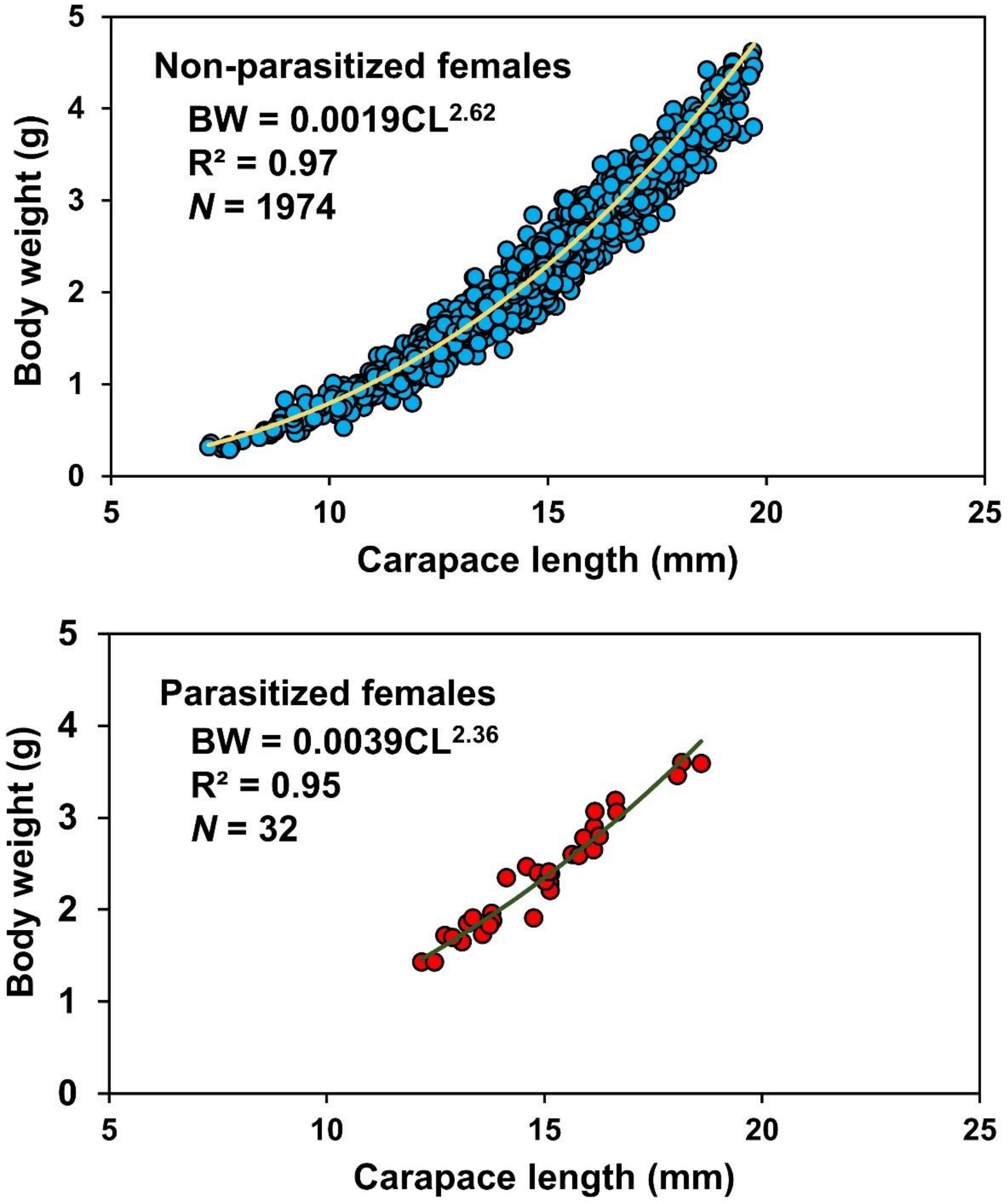
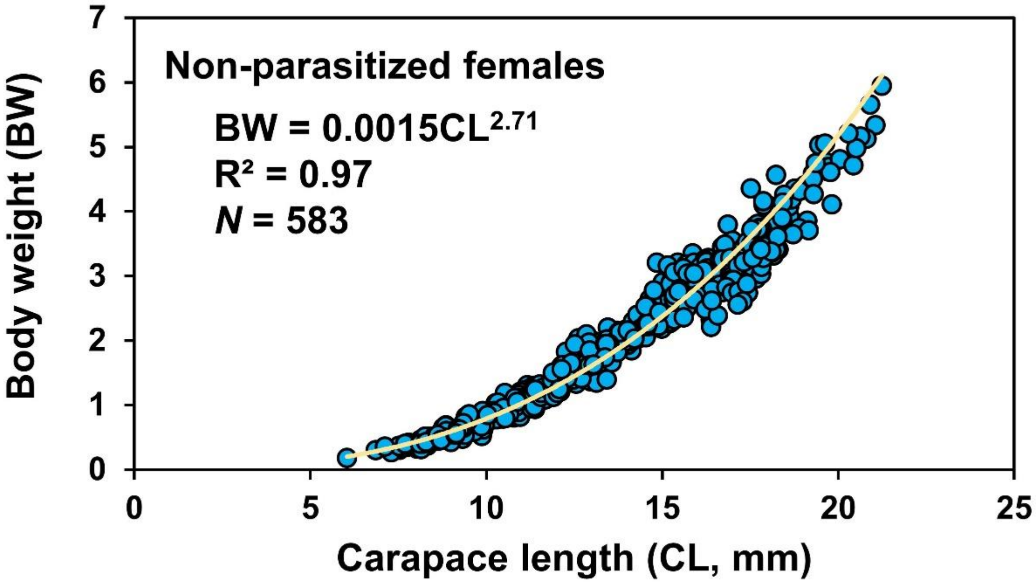


Figure 6.6. Relationship between carapace length (CL) and body weight (BW) of non-parasitized and parasitized females of *Metapenaeopsis kyushuensis* in Kagoshima Bay, Kyushu, Japan.



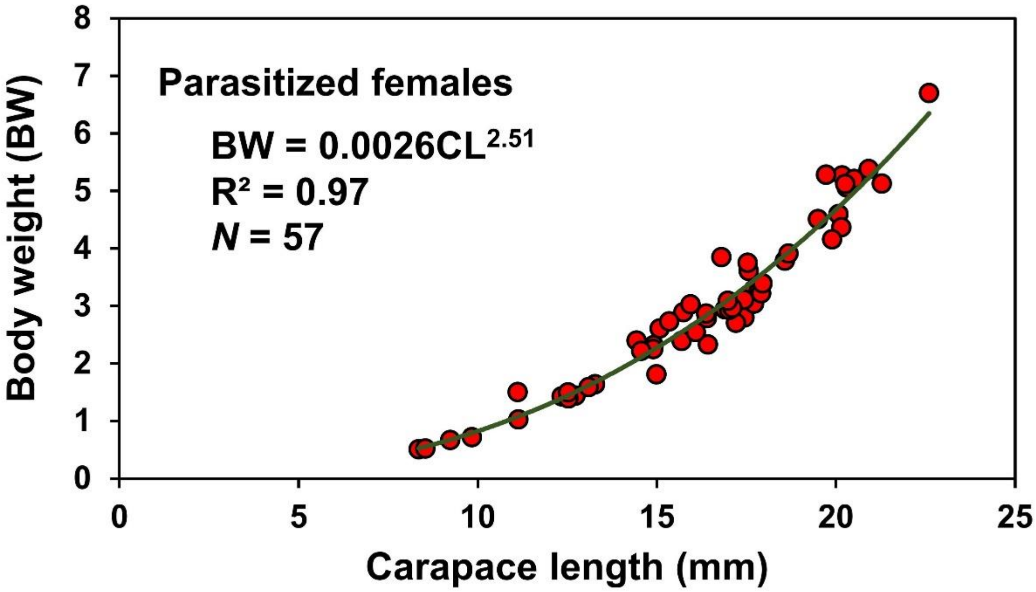


Figure 6.7. Relationship between carapace length (CL) and body weight (BW) of non-parasitized and parasitized females *Metapenaeopsis provocatoria owstoni* in Kagoshima Bay, Kyushu, Japan.

patterns among parasitized and non-parasitized shrimps or to seasonal variations in the reproduction and mortality rates of hosts and parasites (Mathews *et al.*, 1988; Jordá & Roccatagliata, 2002). The bopyrid isopods were found to infest the juveniles of the *Metapenaeopsis* species and can also be traced in the larger specimens. Owens & Glazebrook (1985) showed that bopyrids on penaeid shrimps grow with their hosts and have a similar longevity. Infestation was found to have a negative effect on the reproduction and growth of the hosts as was evident by the histological observation of the ovary and relative growth analysis. Parasitic infestation effectively sterilized the female shrimps as all the infested individuals were found to be immature regardless of their size. Histological observation of the ovaries of parasitized females illustrated that their oocytes cannot develop beyond the oogonium stage. The GSIs of the parasitized females were significantly lower than those of the non-parasitized females. Similar effects of parasitism on reproduction of the host were observed in *M. dalei* (Choi *et al.*, 2004). Abu-Hakima (1984) revealed that the parasitized males of *Penaeus semisulcatus* grew to the same size as the non-parasitized individuals, but the host's reproduction was completely inhibited. The author further reported that the petasma was not formed in parasitized male shrimp. The statistical analysis between parasitized and non-parasitized males was not conducted due to the limited number of parasitized males of *M. sibogae* and the difficulty of distinguishing the males of *M. kyushuensis* and *M. provocatoria owstoni*, but it was evident that the petasma of the parasitized males did not grow, though it was formed. Some studies suggested that the petasma assists in the transfer of spermatophores to the females (Hudinaga, 1942; King, 1948). Thus, parasitic infestation likely affected the reproductive ability of males of the studied species, as was also reported for the parasitized males of *M. dalei* (Choi *et al.*, 2004).

The relative growth rate of BW to CL was significantly lower in the parasitized females of *M. kyushuensis* and *M. provocatoria owstoni* than those of their non-parasitized counterparts.

Similar results were also reported for *M. dalei* (Choi *et al.*, 2004). This could be related to the reduced energy allocation for growth, and/or by the inability of the parasitized hosts to utilize energy properly as a result of parasitic infestation. It was not possible to conduct the relative growth analysis for *M. sibogae* due to the limited number of specimens, and in the males of *M. kyushuensis* and *M. provocatoria owstoni* due to the difficulty of identification, however, similar outcomes to that found for the relative growth analysis of female *M. kyushuensis* and *M. provocatoria owstoni* are hypothesized. The present study provides crucial information on the occurrence of bopyrid isopods in *Metapenaeopsis* species, identification of the parasites, variations in prevalence and parasitic effects of bopyrid isopods on the hosts from Kagoshima Bay. Information provided in this study will enrich the existing knowledge about the bopyrid isopod diversity in Japanese waters, and understanding the host-parasite relationships, which will aid the sustainable management of the fisheries resources.

CHAPTER 7: GENERAL DISCUSSION

The genus *Metapenaeopsis* has a worldwide distribution (De Grave & Fransen, 2011) and its species are important ecologically as well as commercially. Although 5 species and 1 subspecies of *Metapenaeopsis* are found in Kagoshima Bay, *M. sibogae*, *M. kyushuensis* and *M. provocatoria owstoni* are dominant in the benthic community and are commercially important. The present study provided the first information on their reproduction, growth, distribution patterns and population dynamics in Kagoshima Bay using a large number of samples collected over a long period of time.

M. sibogae, *M. kyushuensis* and *M. provocatoria owstoni* showed a wide spatial distribution in the bay, however, the majority of the individuals of all three species were distributed in the central bay, which has a silty sediment. All the species were found to be distributed in areas with water depth ranging from ~80 m to ~230 m. However, *M. sibogae* had relatively higher CPUE in comparatively deeper areas (~180 m), whereas *M. kyushuensis* and *M. provocatoria owstoni* had relatively higher CPUEs in comparatively shallower areas (~130 m) of the central bay. There is no study available on the distribution patterns of any of these species. Apart from Kagoshima Bay, the shallowest habitat record of *M. sibogae* is 247 m in Saleh-Bay, Indonesia (Grave & Fransen, 2011). The present study confirms the presence of *M. sibogae* at a water depth of 77 m, which is thus the new shallowest habitat record of this species. It is important to note that during the present study, no sampling could be conducted at depths < 77 m due to the steep slopes on both sides of the bay (Matumoto, 1943) and a heavy congestion of fixed gear (set-nets, traps, etc.) set by commercial fishers (Ohtomi *et al.*, 2018). The comparatively shallower bathymetric distribution of *M. sibogae* in the bay, could be an adaptation of the local population to cope with the steep slopes of the bay. The bottom-water temperature and salinity of Kagoshima Bay remained stable throughout the study period as also reported in several studies (Rahman & Ohtomi, 2017; Ohtomi *et al.*, 2018), and there were no significant relationships between these parameters and the distribution patterns of the studied shrimps, but the sediment characteristics and water depth might appear to influence their distribution patterns. *M. sibogae* was the most dominant among the studied species followed by *M. kyushuensis*. Analysis of mean CPUE throughout the study period revealed that *M. sibogae* population in the bay started to increase drastically in 2007 and that continued until 2010. Interestingly, the populations of *M. kyushuensis* and *M. provocatoria owstoni* declined in the similar period and afterwards. The main distribution areas of *M. kyushuensis* and *M. provocatoria owstoni* in the bay are similar with those of *M. sibogae*. Therefore, habitat competition might be a factor causing the decline of *M. kyushuensis* and *M. provocatoria* populations in the bay, however, more detail studies have to be conducted.

All the species exhibited asynchronous ovaries, containing oocytes at various developmental stages. This is a common phenomenon in penaeid shrimps including in other species of *Metapenaeopsis* such as *M. aegyptia*, *M. barbata*, *M. dalei*, *M. sinica*, and *M. palmensis* (Sakaji *et al.*, 2000; Sakaji, 2001a, 2001b; Chen *et al.*, 2014). This type of oocyte development is indicative of multiple spawning in a reproductive season. It can, therefore, be generalized that the species of the genus *Metapenaeopsis* are multiple spawners. The ovarian maturity stage (immature, maturing, and mature) was assigned based on the most advanced oocytes present in the ovary. Cortical crypts in the periphery of oocyte cytoplasm, widely recognized as a sign of oocyte prematuration in penaeid shrimps, were not identifiable in the studied shrimps. Previous studies on *M. aegyptia*, *M. barbata*, *M. dalei*, *M. sinica*, and *M. palmensis* have also reported the absence of cortical crypts (Sakaji *et al.*, 2000; Sakaji, 2001b; Chen *et al.*, 2014). The absence of cortical crypts and/or rods in oocyte development may therefore be a characteristic of *Metapenaeopsis* as compared to other penaeid shrimps (Sakaji *et al.*, 2000; Sakaji, 2001b; Chen *et al.*, 2014). In place of cortical crypts and/or rods, species of *Metapenaeopsis* have the characteristic germinal vesicle breakdown (GVBD) indicating maturity. Females containing mature oocytes, i.e., those in which GVBD had occurred, were defined as mature individuals.

The performances of both GSI and macroscopic staging in ovarian maturity classification were histologically evaluated. The GSI exhibited a positive but gradual relationship with ovarian maturation progression. Macroscopic staging, in contrast, showed a sharper relationship with histological stages of ovarian maturity exposing its potential as a maturity index for the studied species, which could also be suitable for other small penaeid shrimps in absence of histological data. The size at sexual maturity was estimated to be 14.3 mm, 13.7 mm and 12.8 mm in CL for *M. sibogae*, *M. kyushuensis* and *M. provocatoria owstoni*, respectively, and individuals of these sizes belong to the 1 year-old age class. *M. sibogae* spawned throughout the year but peaked in September-October. *M. kyushuensis* spawned from April to January with a peak in June-September. The spawning season of *M. provocatoria owstoni* lasted from April to December with May-June being the peak. A shorter spawning season also has been reported for some species of *Metapenaeopsis* inhabiting shallower habitats such as *M. acclivis* and *M. barbata* from Kasaoka Bay in the Ariake Sea and Aki-nada in the Seto Inland Sea spawned from early summer to autumn (Yasuda, 1949; Ikematsu, 1963). A general reproductive pattern can be deduced for *Metapenaeopsis* species. Species of *Metapenaeopsis* inhabiting deeper waters, where the bottom-water temperature remains stable, tend to have an extended spawning season, whereas those inhabiting shallower waters with higher fluctuations in water temperature tend to have shorter reproductive period.

Males and females of *M. sibogae* were recruited during the winter (January) with a modal size of around 9 mm CL. Almost no seasonality in growth rate was detected, therefore, the VBGF was adopted as the best fitting model to describe the growth of both sexes. Growth differed significantly between the sexes. Females had a lower growth rate but reached larger sizes at each age and larger asymptotic size than the males. Longevity was estimated to be 27 months for males and 28 months for females. Females of *M. kyushuensis* were first recruited in winter (December/January) with a modal size of around 10 mm CL. Seasonal oscillation in growth rate was detected in the females of *M. kyushuensis*, therefore, the PGGF was adopted as the best fitting model. The growth rate was the highest in November-December and the lowest in May-June. The longevity was estimated to be about 25 months. Females of *M. provocatoria owstoni* were first recruited individuals in autumn (November) with a modal size of around 10 mm CL. Seasonal oscillation in growth rate was detected in the females of *M. provocatoria owstoni*, therefore, the PGGF was adopted as the best fitting model. The growth rate was the highest in October-November and the lowest in April-May. The difference in seasonal oscillation in growth rete might be influenced by the spawning periodicity. The longevity was estimated to be about 27 months. Shorter longevity has been reported for *M. dalei* in western Korea (water depth ≤ 30 m) (Choi *et al.*, 2005). The difference between the longevities could be attributed to differences in bathymetric distribution, as deep-water shrimp species exhibit longer life cycles compared to shallow water species (King & Butler, 1985).

Analysis of the sum of squared residuals indicated that dimorphic growth existed in all the studied species and that two separate linear BL/CL models fit the data better than a single linear model showing clear transition points, which could be associated with sexual maturity. The estimated morphological sexual maturity sizes of the studied *Metapenaeopsis* species approximate the sizes at sexual maturity estimated though histological observation. It is, therefore, highly convenient for regular monitoring of size at sexual maturity of the studied species through observing critical morphological changes by analyzing relative growth. The relationship between BW and CL showed negative allometric growth in all the studied shrimp.

Similar distribution patterns with progressing age were observed for all the species. The central basin was the main spawning ground for all three species. Recruitment occurred widely in the bay with low CPUE and the recruitment process was completed in spring (April). Individuals belonging to the 0+ age group were distributed throughout their distribution areas. However, an increased tendency of inhabiting the deeper waters was being observed for individuals belong to the ≥1+ age groups. Older individuals living in deeper water areas is a general trend in shrimps (King & Butler, 1985, Ardizzone *et al.*, 1990, Company & Sardá, 2000, Ohtomi *et al.*, 2018).

Bopyrid parasites *M. intermedium*, *M. liuruiyui* and *P. tertium* were identified to infest *M. sibogae*, *M. kyushuensis* and *M. provocatoria owstoni*, respectively. The infestation rate varied significantly among species, with the highest rate recorded for the females of *M. provocatoria owstoni* (8.91%) and lowest for *M. sibogae* (0.07%). The difference in parasitic rates among the species could have resulted from their different habitat preferences, food habits and burrowing behaviors (Sakaji, 1995) apart from other possible influencing factors. Seasonal fluctuations have also been observed with the highest parasitic rates appearing in autumn for all the species. Such fluctuations are ascribed to be caused by the differential migration patterns among parasitized and non-parasitized shrimps, or due to seasonal variations in the reproduction and mortality rates of hosts and parasites (Mathews *et al.*, 1988; Jordá & Roccatagliata, 2002). The infestation inhibited the reproductive ability of the females as all the parasitized females were found to be immature. The relative growth rates of BW/CL relationship of parasitized females were significantly lower than those of their non-parasitized counterparts.

M. sibogae, M. kyushuensis and M. provocatoria owstoni are commercially important fisheries resources in Kagoshima Bay. The present study provided the first information on reproduction, growth, distribution patterns and population dynamics of all these species in addition to reports on parasite infestation and impact assessment of parasite infestation on the host, which will be helpful for their sustainable management. Future studies on male reproduction, spawning frequency, fecundity, as well as a breeding stock assessment, are needed to gain a broader understanding of the reproductive potential of the studied populations, which in turn will form the basis for adequate management regimes. The factors underlying mass mortality of early spawns of the studied species remains unknown, and it deserve greater attention in future studies. The impact of the dominance of the newly recorded species *M. sibogae* on the other major species in the benthic community of the bay needs to be evaluated. Furthermore, revelation of the origin and mode of introduction of *M. sibogae* in Japanese waters will greatly help understanding species invasion and the management of such species and are recommended for future studies.

CHAPTER 8: CONCLUSION

M. sibogae, *M. kyushuensis* and *M. provocatoria owstoni* showed a wide spatial distribution in Kagoshima Bay, however, the majority of the individuals of all three species were distributed in the central bay, which has a silty sediment. All the species were found to be distributed in areas with water depths ranging from ~80 m to ~230 m. However, *M. sibogae* had a relatively higher CPUE in comparatively deeper areas (~180 m), whereas *M. kyushuensis* and *M. provocatoria owstoni* had relatively higher CPUEs in comparatively shallower areas (~130 m) of the central bay.

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Males and females of *M. sibogae* were recruited during winter (January) with a modal size of around 9 mm CL. The VBGF was adopted as the best fitting model to describe the growth of both sexes. Growth differed significantly between the sexes. Females had a lower growth rate but reached larger sizes at each age and a larger asymptotic size than the males. Longevity was estimated to be 27 months for males and 28 months for females. Females of *M.*

kyushuensis were first recruited in winter (December/January) with a modal size of around 10 mm CL. The growth was best described by the Pauly and Gaschütz growth function. The growth rate was the highest in November-December and the lowest in May-June. The longevity was estimated to be about 25 months. Females of *M. provocatoria owstoni* were first recruited individuals in autumn (November) with a modal size of around 10 mm CL. The growth was best described by the Pauly and Gaschütz growth function. The growth rate was the highest in October-November and the lowest in April-May.

Analysis of the sum of squared residuals indicated that dimorphic growth existed in all the studied species and that two separate linear BL/CL models fit the data better than a single linear model. The morphological sexual maturity was calculated as 13.2 mm, 15.0 mm, 13.0 mm, and 15.6 mm in CL for male *M. sibogae*, female *M. sibogae*, female *M. kyushuensis*, and female *M. provocatoria owstoni*, respectively. The relationship between BW and CL showed negative allometric growth in all the studied shrimps.

Similar distribution patterns with progressing age were observed for all the species. Individuals belonging to 0+ age group were distributed throughout their distribution areas. However, an increased tendency of inhabiting the deeper waters being observed for individuals belong to the ≥1+ age groups.

Bopyrid parasites *M. intermedium*, *M. liuruiyui* and *P. tertium* were identified to infest *M. sibogae*, *M. kyushuensis* and *M. provocatoria owstoni*, respectively. The infestation rate varied significantly among species with the highest rate recorded for the females of *M. provocatoria owstoni* (8.91%) and lowest for *M. sibogae* (0.07%). Seasonal fluctuations have also been observed, with the highest parasitic rates occurring in autumn for all the species. The infestation inhibited the reproductive ability of the females as all the parasitized females were found to be immature. The relative growth rates of BW/CL relationship of parasitized females were significantly lower than those of their non-parasitized counterparts.

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