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Original Article

Larval period and molting in the Japanese spiny lobster *Panulirus japonicus* under laboratory conditions

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SUMMARY: A total of 325 pueruli of the spiny lobster *Panulirus japonicus* was successfully reared in the laboratory at Minami-Iku Station of Japan Sea-Farming Association, Shizuoka, Japan, during 1989–1997. Of these pueruli, 136 individuals metamorphosed into the first juvenile stage. The duration of the phyllosoma stage ranged from 231 to 417 days (mean 319.4 days), and it has a tendency to extend as the increase of water volume in the rearing tanks. The number of molting in the phyllosoma stage was 20–31. The body length of the last-stage phyllosoma ranged from 27.9 to 34.2 mm and the duration of the last stage was 11–26 days. The carapace length of the puerulus stage was 6.0–8.0 mm and the duration was 9–26 days. The present data and those of previous studies suggest that the body size and the duration of phyllosoma stage in captivity are affected by environment as in the field. The duration of the puerulus stage is considered to be controlled basically by water temperature and nutritional conditions in the phyllosoma.

KEY WORDS: **growth, larval period, molting, *Panulirus japonicus*, phyllosoma.**

INTRODUCTION

Rearing of Japanese spiny lobster, *Panulirus japonicus*, larvae was reported for the first time in 1899 by Hattori and Oishi.[1] About 60 years later, Nonaka *et al.* succeeded in rearing newly hatched phyllosoma to the second molting stage[†4] by feeding *Artemia* nauplii.[2] In 1981, Inoue succeeded in rearing the larvae from hatching to the last stage.[3] Thus, many experiments had been practiced for about 90 years, but no one succeeded in rearing phyllosoma to puerulus until 1988.

Success in the larval rearing of the spiny lobster, *P. japonicus*, from newly hatched phyllosoma to the puerulus stage, reported in 1989 by Yamakawa *et al.*[4] and Kittaka and Kimura,[5] was the first step in the advancement of seed production techniques for the species.

Following these works, Minami-Izu Station of Japan Sea-Farming Association (JASFA) started to improve techniques for mass seed production of the spiny lobster and succeeded in growing two larvae to the juvenile stage in 1989. During the period 1989–1997, a total of 325 larvae were successfully reared to the puerulus stage, of which 136 individuals metamorphosed into juveniles.

This study describes the growth and development of *P. japonicus* phyllosoma and puerulus based on the results of a series of experiments carried out at the station for 8 years. Comparisons of the duration of the phyllosoma stage and the growth of phyllosoma between laboratory-reared and wild-collected larvae are also discussed.

MATERIALS AND METHODS

The observation in this study was tried on the above-mentioned 325 puerulus and 136 juveniles derived from the puerulus.

Larval rearing methods were tried as follows.

Rearing tanks

Two types of rearing tanks were used: specially designed semispheric containers with diameters of 22, 30, 60 and 100 cm (hereafter referred to as 1, 5, 40, and 150 L tanks, respectively) and commercial *Artemia* incubation tanks with 55 cm in diameter (referred to as 80 L tanks). The semispheric tanks were made from glass (1 L tank) or acrylic resin (5, 40, and 150 L). The 80 L tanks were made from polycarbonate resin.

† We refer to the newly hatched phyllosoma as the first molting stage and each ecdysis advances one molting stage.

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Rearing water

The seawater used for 1 L tanks was filtered with a 0.45 μm-mesh filter (TCG-045; Toyo Roshi Kaisha Ltd, Tokyo, Japan) and sterilized with ultraviolet light (SS-15G; Sen Lights Corp, Osaka, Japan). Phyllosoma in the 1 L tank were transferred daily to another tank containing fresh seawater. For the semispheric tanks of other sizes and 80 L tanks, seawater was filtered through a 0.2 μm mesh membrane filter and then supplied to each tank at the daily water exchange rate of about 20-fold.

In semispheric tanks except for 1 L, rearing water was gently poured by air-tube fitted to air-stones and drained through a central column covered with 1–3 mm mesh net. In 80 L tanks, water was drawn from the drainpipe and drained from the outlet at near surface through 1–2 mm mesh net.

Rearing temperature

Water temperature in 1 L and 5 L tanks was set at three different levels (i.e. 24°C throughout the experimental period, 27°C throughout the period, and 27°C from day of hatching to day 120 and 24°C from day 140 following gradual lowering of temperature for 20 days (27–24 °C level). In 40, 80 and 150 L tanks, the third level of water temperature (27–24°C) was applied.

Feeding

During the initial 4–6 molting stage, larvae were fed with *Artemia* nauplii that had been enriched with *Phaeodactylum* sp. for 24 h. They were fed once a day in 1 L tanks, and twice a day in other tanks. When they were just fed, the densities of nauplii in rearing tanks were 3000–4000 individuals per liter in 1 L tanks. In other tanks, the densities were about 1500–2000 individuals per liter.

In the following stages, minced ovarian tissues of mussel were given together with *Artemia* reared with *Phaeodactylum* sp. for 2–14 days.

The minced tissues of mussel were cubic and the lengths of pieces were 0.5–2 mm. They were fed once a day and the numbers of tissues were four–six pieces per one phyllosoma. The reared *Artemia* were grown up to 0.7–3 mm and fed once a day with the densities of about 50–1500 individuals per liter.

Rearing procedure

In 1 L tanks, one to 20 newly hatched phyllosoma were kept. When they attained the 6–16 molting stage, some of the phyllosoma larvae were transferred into 5 L tanks at a stocking density of 1.6–3 individuals/L. In 40, 80 and 150 L tanks, newly hatched phyllosoma were kept at densities of 10–20 individuals/L. In the 150 L tanks, the larvae rearing was discontinued from day 41 because the large water volume of the tanks was difficult to manage for the rearing, and the remaining larvae in the tanks were transferred into 40 L tanks. They were moved again to 5 L tanks because of decrease in number in the 40 L tanks. The number of molting was counted with a casted crust.

When the phyllosoma attained the puerulus stage, each puerulus was immediately transferred to a 3 mm mesh cage (10 × 10 × 7 cm), and reared at the same temperature as in the water in which they metamorphosed into puerulus.

Collection of wild puerulus

Wild pueruli were collected daily with five C-type collectors[6] at the wharf at Minami-Izu Station from December 1989 to December 1993. They were measured on carapace length and reared until first juveniles to confirm their species.

RESULTS

Duration of the phyllosoma stage

The number of puerulus obtained every year from 1989 to 1997 was 2, 5, 67, 1, 144, 6, 59, and 41, respectively, totaling 325 individuals. Of those, the duration of the phyllosoma stage was determined precisely for 271 individuals. The duration ranged from 231 to 417 days (mean 319.4 ± 40.9 SD days).

Table 1 shows frequency distribution of stage duration at a 20-day interval by tank size. In the 1 L tanks, 53 individuals metamorphosed into puerulus. The duration of the phyllosoma stage was 231–315 days with a mean of 279.6 days. The 35 individuals developed into the puerulus stage in the 5 L tanks and the duration ranged from 234 to 356 days (mean, 299.8 days). In the 40 L tanks, the 178 individuals reached the puerulus stage, and the duration was 256–417 days (mean 336.1).

Thus a trend was observed toward an increase in duration of the phyllosoma stage with increase in tank size ($P < 0.01$ by t-test). The variation range of the duration of the phyllosoma stage, being 84, 122 and 161 days in the 1, 5 and 40 L tanks, respectively, showed a trend toward increase in proportion to the increase in tank size except for the result in the 80 L tanks.

Relationship between rearing water temperature and the duration of the phyllosoma stage is shown in Table 2. In the group maintained constantly at 27°C, the duration was 291.0 days. In the group in which temperature

Table 1 Frequency of the duration of phyllosoma stage in *Panulirus japonicus*

Duration (days)	Capacity of rearing tank				
	1 L	5 L	40 L	80 L	Total
221–240	3	1			4
241–260	7	3	1		11
261–280	15	5	6		26
281–300	18	10	19	2	49
301–320	10	8	31	1	50
321–340		2	43	1	46
341–360		6	36		42
361–380			28	1	29
381–400			11		11
401–420			3		3
n	53	35	178	5	271
$\bar{x}$	279.6	299.8	336.1	320.2	319.4
Range	(231–315)	(234–356)	(256–417)	(290–366)	(231–417)

Table 2 The duration of phyllosoma stage relating to rearing temperature

Rearing temperature	*n*	Duration of phyllosoma (days) Average	Min.	Max.	SD	CV (%)[1]
27°C	2	291.0	251.0	331.0	40.0	13.7
27–24°C	302	321.0	234.0	417.0	36.7	11.4
24°C	5	328.2	231.0	379.0	27.0	8.2

[1] Coefficient of variance.

was set initially at 27°C and later lowered to 24°C, the duration was 321.0 days. At a constant temperature level of 24°C, the duration was 328.2 days. These results clearly indicate that the duration of the phyllosoma stage increased as the water temperature declined.

Number of molt during the phyllosoma stage

The number of molting ranged 20–31 (mean 26.9) in the 1 L tanks and 26–30 (mean 27.4) in the 5 L tanks, suggesting no obvious difference associated with the tank size.

Relationship between the duration of the phyllosoma stage and the number of molting is shown in Fig. 1. The correlation coefficient was 0.92 for phyllosoma reared constantly at 24°C and 0.72 for those reared at 27°C initially and 24°C finally, suggesting a positive relationship between the two factors. Individuals with long periods of the phyllosoma stage underwent more numerous molting, and the interval of molting became longer at the lower temperature so that the number of molting decreased.

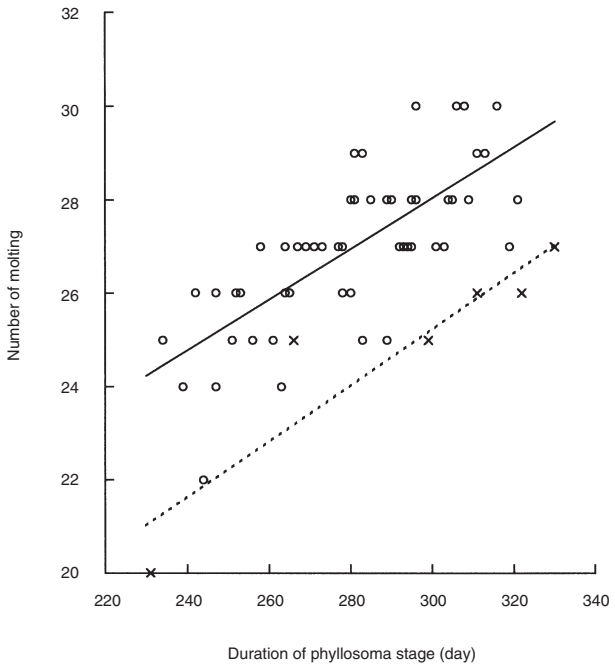

Fig. 1 Relationship between number of molting and duration of phyllosoma stage in *Panulirus japonicus*, at two different water temperatures. Data (○) and regression line (—) at 27–24°C where $y = 0.0544x + 11.73$, $r = 0.723$, $n = 63$. Data (×) and regression line (– –) at 24°C where $y = 0.060x + 7.137$, $r = 0.919$, $n = 6$.

Phyllosoma in the last molting stage

As shown in Fig. 2, the body length of 12 individuals of the last phyllosoma stage ranged from 28 to 34 mm (mean 31.3 ± 1.9 mm), with a size difference of 6 mm between the smallest and the largest individual. The

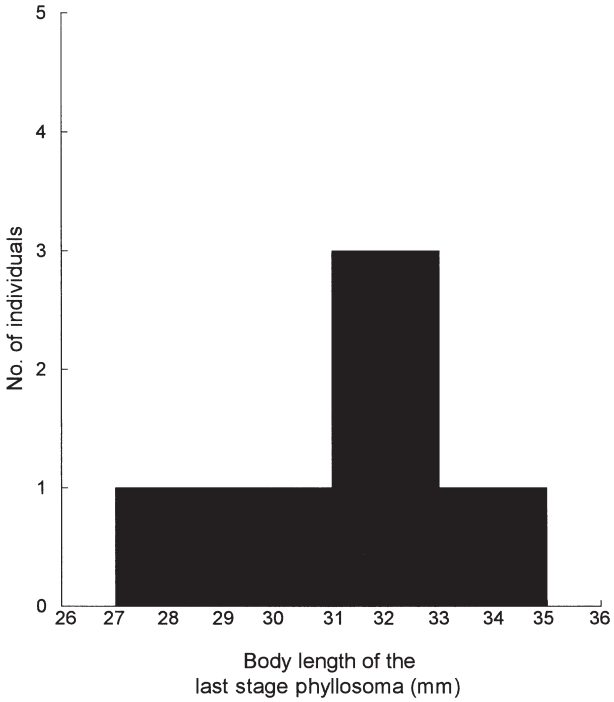

Fig. 2 Frequency distribution of body length in the last stage phyllosoma stage in *Panulirus japonicus*. $n = 12$.

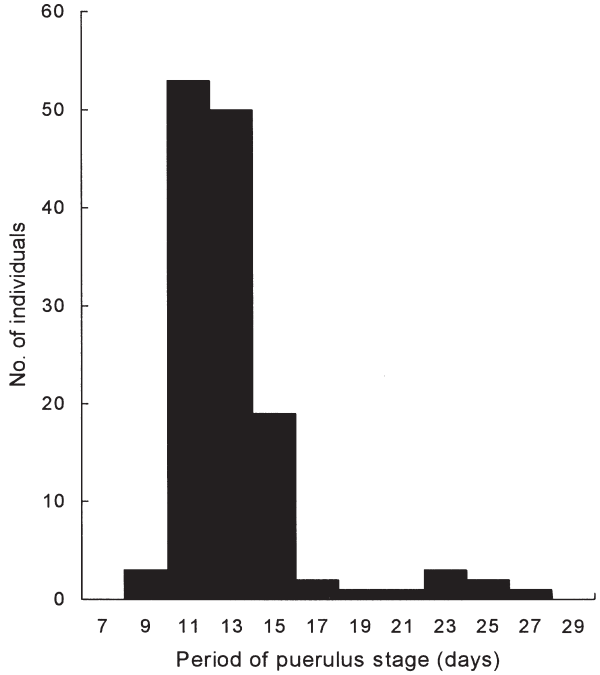

Fig. 3 Frequency distribution of duration of the puerulus stage in *Panulirus japonicus*.

duration of last phyllosoma is shown in Table 3 for 48 individuals reared in 1, 5, and 40 L tanks. It ranged from 11 to 26 days with a mean of 17.1 days.

Puerulus stage

The duration of the puerulus stage in 135 individuals was 9 to 26 days with a mean of 12.6 days (Fig. 3). Ninety-three per cent of these individuals metamorphosed into juveniles for 9–15 days, and the remaining required 16–26 days to attain the stage. The puerulus stage was less than 17 days for individuals which were reared in 40 L tanks, while it was more than 17 days in some individuals reared in 1 L or 5 L tanks during the phyllosoma stage.

The carapace length of 93 puerulus was 6.0–8.0 mm with a mean of 7.0 mm (Table 4). The carapace length of 105 wild puerulus, which were collected daily with settled collectors at the wharf at Minami-Izu Station from December 1989 to December 1993, was slightly greater than that of the reared specimens, ranging from 6.4 to 8.0 mm with a mean of 7.31 mm.

Table 3 Frequency of the duration in the last molting stage of phyllosoma in *Panulirus japonicus*

Duration (days)	Capacity of rearing tank			
	1 L	5 L	40 L	Total
11	1			1
12				
13	2			2
14	1			1
15	3	1	2	6
16	5	5	1	11
17	6	3	1	10
18	1	3		4
19	2	1	1	4
20	1	2		3
21		1		1
22		1	1	2
23	1	1		2
24				
25				
26	1			1
n	24	18	6	48
$\bar{x}$	16.8	18.1	17.3	17.1

DISCUSSION

The number of molting, durations and body sizes of phyllosoma and puerulus from the data obtained in this study and those given in the previous study are shown in Table 5. The duration of the phyllosoma for reared individuals was 307 days by Yamakawa *et al.*[4] and 340 and 391 days by Kittaka and Kimura.[5] Oshima suggested that, on the basis of the occurrence season of puerulus in the wild, the phyllosoma stage would span at least half a year.[6]

These are not contradictory to the results of the present study.

According to Booth and Phillips the duration of the phyllosoma stage has been reported for the following nine palinurid species/subspecies: *P. argus*, *P. japonicus*, *P. cygnus*, *P. interruptus*, *P. penicillatus*, *P. homarus rubellus*, *P. homarus homarus*, *P. polyphagus* and *P. marginatus*.[9] The duration has been assumed to be 4–6 months for *P. homarus rubellus* and 6–11 months for the remaining seven species/subspecies without *P. polyphagus*. In the present study, the duration of *P. japonicus* was 7.7 to 13.9 months (231–417 days) with a mean of 10.6 months (319 days).

Although the relation between the duration of phyllosoma and tank size is not clearly described, the duration seems to increase as the tank size becomes larger, except for 80 L tanks as shown in Table 1. In this study, the rearing methods were basically the same in each size of tank, but we could not use entirely the same methods in all trials. Therefore, more experiments will be needed to confirm this tendency on the relationship between the tank size and phyllosoma duration.

The duration of tank-reared phyllosoma is longer than those estimated for wild-collected samples in the previous studies.[6] Further investigations are needed to confirm whether a definite difference exists between individuals in captivity and those occurring in the sea, although the great variation in duration of the phyllosoma stage found in both reared and wild may make it difficult to determine the period clearly.

Johnson classified the phyllosoma of *P. interruptus* collected from the wild into 11 stages.[10] The work has been used as a standard reference in the studies of the development of palinurid phyllosoma, of the nine species/subspecies mentioned above. His classification is based on morphological characters, and permits size variations within a stage and size overlapping between stages. Inoue estimated 11 stages for *P. japonicus* phyllosoma on the basis of his rearing experiments from hatching to the last stage.[3] He pointed out that a single molting did not necessarily result in development of the next stage and

Table 4 Frequency of carapace length in reared and wild* puerulus

Carapace length of puerulus (mm)	Reared	Wild
6.0	1	
6.2	2	
6.4	10	2
6.6	8	1
6.8	9	7
7.0	17	11
7.2	24	16
7.4	13	25
7.6	3	23
7.8	3	16
8.0	3	4
n	93	105
$\bar{x}$	7.00	7.31

*Collected from the site of Minami-lzu Station JASFA.

Table 5 Comparison of the duration and size of phyllosoma/puerulus in palinurid lobsters

Species	Authors Reviewers	No. of stages	No. of molts	Duration of phyllosoma stage (month)	Duration of phyllosoma stage (days)	Body length of the final stage of phyllosoma (mm)	Duration of puerulus stage (days)	Carapace length of puerulus stage (mm)
Panulirus japonicus	This study		23 ~ 31*		234 ~ 417*	27.9 ~ 34.2*	9 ~ 26*	6.0 ~ 8.0*
	Oshima[6]			> 6				–
	Inoue[3]	11		6 ~ 9		29.64*		–
	Yamakawa *et al.*[4]		29*		307*	30.3*	10*	–
	Kittaka and Kimura[5]				340 391*	32*	13*	–
	Nonaka *et al.*[7]						1 ~ 20	6.0 ~ 9.0
	Kanamori[8]						1 ~ 9	–
Panulirus argus	Booth and Phillips[9]	11		> 6			> 4 ~ 8	
Panulirus cygnus	Booth and Phillips[9]	9	15	9 ~ 11			15	
Panulirus interruptus	Booth and Phillips[9]	11		7.75			75	
	Booth and Phillips[9]			> 5*				
Panulirus penicillatus	Booth and Phillips[9]	11		> 7 ~ 8				
Panulirus homarus homarus	Booth and Phillips[9]	10						
Panulirus homarus rubellus	Booth and Phillips[9]	9		4 ~ 6				
Panulirus polyphagus	Booth and Phillips[9]						> 2 ~ 4	

*Reared individuals.

that one stage might have several molting stages which did not differ from each other in their morphological traits.

Classification of phyllosoma into stages can certainly be made based on morphological characteristics. In contrast, the relationship between the stage of phyllosoma and the molting stage remains largely unclear, as the great variation in number of molting seen in this study. Further examination is necessary in order to clarify whether there is a constant relationship between stages and molting stages. Characteristics that can represent the growth and development of phyllosoma are at present limited to the body length and molt-stage, yet these two factors seem to be sufficient at least for practical purposes for the time being.

The duration of puerulus stage in laboratory rearing was 10 days by Yamakawa *et al.*,[4] 13 days by Kittaka and Kimura,[5] and 9 to 26 days in this study. However, the duration of wild-collected puerulus was reported to be 1–20 days by Nonaka *et al*.[7] and 1–9 days by Kanamori.[8] According to Booth and Phillips, the duration of puerulus on the other palinurid species has been reported as following: over 4–8 days (*P. argus*), 15 days (*P. cygnus*), 75 days (*P. interruptus*) and 2–4 days (*P. polyphagus*).[9]

The much shorter puerulus period in wild-collected puerulus seems to be attributable to the timing of their collection immediately before metamorphosis. The maximum duration of puerulus stage of *P. japonicus* was 26 days for reared individuals in this study and 20 days for wild-caught ones.[7] These indicate that some individuals take a long period of the puerulus stage both in captivity and in the natural environment.

It is very likely that the body size of puerulus is influenced solely by the environment they have lived in during their phyllosoma stage, because puerulus do not feed throughout the stage.[4,5,11] Consequently, the duration of the puerulus stage may be controlled by two factors (i.e. water temperature and the nutritional conditions during the phyllosoma stage).

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