博士論文要約 (Summary)

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CHAPTER 1: GENERAL INTRODUCTION

The genus *Charybdis* De Haan, 1833 is one of the largest genera in this family, with 6 subgenus, 76 species, 2 subspecies, and 4 varieties (WoRMS 2020). Japanese waters are inhabited by 19 species of *Charybdis* (Miyake 1998). Among these, *Charybdis bimaculata* (Miers 1886) has the most diverse bathymetric habitats (from the intertidal to a depth of 439 m) (Miyake 1998). This crab has been reported in Australia, China, India, Japan, Korea, South Africa, and Taiwan (Miyake 1998). This species is reported as one of the dominant decapods in Kagoshima Bay, southern Japan (Ohtomi et al. 2004). Despite its broad distribution and ecological significance, studies on the population ecology of this species are scant. Existing studies have investigated few aspects of this species' reproductive biology in the shallow waters of the Zoea and megalopa (Hwang and Kim 1995), the stomach contents in Ise Bay (Narita and Sekiguchi 2002), cheliped handedness (Lee 1995; Yamaguchi and Tokunaga 1995), and genetic relationships with other species (Heo et al. 2003). The only populations studied so far are those of in the shallow water inner bays; Ise Bay (Narita et al. 2008), Tokyo Bay (Doi et al. 2008), and Seto Inland Sea (Ogawa 1998).

Kagoshima Bay, south Kyushu, Japan (31°25' N, 130°38' E), is a semi-enclosed deep-water bay with a maximum water depth of 237 m (Ministry of the Environment 2020). *C. bimaculata* is one of the dominant decapods in Kagoshima Bay, southern Japan in terms of catch per haul (Ohtomi et al. 2004) and considered as an emerging commercial species based on the rising consumption demand (FN and JO unpublished). There is, however, no study available on the population ecology of *C. bimaculata* in this unique representative deep-water habitat. The population ecology of this emerging commercially important crab in this unique bay environment, is particularly interesting ecologically and important for sustainable management and conservation protocols. The present study, therefore, aimed at providing the intensive information on the reproductive biology, recruitment, growth patterns, longevity, and spatiotemporal distribution of *C. bimaculata* in Kagoshima Bay, southern Japan.

CHAPTER 2: REPRODUCTIVE BIOLOGY OF *CHARYBDIS BIMACULATA* IN KAGOSHIMA BAY, SOUTHERN JAPAN

Background and purposes

Earlier studies assessed a few aspects of this species' reproductive biology in the shallow waters of the Seto Inland Sea (Ogawa 1998), Tokyo Bay (Doi et al. 2008) and Goheung Bay (Hong et al. 2022). Thus far, no study has thoroughly described an estimation of ovarian maturity nor offered the histologically confirmed

size at sexual maturity of this species. There have been no precise studies on the oocyte development or the ovarian maturity staging of this crab which are imperative to form a base on the reproductive biology of a species. This study aimed to fill these gaps in literature by describing the oocyte development, ovarian maturation, embryonic development, histologically confirmed size at sexual maturity, and spawning and hatching season of *C. bimaculata* in Kagoshima Bay, southern Japan as a representative deep-water body. Moreover, the suitability of the gonadosomatic index and macroscopic staging was tested for their utility as simple and reliable indices of ovarian maturity conditions. In this study, a rapid and reliable embryonic developed.

Materials and methods

Monthly sampling was carried out in the central area of Kagoshima Bay at depths ranging from 132 m to 227 m, from October 2018 to January 2020. Sampling was conducted with the training vessel Nansei-Maru (175 t) of the Faculty of Fisheries, Kagoshima University by a simple trawl net. The net was towed for a 10 min. pre-set tow duration. The effective tow duration was determined and the catch per unit effort was calculated following Fulanda & Ohtomi (2011). CPUE = Number of individuals collected per haul \times 10 / Effective tow duration (min). The CPUE was regarded as a measure of relative abundance of *C. bimaculata*. At each haul, target species were sexed based on the shape of the abdominal flap (i.e., wider, horseshoe-shaped in females and narrower, pointed-shaped in males). Ovaries were macroscopically classified as one of three maturity stages based on the shape and color of the ovary. Ovarian maturity status was then confirmed by histological observation. Size at sexual maturity (L_{50}) was stated as the Carapace width (CW) at which 50% of female individuals turn mature following King (2007). All of the females at each haul were checked for the presence of egg mass attached to the pleopods. Initially, the embryos of ovigerous females were categorized into one of four stages by visual observation depending on brood color. Furthermore, embryos were examined microscopically and categorized into one of four stages following Kobayashi and Vazquez Archdale (2017).

Results

Six sequential oocyte developmental stages were confirmed by histological examination. *C. bimaculata* exhibited asynchronous-type ovarian development. Therefore, ovarian maturity status was determined from the most advanced oocyte found in the ovary. Females having oocytes evidencing either germinal vesicle breakdown or migratory nucleus were defined as mature. The L_{50} was estimated to be 21.8 mm in carapace width. The spawning season was determined from May to November when mature females occurred. Microscopic observation confirmed four sequential embryonic developmental stages. The majority of females with final-stage embryos had mature ovaries signifying continuity of reproduction. Asynchronous-type ovaries, continuous reproduction, and consecutive broods suggest multiple spawning in a single spawning season. The incubation period was estimated to be under one month.

Conclusion and consideration

The findings of this study should assist in developing an in-depth knowledge of reproductive biology

of this species. Explication of the spawning and hatching season and the incubation period of *C. bimaculata* should support fishery management protocols to protect the hatching population and to permit an ample portion of the hatching stock to reproduce (Van Overzee and Rijnsdorp 2015), as fisheries may have an adverse impact on the ecosystem with the bycatch of this emerging commercial species, habitat damage from towed bottom gear, and the application of evolutionary pressure (Dayton et al. 1995; Jennings and Kaiser 1998). Further detailed studies on spawning frequency and breeding stock assessments will offer broader knowledge of *C. bimaculata*'s reproductive potential and, in turn, continue to improve the management regimes for this developing commercial species.

CHAPTER 3: RECRUITMENT AND GROWTH PATTERNS OF *CHARYBDIS BIMACULATA* IN KAGOSHIMA BAY, SOUTHERN JAPAN

Background and purposes

The study of growth has uttermost importance as the biological parameters attained through the growth model are the base for fisheries management (Shinozaki-Mendes et al. 2012). Estimation of growth in crustaceans is tough as its exoskeleton is replaced periodically during ecdysis, making age determinations impossible by periodical markings on the body surface (Hartnoll 2001; Castilho et al. 2015). Length-frequency analysis is the most extensive method for determining the age of wild crustacean populations (Oh et al. 1999). The existing study on recruitment and growth of this species was conducted in shallow water, Tokyo Bay (Doi et al. 2008). Detailed studies on the growth, recruitment, and longevity of this species, however, are unveiled in deep water like Kagoshima Bay. Information on biological aspects including recruitment, growth patterns etc. are indispensable for fisheries management. Lack of adequate knowledge on recruitment and growth of *C. bimaculata*, therefore, impedes the definition of proper management schemes for this promising fishery and other deep-water habitats. The present study aims to provide information on recruitment, longevity, and growth patterns of *C. bimaculata* in deep water using length-frequency method from monthly samples collected over a three study periods that will certainly aid in stock assessment.

Materials and methods

Monthly sampling was done over three study periods: 1990s, 2010s, and 2020s. at the central basin (174–229 m deep) and one study period 1990s (April 1995 to February 1996) at the channel part (122–136 m). In the 1990s study period, sampling was attained using commercial small-scale seiners at the central basin and at the channel part using 25 min towing. In the case of 2010s and 2020s, sampling was carried out with the training vessel Nansei-Maru at the central basin with a 10 min preset tow duration. All the individuals are sexed by the shape of the abdomen. CW was measured as the distance between the lateral spines. The sex ratio was calculated as the percentage of males to females for each study period and depth. Monthly length-frequency distributions were constructed using CW histograms with 1 mm class intervals for each sex. Data were modeled as mixed normal distributions where there were 30 or more crabs of each sex. Each identified normal distribution was assumed to represent an age group. In cases where two normal

distributions were identified, judging from modal changes in histograms, the smaller age group was considered as newly recruited group. The age of individuals in months was assigned to mean CW belonging to each normal distribution using 1 August as an arbitrary birth date (Nawer et al. 2022). This date is the approximate midpoint of the period when zoeae hatch. Their monthly ages were calculated by subtracting the date of hatching (1 August) from the date of collection. Longevity was estimated from the time series of CW frequency distributions enumerating the monthly age of the same age group consistently. Growth models were constructed using the von Bertalanffy growth function. The growth parameters within the same sex of male and female *C. bimaculata* between two different depths (channel area vs. central basin of 1990s study period) as well as between two different time series (2010s vs. 2020s) were compared to check if there is any significant difference between growth parameters, using the likelihood ratio test by Kimura 1980.

Results

A total of 8,265 individuals of *C. bimaculata* were examined, of which 46.7% were males and 53.3% were females. The overall sex ratio was biased greatly towards females and varied significantly from 1:1 ($\chi^2 = 0.53$; *P* < 0.001). Recruitment was estimated to be occur between September to November in this bay. Recruitment seemed to be synchronized with the peak spawning season with a time lag of 2–4 months. Growth was appositely delineated by the von Bertalanffy growth function for both sexes. The growth equations revealed that males had lower K values and higher asymptotic sizes than females. Males attained larger size than females of the same age. Spatial and temporal comparison in growth parameters between the same sexes found no statistical differences except in one case. Longevity was estimated to be around 19 months for males and 18 months for females at the central basin and 15 months for both sexes at the channel area.

Conclusion and consideration

Clarification of biological aspects of any species is indispensable for fisheries management. Findings in this study, thus, will be useful for the subsequent management of this species. Future research on the phases prior to the juvenile phase together with population dynamics of this species is recommended.

CHAPTER 4: SPATIOTEMPORAL DISTRIBUTION OF *CHARYBDIS BIMACULATA* IN KAGOSHIMA BAY, SOUTHERN JAPAN

Background and purposes

Kagoshima Bay is a unique habitat of *C. bimaculata*. Understanding the causes and mechanisms of alteration in the abundance of species over time and space is a crucial issue in marine ecology. There is a growing interest in describing the spatial distribution of fishery resources by the habitats essential to complete the resources' life cycle. However, study on the distribution of *C. bimaculata* is evidently lacking in Kagoshima Bay and other deep waters of the world. The present study aimed to provide information on spatiotemporal distribution of this species in Kagoshima Bay using a large number of specimens.

Materials and methods

Kagoshima 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 237 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. According to Ohtomi et al. (2018), the proportion of clay content was low and did not vary considerably among the stations. 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 *C. bimaculata* were collected by experimental trawl surveys Nansei-Maru from January 2006 to October 2021 seasonally from pre-established 8 stations. A total of 498 trawls were conducted using a simple trawl net with a 10 min. pre-set tow duration. The effective tow duration was determined and the catch per unit effort was calculated following Fulanda & Ohtomi (2011).

Results

A total of 20,641 individuals of *C. bimaculata* were collected. Spatial distribution result showed that *C. bimaculata* has a wide spatial distribution in the bay. High variations in CPUE values were observed among the stations where highest mean CPUE was found at St. 4 (147 \pm 203) followed by St. 7 (58 \pm 98) in the central basin, while the lowest mean CPUE was recorded at St. 8 in the bay mouth (0.68 \pm 2). Analysis of seasonal CPUE throughout the study period revealed that *C. bimaculata* population in the bay decreased in Autumn noticeably every year and started increasing again in winter with peak in spring. The new cohorts of *C. bimaculata* first appeared in autumn; however, these cohorts were fully recruited in the following winter and continued to thrive and grow in size through the spring and summer, which was reflected in the higher CPUE of the crab in these seasons. On the other hand, in autumn, the older age group drastically reduced after spawning, whereas the new one just started to be recruited in the fishery (see result of Chapter 3).

Conclusion and consideration

The present study has revealed the spatiotemporal distribution of *C. bimaculata*, which would clarify the life history of this crab and thereby formulating effective strategies for its sustainable management. Studies on the influence of primary food resources and light intensity on the distribution patterns of this crab are recommended in order to make the picture of its life cycle in Kagoshima Bay complete.