Occurrence pattern and reproductive ecology of the leucosiid crab *Pyrhila pisum* (De Haan) in tidal flats in Hakata Bay, Fukuoka, Japan

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Abstract.— Occurrence pattern and reproductive ecology of a leucosiid crab, *Pyrhila pisum* were surveyed in tidal flats in Hakata Bay, Fukuoka, northern Kyushu, Japan. Size composition of each sex (male, juvenile and adult females) including carcasses was analyzed, and developmental stages of embryos and combination of mating pairs were checked. A mark-recapture experiment on adult crabs was conducted. In the study site, *P. pisum* was observed during 5 months only (late April to mid September). Carcasses were rarely found, and many adult crabs probably survived and migrated to the deeper subtidal zone after September. Four cohorts were detected in both sexes, and adults did not molt during their reproduction. Reproduction started after May in two large-sized adult cohorts. Female adults could spawn eggs three times. Two juvenile female cohorts were detected in May, and they joined reproduction after most of them had attained maturity by a puberty molt in late June and late July. Adult females copulated regularly in their hard-shell condition, and mating was observed even for ovigerous females.

Key words: leucosiid crab, *Pyrhila pisum*, mating behavior, reproductive ecology, tidal flat

■ Introduction

Among brachyuran crabs, leucosiid crabs have some discriminative characteristics, such as a round carapace with a short cephalic region where small eyes frontally protrude, a very hard and thick exoskeleton, elongated chelipeds and short walking legs by which they slowly walk forward. However, there have been very few studies about the ecology of leucosiid species, probably because many species are small or rare, inhabit the subtidal area and are not economically important. There have been some descriptive studies about this family, on their taxonomy and larval development, but ecological ones are limited to *Ebalia tuberosa* (Pennant), which is distributed in the subtidal area of the northern Atlantic Ocean (Schembri, 1979, 1981a, b, 1982a, b, c, 1983), three *Persephona* species (*P. lichtensteinii*

Leach, *P. mediterranea* Herbst, and *P. punctata* (Linnaeus)) that inhabit the south-eastern Brazilian coast (Almeida et al., 2013; Pereira et al., 2014), and brief reports on a few other species (Hale, 1926; Naidu, 1954).

Pyrhila pisum (De Haan), which was previously known as *Philyra pisum* De Haan, is a leucosiid crab occurring in tidal flats in eastern Asia, including the continental coastal zone of the Yellow Sea and East China Sea, Taiwan, and the temperate and subtropical coasts of Japan from Aomori Prefecture to Amami Island, in Kagoshima Prefecture (Galil, 2009, Suzuki, 2012). As an unusual case from this family, *P. pisum* abundantly occurs in the intertidal areas of some tidal flats in temperate Japan. However, this crab is often regarded as an endangered species similarly with cohabiting brachyuran crabs, because their ideal habitat environment (wide tidal flats) has been rapidly decreasing

by reclamation projects in Japan (Suzuki, 2012). On the other hand, recently *P. pisum* has been unintentionally introduced to some areas and is expanding its distribution as an invasive species along the coast of Japan, in conjunction with the release of the Manila clam *Ruditapes philippinarum* (A. Adams & Reeve) imported from China or Korea into tidal flats for aquaculture operations, similarly to the cases of expansion of the clam-eating moon snail *Laguncula pulchella* Benson (Okoshi, 2007). It is necessary to recognize the occurrence patterns and ecological niche of *P. pisum* for the suitable maintenance of tidal flats in Japan. However, its ecological information has been very scarce when compared to other brachyuran crabs co-occurring with them (e.g., varunid and ocypodid crabs). Only in the rivermouth of Obitsu River, Tokyo Bay (35°24' N, 139°54' E) their occurrence and reproductive patterns have been reported by Higashi and Furota (1996). Thus, Kobayashi (2013a) recently reported the foraging behavior and carnivorous food preference of *P. pisum* in Hakata Bay.

Observation and collection of *P. pisum* is comparatively easy among brachyuran crabs in the tidal flats of Japan; *P. pisum* is found during the daytime actively wandering on sandy or muddy substrates along the shoreline, and often expose their mating behavior without using shelters or burrows. This is probably derived from the rareness of specific predators inhabiting their habitat, and because they are protected by their very hard exoskeleton. In addition, they do not occupy a limited habitat area, and they often wander for long distances along the edge of the surf. Therefore, their modal distribution area temporally changes with the tidal cycle (Higashi & Furota, 1996). But some crabs remain in pools on the dried-up tidal flat during the ebb tide.

Evident sexual dimorphism is present in adult crabs, and males embrace females from the rear with their elongate chelipeds as they guard them. These evident sexual dimorphism

and exposed mating behaviors may be observed similarly among other leucosiid crabs. However, the guarding behavior has not been reported in the mating of *Ebalia tuberosa* (Schembri, 1983), and pronounced sexual dimorphism is absent in *Persephona lichtensteini* among three *Persephona* species (Almeida et al., 2013), suggesting mating behavior variations within this family.

Therefore, the ecology of *P. pisum* was surveyed in the intertidal area of tidal flats in Hakata Bay, focusing on their pattern of occurrence and reproduction.

Materials and Methods

Collection sites

The two sites selected for the survey in Fukuoka, Kyushu, western part of Japan (Fig. 1) were a tidal flat along the river channel of the west side of Tataru River at the Hakozaki pier (Site 1), located in south-eastern Hakata Bay (33° 38'N, 130° 25'E, nearly 3,000 m²); and the other the sandy beach of Gan-no-su (Site 2), located in north-eastern Hakata Bay (33° 40'N, 130° 24'E, nearly 20,000 m²). Within Hakata Bay, *P. pisum* abundantly occurs in the tidal flats in the coastal area and lower tidal river area; Site 1 is only a small portion of their distribution area in the tidal area of Tataru River, and Site 2 is also a small portion of the wide distribution area along the north-eastern coast of Hakata Bay. Ecological surveys of *P. pisum* started in Site 1 in 2005, but the population drastically decreased after dredging occurred in the rivermouth of the Tataru River in 2007. Thus, from then on, their main collecting site was shifted to Site 2, which covers a wide area where crabs are abundantly distributed. Environmental fluctuations in salinity and water flow rates were larger in Site 1, but disturbance of environment by non-commercial shellfish gathering (anthropogenic effect) was stronger in Site 2; shellfish gatherers tread and dig out a wide area of the surface layer of the substrate,

Fig. 1. Map of Hakata Bay, Fukuoka, Japan showing collection sites.

change the composition of the benthos community by selectively gathering the Manila clam *R. philippinarum*, and often capture or carry out crabs, including the research target of the present survey.

Occurrence pattern of *Pyrhila pisum* on the tidal flat

Pyrhila pisum, including copulating and guarding mating pairs were collected by hand during the daytime for 1–2 hours during the ebb tide in Site 1 from April to September, 2005 (Survey 1), and from May to September, 2006 (Survey 2). In Survey 1, sampling was conducted as frequently as possible, to detect the exact date when *P. pisum* occurred in the tidal flat. While in Survey 2, the number of sampling days was decreased and the collecting effort was increased for each day to gather a larger number of crabs for statistical analysis. In addition, carcasses were also collected. In the present survey, quantitative sampling was difficult and all collection was qualitative, because the sampling area where the sampler can

walk varies with tidal phase, river flow rate and substrate condition, and the crabs wander within the wide intertidal range and their distribution pattern widely changes with the tidal condition according to time and day.

The collected crabs were sexed according to the shape of their abdomen (Higashi & Furota, 1996). The number of crabs in each sex was counted and the bias of the sex ratio was detected in each day using the binomial test. The maximum carapace width (CW) of each crab was measured to the nearest 0.1 mm using an electronic caliper. Females were categorized into juveniles (immature stage) and adults (reproductive stage), based on their morphological differences (Higashi & Furota, 1996); where juveniles showed nearly flattened bell-shaped abdomens and exposed edges of the thoracic sternum similar to males, while in adults the abdomen was sterically swollen, nearly oval and all parts of their thoracic sternum were covered. Thus, these two female stages can be discontinuously distinguished by their appearance; the whole body of the adult female was

almost spherical, while those of juvenile females and males were nearly semi-spherical. Presence of puberty molt, which means molting from juvenile stage to adult stage (Hartnoll, 1978; 1982), can be also easily confirmed in female *P. pisum*. As for males, later it became clear that adult males could be distinguished from juveniles by their elongated chelipeds, which are used to grasp mates during guarding, but this was not applied at the time of Surveys 1 and 2.

Ovigerous adult females were recorded (Surveys 1 and 2) and their eggs (embryos) were observed under a binocular microscope; their condition, showing the developmental stages of embryos, was recorded (Survey 1). These stages were classified according to Kobayashi and Matsuura (1995) into the 19 stages used for *Eriocheir japonica* (De Haan), and gathered into larger categories of 6 stages (Stage I–VI), defined as follows. Stage I: no cleavage, just after spawning (stage 1). Stage II: cleavage is present in the yolk (stages 2–5). Stage III: transparent embryo with no pigmentation developed in half of the total egg volume (stages 6–9). Stage IV: crescent-shaped compound eyes are present, the yolk has four lobes and occupies more than one third of the total egg volume (stages 10–14). Stage V: oval-shaped compound eyes, the yolk occupies less than one third of the total egg volume, and is composed of two lobes or separated into two small particles (stages 15–18). Stage VI: just after hatching, where only membrane remains (stage 19). Non-ovigerous adults, except for stage 19, were also classified into Stage N (stage 0).

The numbers of paired crabs (in copulation or guarding) were also recorded (Survey 1). Copulation was confirmed from the posture of the pairs; when they were facing each other with their abdomens extended and the males' pleopods attached to the females' genital apertures. This was easily confirmed, because the pairs were locked, maintained this posture and they remained immobile even when they were

picked up. Although transfer of semen into females could not be confirmed in the present survey, crabs that maintained their copulatory posture and remained immobile for at least one minute were regarded as copulating pairs, because males struggled for a while and separated from females within one minute in the unsuccessful case, while they kept copulatory posture for about 1–2 hours without moving in the successful case (Kobayashi, unpublished data). Males guarded their mates by grasping them from behind with their elongated chelipeds and first ambulatory legs. The pairs often walked in tandem along the shore in the intertidal area. These pairs were classified into four combinations according to their sex and the condition of the guarded mates (male, juvenile female, non-ovigerous adult female or ovigerous adult female).

Size-frequency histograms were constructed to detect the size composition. As for Survey 2, cohorts in each population were identified using the Electric Length Frequency Analysis (ELEFAN I) routine of the FISAT II package (FAO, 2002), based on modal progression analysis of size-frequency data using Bhattacharya's method. Mean CW was calculated for each identified cohort. The word 'cohort' is usually defined as "year class born in the same reproductive period", but in this study we simply use it to describe the different groups detected by the cohort analysis method in order to facilitate the understanding, because evident polymodal distribution was confirmed in each histogram.

Handling of each crab in the measurement above the water seemed to have little effect on the vitality of crabs; they often dried up in the tidal flat or wandered above the water in the natural condition. After measurement, all or most crabs were soon released in their collection sites except for a few individuals that were used for other behavioral experiments during each period.

Mark-recapture experiment

Adult *P. pisum* were collected in Site 2, where they were abundantly distributed. After marking each crab in the laboratory, 130 females and males (75 crabs in May 25, 35 in June 4, 10 in June 17 and 10 in August 5, 2009) were released into Site 1, where the anthropogenic effect was small. Crabs were marked by gluing a letter-notched colored plastic plate with an identity number (001–130 in a different color for each sex) to their carapace. Searches for the marked crabs took place 25 times during the ebb tide from June 3 to August 21. Crabs were recaptured, and the presence of pairs and egg-laying with the developmental stages of embryos were recorded. The stages were classified according to the 20 stages (stage 0–19) described in Survey 1. The time of oviposition in each female was confirmed according to the change in the developmental stage of the embryos present on the recaptured crab, following the literature (Higashi & Furota, 1996) that states that complete embryonic development needed ca. 20 days in May under rearing condition.

Results

Survey 1

Six hundred and sixty two males (7.2–24.0 mm CW), 77 juvenile (5.9–17.1 mm CW) and 191 adult females (12.0–22.7 mm CW) were collected from April 30 to September 16, 2005 (total sex ratio is male : female = 1 : 0.29), and no crabs were found in Site 1 before and after the observations (Fig. 2). In the later period of this survey (after August 30), crabs were rare (<10 individuals). From the middle of August to mid-September dense accumulation of stranded green algae (*Ulva* sp.) littered the intertidal area, and this mass of rotten algae transformed the bottom sediment into an inhospitable condition (black coloration and noxious odor), causing mass mortality of benthos animals. Under such a condition (green tide), *P.*

pisum numbers gradually decreased and no crabs were found even after the green algae disappeared in October.

The number of males was larger than that of females in 27 days (93.1%) and significantly male biased (binomial test, $p < 0.05$) in 16 days (55.2%) among 29 days in which crabs were collected.

In May, large crabs (mostly 14 mm < CW) were dominant in both sexes. In June, the size frequency distribution was polymodal, with at least 2 modes in both sexes; 12–14 mm CW and 18–20 mm CW in males, and 12–14 mm CW in juvenile females and 18–20 mm CW in adult females in June 8. Juvenile females of 10–16 mm CW occurred from April 30 to July 11, but totally disappeared later in summer. Abundant small adult females (12–16 mm CW), including ovigerous ones, alternatively appeared in the later summer season (August 20), and this suggested the presence of a puberty molt in small crabs around July. Adult females occurred during all this period, but ovigerous crabs occurred only from May 18 to September 16. Ovigerous crabs occurred in all adult female size ranges (12–24 mm CW). Very small juveniles (8 mm > CW) were found in small numbers in both sexes in August 5 and 22.

The change in the adult females' condition, including the developmental stages of their embryos, is shown in Fig. 3. Until late May, most adult females were in a non-ovigerous condition (Stage N), or the embryonic stages remained in the earlier stages (Stage I–II). After June, embryos developed into the later stages after pigmentation (Stage V) and the first hatching (Stage VI) was confirmed in June 25. Various embryonic stages occurred until September, suggesting that spawning and hatching still continued in the intertidal area from mid-May to mid-September. From the developmental process of the embryo, at least two groups can be detected before and after mid July; there is a group showing development from May to July, and another group showing development

Ovigerous condition and developmental stages of embryos

Fig. 3. Frequency distributions showing ovigerous conditions of adult females and developmental stage of embryos per day from April to September, 2005 (Survey 1). For the explanation of Stages N–VI, see text. The darkness of the bars indicates the degree of embryonic development; white: before spawning, light to dark gray: ovigerous, black: just after hatching.

after mid July. Especially, a modal developmental stage in July 12 suggested that a substantial number of crabs belonging to the later group spawned eggs in early July. Following the size histogram (Fig. 2), this season coincided with the puberty molt presumed from the disappearance of juvenile females. This suggests that adult crabs began reproduction and spawned eggs after their puberty molt around July.

The combination of mating pairs observed from May 18 to September 16 is shown in Fig. 4. The number of pairs was small compared to the total number of crabs collected (Fig. 2); and ranged from 1–6 per day. The number of guarding pairs was much larger than those copulating (46:2), suggesting that the duration of guarding was much longer than that of copulation. Copulation and guarding were observed both in non-ovigerous and ovigerous adult females; especially after mid July, when most adult crabs were ovigerous (Fig. 2) and most pairing females were ovigerous (77.8%). Males did not show a predilection in the condition of their mates for mating, and adult females mated regardless of their ovigerous condition. However, neither juvenile females nor males were found among the mates. These crabs were sometimes captured by approaching males, but rapidly escaped from them. Wandering adult males often approached and contacted other crabs regardless of whether they were adult females, but these males were frequently rejected and it was rare for them to start mating.

Survey 2

Eight hundred and seventy nine males (7.3–23.2 mm CW), 133 juvenile (9.4–17.2 mm CW) and 337 adult females (10.8–22.4 mm CW) were collected from May 9 to September 19, 2006 (total sex ratio is male : female = 1 : 0.38) (Fig. 5). At most four cohorts were confirmed in both sexes. These cohorts were nearly similar in size in both sexes (Fig. 6). In females, two large-sized cohorts (mean CW > 15 mm) had

already reached maturity in May, but two small-sized cohorts (15 mm > mean CW) were still juveniles (Fig. 5). These juveniles attained maturity after a puberty molt in late June or late July as shown in Survey 1, suggesting that they joined reproduction for the first time after that.

The number of males was larger than that of females in 14 days (100%) and significantly male-biased (binomial test, $p < 0.05$) in 12 days (85.7%) out of the 14 days lasting from May 9 to September 2.

Dead crabs were rarely collected in the study site, and consisted of only a few carcasses (1–3 per day, 11 in total) compared to the number of living crabs found (Fig. 5). Carcasses of both sexes (male and non-ovigerous female adults) were found only in late summer after July 30. Their sizes ranged from 15.2–22.3 mm CW in males and 15.0–21.6 mm CW in females, which belonged to three large-sized adult cohorts (Fig. 6).

Mark-recapture experiment

From the 130 crabs released, 63 males were recaptured for a total of 97 times (Table 1) and 33 females were recaptured for a total of 37 times (Table 2). Males were more easily recaptured, suggesting that they tended to wander actively in the shore, while females were cryptic in the substrate.

Most recaptured females were ovigerous (94.6%), suggesting that females did not decrease their activity considerably in their ovigerous condition. Changes in the developmental stages of the embryos showed that adult females could oviposit up to three times during 2 months; confirmed number of the oviposition time was once (12 crabs), twice (18 crabs) and three times (2 crabs, crab No. F11 and F95). After the cohort analysis in Fig. 6 and determining the developmental stages of embryos in Fig. 3, the 75 and 35 females released in May 25 and June 4, respectively belong to the larger 2 cohorts (17 mm ≤ CW), and the embryos

Fig. 4. Frequency distributions for each combination of mating pairs in each day observed in Site 1 in Hakata Bay, per day from May to September, 2005 (Survey 1). M: male, NOAF: non-ovigerous adult female, OAF: ovigerous adult female. The pairings with NOAF were represented with white bars, while those with OAF were with black bars.

Fig. 5. Carapace width frequency distributions of live and dead specimens of *Pyrhila pisum* collected from Site 1 in Hakata Bay, per day from May to September, 2006 (Survey 2), showing normal distributions (curves) and the mean value of each cohort detected (triangular symbols). Same triangular symbols that continue over collection dates belong to the same cohorts that have settled in the same season and year.

Fig. 6. Temporal change in the mean carapace width of each *Pyrhila pisum* cohort collected from Site 1 in Hakata Bay from May to September, 2006 (Survey 2).

found on these days were the first brood for each female. This result suggested that adult females in the tidal flats oviposited at most three times during their reproductive season.

Only one dead crab was collected in mid August in the study site, but very few crabs could be recaptured in late August. Males released in May 25 were not recaptured after July 19, and crabs released in June 4 and 17 were not recaptured after August 14, although they were frequently recaptured in June and early July. In contrast, females released in May 25 and June 4 and 17 were recaptured alive until in August 14. This suggests that males tend to disappear from the intertidal area earlier than females. In addition, the time when crabs were recaptured coincided when crabs ceased molting. Exuviae

from adults were not found during the survey in the study site, which suggests that adults remain in their hard-shell condition and do not molt during most of their reproductive season in the intertidal zone.

Although released crabs were frequently recaptured, paired crabs were rare; 7 males (7 in a total of 97 recaptured times, 7.2%) and 5 females (5 in a total of 37 recaptured times, 13.5%). In both sexes, pairing was observed only once in each crab. The recaptured paired females included three ovigerous ones at stages 11, 12, and 15 of the 19 embryonic stages, which needed at least several days for the next hatching and oviposition.

The male-biased recapture rate and the low proportions of pairing crabs and carcasses co-

Table 1. Male crab *Pyrhila pisum* recaptures in Site 1 in Hakata Bay and condition of each crab. R: released, S: single, P: pair.

Crab No.	Date																				
	2008																				
	May	June										July									
	25	3	4	5	6	10	16	17	18	23	25	30	1	2	4	6	16	19	24	31	
M1	R							S													
M2	R														P						
M5	R												S								
M6	R	S													P						
M7	R	S																			
M8	R							S													
M10	R	S																			
M12	R	S			S																
M15	R	S																			
M19	R												S								
M20	R						S		S				S								
M23	R						S														
M24	R	S				S															
M25	R													S							
M26	R																S				
M30	R								S												
M31	R												S	P							
M33	R						S														
M34	R	S																			
M38	R	S																			
M39	R					S	S														
M41	R								S				S								
M45	R			S											S						
M47	R						S														
M48	R					S					S	S									
M52	R				S				S		S										
M53	R					S	S	S		S											
M55	R					S	S														
M56	R								S												
M62	R	S					S														
M63	R	S											S								
M64	R												S								
M68	R												S								
M69	R						S														
M75	R										S										
M77		R								S											
M78	R		R	S	S	S		S							S						
M79	R					S		S					P								
M80	R			S				S										S		S	
M81	R			S																	
M84	R					S															
M86	R			S																	
M89	R			S										S							
M90	R					S															
M91	R			S		S															
M92	R					S															
M93	R							P													
M95	R					S			S								S				
M97	R					S															
M98	R			S																	
M100	R					S															
M101	R					S				S											
M102	R													S	S						
M104	R																	S			
M106	R								S	S			P								
M112								R												S	
M113								R							S						
M114								R							S						
M115								R									S				
M116								R					P								
M123																		S			
M124																		R			S
M125																		R	S		

Table 2. Female crab *Pyrhila pisum* recaptures in Site 1 in Hakata Bay, condition of each crab, developmental stage of embryo (0–19) and estimated times of oviposition. *R*: released, *S*: single, *P*: pair. *D*: death.

Crab No.	Date																												Times of oviposition
	2008																												
	May			June										July										August					
	25	3	4	5	6	10	16	17	18	23	25	30	1	2	4	6	16	19	24	31	1	5	14	18	20	21			
F1	R 0												S 7														1		
F3	R 5								S 2																		2		
F9	R 0														P 15												1		
F11	R 5												P 11								S 11						3		
F14	R 5							S 7																			2		
F16	R 1								S 12																		2		
F21	R 0								S 5																		1		
F30	R 5														S 6												2		
F32	R 1					S 7																					2		
F38	R 0								S 8																		1		
F41	R 5							S 7																			2		
F43	R 1								S 7																		2		
F48	R 5					S 7																					2		
F52	R 0																		S 11								1		
F53	R 0							S 15																			1		
F56	R 0																					S 12					1		
F59	R 0														S 7												1		
F64	R 0		S 1																								1		
F66	R 1							S 7																			2		
F67	R 0																					S 5	D 8				1		
F70	R 0																		S 16								1		
F78		R 10						S 7																			2		
F82		R 8																				S 5					2		
F92		R 1						P 0								S 7											2		
F95		R 8							S 3						S 6												3		
F97		R 11						S 7																			2		
F100		R 8						S 7																			2		
F110		R 5														S 12											2		
F112								R 0								P 0											0		
F117								R 7														S 8					2		
F119								R 7							S 6												2		
F120								R 7													S 1						2		
F125																						R 7	P 12				1		

incided well with the collection results of Survey 1 and 2.

Discussion

Life history of *P. pisum*

In Hakata Bay's tidal flats, *Pyrhila pisum* occurred from late April to mid September (5 months) while adult crab carcasses were rarely found in the study site. Only one marked crab carcass was found in the late reproductive season from 260 released crabs. In addition, large adult crabs occurred numerously in the spring, which indicates that at least these adults had overwintered in the other site. Although carcasses might have been swept downstream easily or buried when compared to Site 2, because in Site 1 the bottom substrate is often disturbed

by swollen river flows and mud deposits, this result suggested that some adult crabs certainly died after reproduction in the late reproductive season, but most crabs of small-sized adult cohorts did not die during the reproductive season in the intertidal zone and probably migrated to a deeper subtidal zone. In addition, very young crabs ($CW < 5$ mm) were not found at the collection site. Therefore, *P. pisum* does not exclusively inhabit tidal flats, but has an active seasonal migration habit to deeper subtidal areas like some other crabs (Naylor, 1962; Shiota and Kitada, 1992; Hines et al., 1995; Kobayashi, 2013b) and hermit crabs (Fotheringham, 1975; Rebach, 1978; Miyagawa and Koga, 2017). *Pyrhila pisum* may spend a long part of their life in the deeper area, where they can grow, molt and die, and limitedly occur in

the intertidal area of Hakata Bay's tidal flat. Cues of their migration are not merely changes in water temperature in this case, even if the deep subtidal area is their hibernation area, and probably the inhospitable environmental conditions in September might facilitate their migration.

In Hakata Bay it is impossible to observe the complete life cycle of this crab. However, in Tokyo Bay, the occurrence of *P. pisum* in the tidal flats was a little longer than in Hakata Bay, lasting from April to November (Higashi and Furota, 1996). Some very young crabs (1.6–4.6 mm CW) were also collected in Tokyo Bay, suggesting that the duration of their stay in the intertidal tidal flat changes with some environmental conditions. It is possible to estimate the outline of their life cycle with the help of the data observed in Tokyo Bay.

Four cohorts were detected in both sexes, and adults did not molt during their reproduction. Reproduction was initiated after May by two large adult cohorts. These adult females could spawn eggs up to a maximum of three times. Most crabs of the two juvenile cohorts in May became adults (puberty molt) and joined reproduction after late June. This process coincided with the observation by Higashi & Furota (1996), who found that molting from April to August was only puberty molts among females under rearing conditions. These young adult crabs might continue to reproduce until September, but they disappeared from the intertidal area in Hakata Bay. In the case of Tokyo Bay, all adult females were in their non-ovigerous condition in early October, suggesting that reproduction had already finished (Higashi & Furota, 1996). The beginning of reproduction was May, when the atmospheric temperature reached 20°C (Japan Meteorological Agency). If temperature is an important factor for *P. pisum*'s reproduction, as often seen in temperate or tropical animals which have their critical temperature of reproduction at around 20°C (Yonge, 1940; Pearse, 1968), re-

production may have ceased in October, when the temperature drops below 20°C, as it does in Hakata Bay (Japan Meteorological Agency), and it can be presumed that reproduction lasts there from May to September (5 months). Early in their reproductive season (before July) larvae hatched only from large old crabs, which had joined reproduction in their second year, and small young adult crabs first join reproduction mostly later in the season (after July). Both the large old adults and the young small adults were composed of two cohorts. Because the size range of each cohort partially overlapped, the two cohorts which attained maturity in the same year might originate from the larvae that hatched in the anterior (before July) and posterior season (after July) of the same year, respectively.

Recruitment of young crabs (CW < 8 mm) into the tidal flat was observed from late July to early November in Tokyo Bay (Higashi & Furota, 1996). In Hakata Bay very young juveniles (CW < 8 mm) were similarly collected in August (Fig. 2), and the hatching season was estimated to last from late June to September (Fig. 3). The duration of larval development in *P. pisum* is short; 2 or 3 zoea stages and 1 megalopa stage, which probably take approximately 2 weeks to develop under natural conditions, because zoea stages needed 11 days under a rearing condition of 25°C (Ko, 1996). The minimum size of the young crabs collected in Tokyo Bay was 1.6 mm CW, which suggests that these young crabs included 0-year aged ones, because the 1st instar crab is approximately 1 mm, which can be estimated from the size of megalopa (Ko, 1996). However, the exact ages of all these young crabs cannot be confirmed (only 0-year age or 0 and 1-year ages), unless their growth processes are detected.

Mating behavior of P. pisum

From the mark-recapture experiment, it was presumed that adult females are cryptic, do not

wander actively and bury themselves in the sediment compared to adult males. Such a behavioral difference between the sexes suggests that the male-biased sex ratios in Survey 1 and 2 are not exactly those of the natural population. Probably adult males actively search for mates during wandering. However, the proportion of successful pairing in the population was low and both ovigerous and non-ovigerous adults were found mostly in a non-pairing condition, as shown in Survey 1 and the mark-recapture experiment. The pairing activity did not always succeed nor continue for such a long time, and males often lost guarding mates.

Adult females can mate and oviposit in their hard-shell condition (intermolt phase), but juvenile females are not subject to the guarding by males, suggesting that at least their guarding is not the same as the pre-copulatory guarding found before molting in some Portunidae and Cancridae, where males often start guarding juvenile females just before their puberty molt (Edwards, 1966; Berrill & Arsenhault, 1982; Savage, 1971). Although recaptured crabs did not show repeated pairing in the mark-recapture experiment, crabs of both sexes probably mated several times, because ovigerous females which needed at least several days for their next oviposition exhibited pairing in their natural condition. A similar mating behavior in females carrying eggs has been reported in other leucosiid crabs, *Ebalia scabriuscula* Ortmann (Naidu, 1954) and *E. tuberosa* (Schemberi, 1983), but this behavior is rare in most brachyuran crabs, except for some majiid crabs (Schemberi, 1983).

It was estimated that the duration of guarding was much longer than that of copulation, but detailed relationships between female conditions (e.g., timing of the softening of genital openings, ovarian maturity, and spawning), copulation and the guarding behavior cannot be determined from the present data. Additional observations of their mating behavior in the field and experiments in the laboratory may be

necessary to confirm the significance of their mating behavior. Further study will reveal the reproductive ecology of this unique species and family.

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