

Larval Development of the Grooved Shrimp (*Penaeus kerathurus* Forskal, 1775) Under Laboratory Conditions

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Abstract

The larval stages of the grooved shrimp *Penaeus kerathurus* (Forskal, 1775) were described and photos were taken based on the materials hatched and reared in the laboratory. The larval development of *P. kerathurus* consists of 6 naupliar, 3 protozoal, and 3 mysis stages before the first postlarva. Differences in the number of setae on 2nd antenna exopod and spine formation on caudal furca were described for naupliar (N1-N6) stages of *P. kerathurus* and the chronological pattern was compared with previous and relevant studies on other *Penaeus* species.

Key Words: Grooved shrimp, Penaeidae, *Penaeus kerathurus*, larval development

Introduction

Penaeus kerathurus is found from the South coast of England down the whole Eastern Atlantic seaboard as far south as Angola, and throughout the Mediterranean Sea (Dore and Frimodt, 1987). It is also distributed along the coast of Aegean Sea and is one of the most important commercial species in this region. Heldt (1938) first described the larval development of *P. kerathurus* and reported eight naupliar, three protozoal and three mysis stages. Although, most studies described six naupliar stages (San Feliu, 1966; Lumare *et al.*, 1971; Lumare and Gozzo, 1973; Lumare, 1979; Alpbaz, 1980), Haliki (1981) and Uçal and Hoşsucu (1987) reported five naupliar stages, with the number of protozoal and mysis stages being identical. Morphological details of the different larval stages, however, have not been described in these studies.

The purpose of the present study was to describe the larval development of *P. kerathurus* with an emphasis on naupliar stages and to compare its larval development with that of other known *Penaeus* larvae.

Materials and Methods

Three matured females of *P. kerathurus* were caught at night by a shrimp boat off Sahil Evleri of Izmir Bay. The next morning, they were transferred to the hatchery of the E.U. Fisheries Faculty in Urla in a 50 l plastic bucket, and stocked in three fiberglass

tanks (Volume: 2m³) with aerated seawater. Only one female in tanks spawned during the night, and the fertilized eggs in the tank were used for this study. Spent spawner was removed to avoid a second spawning in the same tank. Before the experiment, the tank was filled with 20 µm-filtered seawater at 37‰ and 28°C containing 20 to 40 ppm disodium salt of ethylene-diamino tetra-acetic acid (EDTA).

The diatom, *Chaetoceros calcitrans*, was added at about 1 x 10⁵ cells ml⁻¹ and this concentration was maintained daily at protozoa stage. When the shrimp reached mysis I, freshly hatched *Artemia* nauplii were added daily at a density of 1-4 individuals ml⁻¹. During larval rearing, half of the water in the tank was changed every 48 h. The water temperature ranged 29.0-30.0°C, and the salinity was 37.0-38.0‰.

Observations were made on all larval stages and the first postlarval stage. Animals were dissected and their photos were taken under the light microscope. Measurements were made according to the definitions adopted by Motoh (1979) and a micrometer eyepiece was used for length measurement. At nauplius stage the length of the body (BL) was measured between the apical and caudal ends excluding the furcal spines, and for the width of the body (BW), at the point of greatest width where the 2nd appendages are located. The total length (TL) of a larva was taken from the tip of rostrum, if present, to the posterior end of telson excluding the furcal spines. Carapace length (CL) was measured from the tip of rostrum, if present, to the mid-posterior end of the carapace. The measurements are expressed as 95% confidence limit ($\bar{X} \pm 2SE$) of 30 individuals.

Results

The chronology of larval development is shown in Table 1.

Table 1. Chronology of larval development of *P. kerathurus* 31 July - 11 August, 2000

Stage	Date	First detected time	Duration (Hrs)
Egg	31 / July	21:00	12:30
N1	01 / August	09:30	05:00
N2		14:30	05:00
N3		19:30	06:00
N4	02	01:30	06:00
N5		07:30	05:00
N6		12:30	09:00
PZ1		21:30	32:00
PZ2	04	05:30	43:30
PZ3	06	01:00	36:30
M1	07	13:30	28:00
M2	08	17:30	33:00
M3	10	02:30	28:00
PL1	11	06:30	

Water temp.: 29.0-30.0°C, salinity: 37.0-38.0‰,

N: Nauplius, PZ: Protozoa, M: Mysis, PL: Postlarva

Egg

The viable eggs are spherical, and the diameters were 0.256-0.272 mm. The first cleavage occurred at 30 min after spawning. The embryonic nauplius bearing setae on each appendage was observed about 4:30 hours before hatching and had a vibrating movement about 2 hours before hatching. The larvae reached at nauplius stage in 12:30 hours after spawning.

Nauplius

In *P. kerathurus*, there are 6 naupliar (N1-N6) stages (Figure 1) and all instars bear 3 pairs of ventrally located appendages; namely, 1st antennae, 2nd antennae and mandibles. The setal formulae of each appendage in the first to the sixth nauplius are shown in Table 2.

First Nauplius (N1): BW = 0.186 ± 0.001 mm; BL = 0.368 ± 0.001 mm.

Body is pyriform, unsegmented. The anterior half of the body is somewhat larger than the posterior. An ocellus is present at the anterior part and remains unchanged during the naupliar stage. The posterior margin of the body bears one pair of furcal spines, which is slightly curved. There are three pairs of unsegmented appendages. The first antenna is uniramous, while the second antenna and the mandible are biramous. The setae on these are smooth and not plumose.

Second Nauplius (N2): BW = 0.190 ± 0.001 mm; BL = 0.388 ± 0.001 mm.

The body shape is similar to that of the first nauplius. The most distinguishable change from the preceding stage is that the longer setae on each appendage become plumose. A pair of furcal spines is straight. Compared with that of the preceding nauplius, the labrum is somewhat pointed, the labium being rather distingly below the labrum.

Third Nauplius (N3): BW = 0.194 ± 0.001 mm; BL = 0.400 ± 0.001 mm.

Posterior part is slightly bifurcated, bearing 3 pairs (sometimes 2 pairs) of furcal spines. The number of setae on the second antenna is increased. With the new seta at the tip of the exopod, the number increases to 7 and the small new seta which grew in the preceding nauplius becomes somewhat longer and plumose. The mandible has no change from the N2. Rudiments of ventral appendages are observed, and develop gradually in subsequent naupliar stages.

Fourth Nauplius (N4): BW = 0.198 ± 0.000 mm; BL = 0.420 ± 0.001 mm.

The body becomes longer, the posterior end of the body is clearly bifurcated, and the furcal spines become 4 pairs. The number of setae on the endopod of the second antenna is increased. With the appearance afresh of one small seta at the tip and another small one at the side of the exopod, their number increases to 9. Sometimes, these small setae are missing. Rudimentary appendages more prominent with 4 pairs of biramous appendages are clearly seen. The basal part of each mandible becomes slightly swollen.

Fifth Nauplius (N5): BW = 0.199 ± 0.000 mm; BL = 0.463 ± 0.002 mm.

The posterior of the body becomes more slender than that of the preceding stage, and two rounded furcal processes are formed. The furcal spines increase to 6 pairs, and the longer spines bear many minute spinules on the surface. With the new seta at the tip of the exopod, the number increases to 10. Sometimes this small seta is missing. An articulation on the exopod of the second antenna can be observed slightly. Rudimentary ventral appendages have developed further. The swellings at the base of the mandibles are more distinct.

Sixth Nauplius (N6): BW = 0.201 ± 0.001 mm; BL = 0.527 ± 0.002 mm.

Posterior end is deeply bifurcated, bearing 7 pairs of furcal spines. A carapace and a digestive tract are rudimentary. Adding 2 small setae on the outside the exopod has 12 setae with 9 articulations. These 2 small new setae on the outside, however, are sometimes lacking. Ventral appendages bear a few small terminal setae. The swellings at the base of the mandibles are larger, and small teeth can be observed.

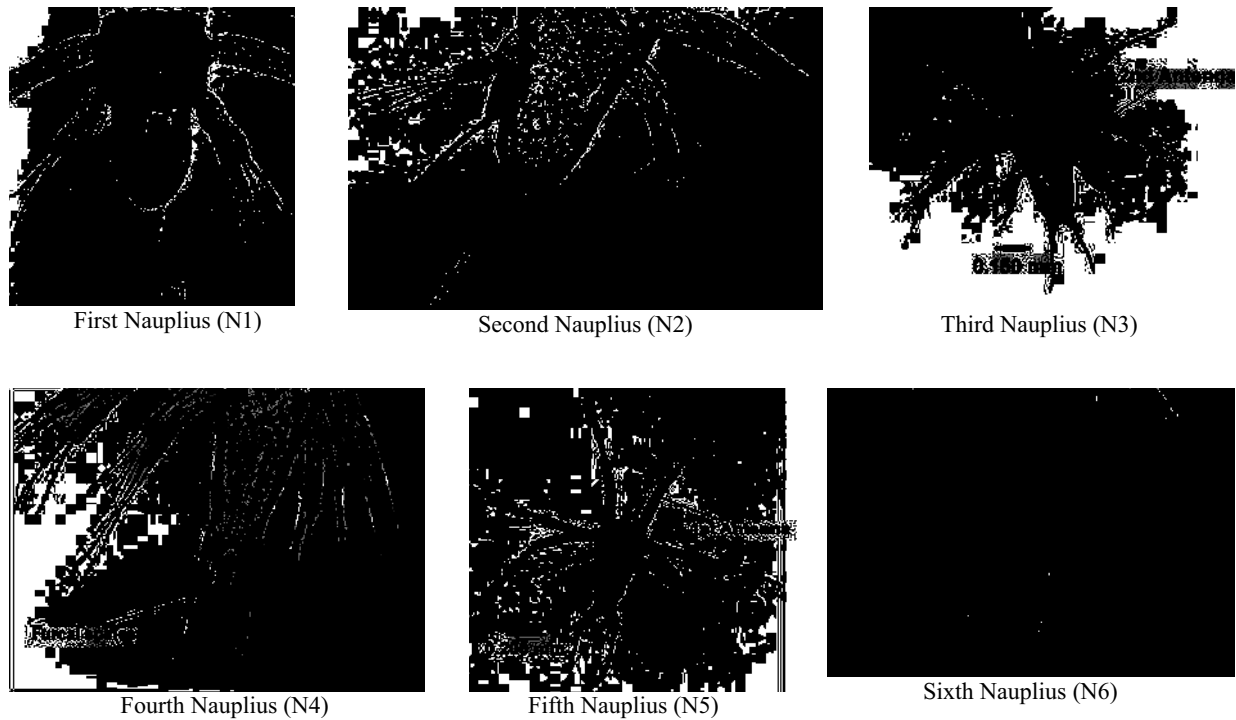


Figure 1. Naupliar stages of *Penaeus kerathurus*.

Table 2. Setal formulae of appendages in naupliar stages of *P. kerathurus*

Stage	First antenna			Second antenna						Mandible	
	Inn.	Ter.	Out.	Endo.		Exo.			Endo.	Exo.	
				Inn.	Ter.	Inn.	Ter.	Out.			
N1	2	(1)2	1	2	2	3	2	0	3	3	
N2	2	3	1	2	2	3	2(1)	0	3	3	
N3	2	3	1	2	(1)2	4	2(1)	0	3	3	
N4	2	3	1	2	3	(1)4	3(1)	0	3	3	
N5	(1)2	3	1	2	3	(1)5	3(1)	0	3	3	
N6	3	3	1	2	(1)3	(1)5	3(1)	(2)	3	3	

Endo.: Endopod, Exo.: Exopod, Inn.: Inner, Ter.: Terminal, Out.: Outer, (): Minute setae

Protozoa

First Protozoa (PZ1): CL = 0.455 ± 0.003 mm; TL = 0.961 ± 0.011 mm.

The body is divided into 2 parts. The anterior part is covered by the well-rounded carapace. The posterior part consists of a six-segmented thorax and an abdomen (Figure 2). Feeding appendages are functional and antennae and caudal spines are even further developed. The furcal spine formula is 7+7.

Second Protozoa (PZ2): CL = 0.721 ± 0.004 mm; TL = 1.580 ± 0.019 mm.

The second protozoa is distinguished from the first by the presence of a pair of stalked compound eyes, a rostrum with spines, and a further elongated abdomen (Figure 2). The furcal spine formula remains 7+7.

Third Protozoa (PZ3): CL = 0.946 ± 0.006 mm; TL = 2.476 ± 0.013 mm.

Each of the first five segments has a dorso-median spine at the posterior margin (Figure 2). Vestigial pereopods or walking limbs posterior to the feeding appendages can also be seen. The most obvious characteristic of PZ3 larvae is the presence of

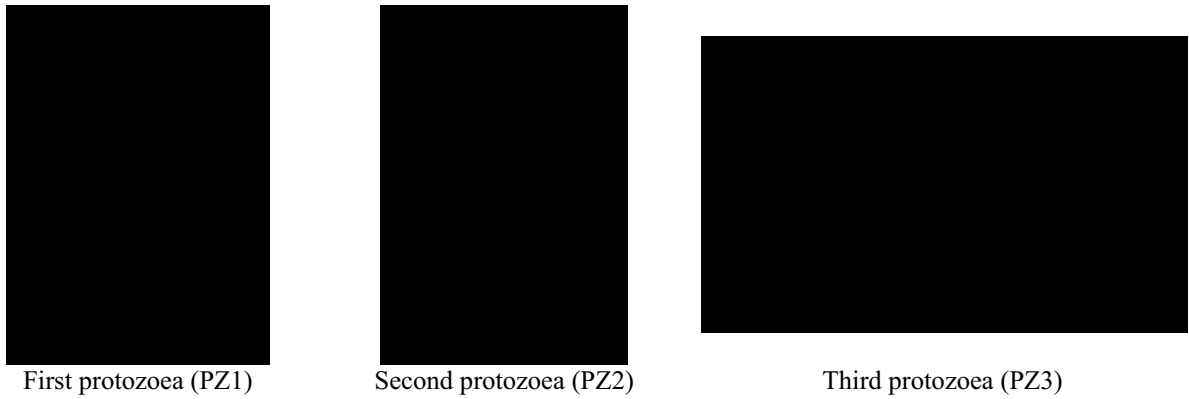


Figure 2. Protozoa stages of *Penaeus kerathurus*.

uropods, flat fan-like swimming appendages, anterior to the tail. The furcal spine formula is 8+8.

Mysis

First Mysis (M1): CL = 1.064 ± 0.060 mm; TL = 3.090 ± 0.016 mm.

The first mysis is easily distinguished from the last protozoal substage by an even further elongation of the body, development of functional pereopods, and the appearance of another fan-like swimming appendage, the telson. No pleopods are present (Figure 3).

Second Mysis (M2): CL = 1.172 ± 0.005 mm; TL = 3.680 ± 0.024 mm.

The M2 is characterized by the developed uniramous pleopods, the outer-distal spine on the exopod of the second antenna, and a rostral tooth on the carapace. The pereopods are almost unchanged, but the unsegmented pleopods, forming the shape of "J" letter, have elongated (Figure 3). The furcal spine formula is 8+8.

Third Mysis (M3): CL = 1.212 ± 0.007 mm; TL = 4.217 ± 0.015 mm.

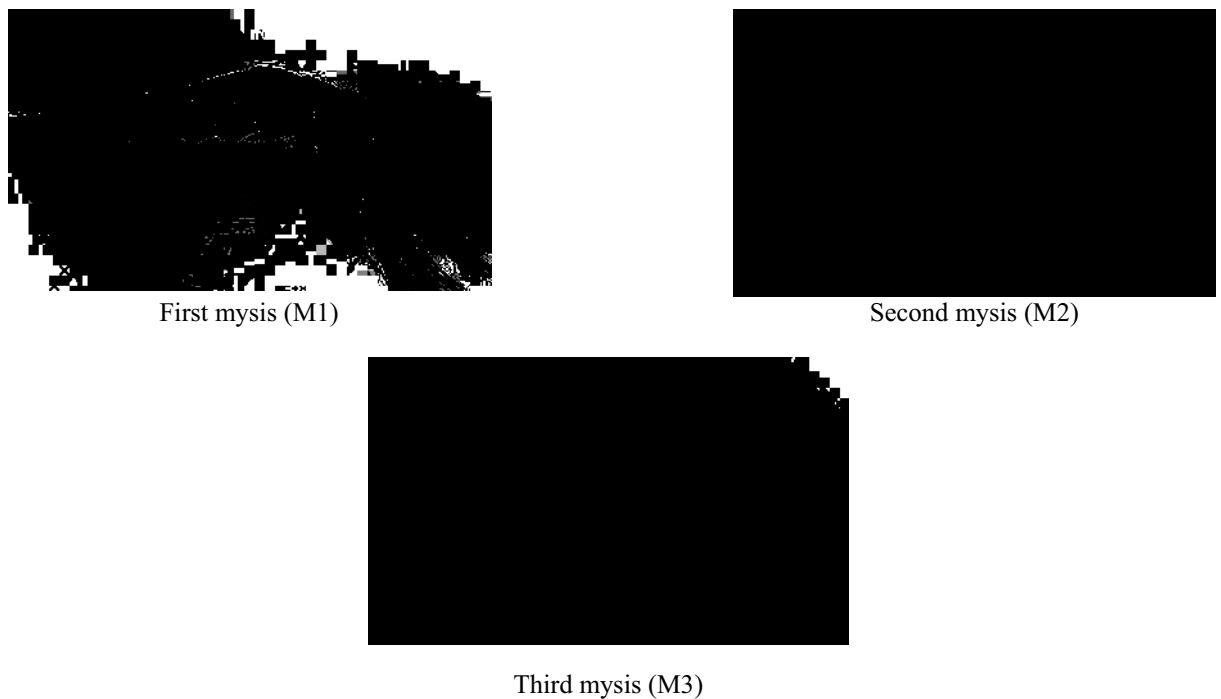


Figure 3. Mysis stages of *Penaeus kerathurus*.

Substage M3 larvae can be distinguished from M2 larvae by appearance of the pleopods. These pleopods are composed of two segments and nearly twice as long as those of M2 larvae (Figure 3). The furcal spine formula is 8+8.

Postlarva

First Postlarva (PL1): CL = 1.394 ± 0.011 mm; TL = 4.841 ± 0.0317 mm.

The first postlarva can be distinguished from M3 by functional chelae of pereopods, and setose pleopods (Figure 4). Being different from the mysis, they swim normally balancing the body horizontally. Rostrum with 2 forward-pointing rostral teeth on dorsal side, one on rostrum proper and another on carapace. The caudal furca still bears 8+8 setae.

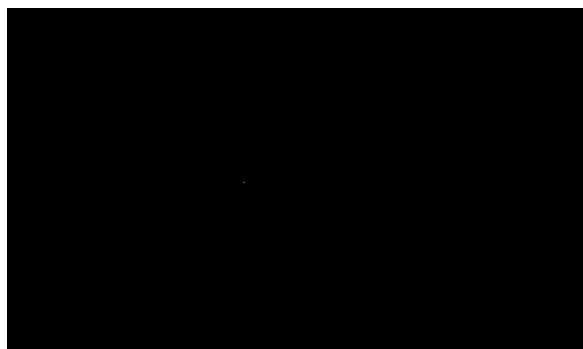


Figure 4. First postlarva (PL1) stage of *Penaeus kerathurus*.

Discussion

Hatching duration of eggs was determined to be 12 h 30 min, which is similar to that of *P. brevivirostris* showing 13 h while that of other *Penaeus* shrimps ranges from 14 h to 18 h. In this study, water temperature is suggested to be the main factor

affecting the duration of egg and naupliar stages. The naupliar chronology took progressively longer and the sixth nauplius took twice as much or longer than that of the preceding stages, a tendency that was also reported in other *Penaeus* species however further studies will be required for clarifying the phenomenon (Table 3).

The larval development of *P. kerathurus* has been studied previously, (Lumare *et al.*, 1971; Lumare and Gozzo, 1973; Lumare, 1979; Alpbaz, 1980; Haliki, 1981; Uçal and Hoşşucu, 1987), but these studies did not provide a detailed morphological description of the appendages compared to the present study. A major difference among the six previous studies is in the number of naupliar instars recorded. Only five naupliar instars are reported by Haliki (1981) and Uçal and Hoşşucu (1987). We considered that the main criteria for determination of substages of nauplius were the number of setae on the second antenna and spine formulation on caudal furca as in previous studies. In contrast, Lumare *et al.*, 1971; Lumare and Gozzo, 1973; Lumare, 1979; and Alpbaz, 1980 recorded six, which is consistent with our observation. The setal numbers on second antenna exopod and furcal spine formula in naupliar stages of *P. kerathurus* are similar to those of *P. brevivirostris*, *P. occidentalis* and *P. japonicus*. In the previous studies with *P. kerathurus*, there are reports of different furcal spine formula between N2 and N4 stages. Uçal and Hoşşucu (1987) observed that furcal spine formula was 1-2 at N2, 4-5 at N3, and 6+6 at N4, whereas in the present study the furcal spine formula was found to be 1+1 at N2, 2-3 at N3, and 4+4 at N4. Comparisons related to the number of setae on second antenna was presented in Table 4 and spine formulation on caudal furca was given in Table 5.

In this study, we observed the similarities in morphological characteristics between *Penaeus kerathurus* and *Penaeus japonicus*, which were also reported by Lumare and Gozzo (1973) and Alpbaz (1980). As to body formation, PZ1 had abdominal segments at the end of the stage and the abdomen

Table 3. Comparison of hatching duration of eggs and N1-N6 stages between *P. kerathurus* and other *Penaeus* sp.

Egg / N1-N6 (Hrs)	References	Egg	N1	N2	N3	N4	N5	N6
<i>P. monodon</i> (25-28°C)	Kungvankij, 1976	15	5	6	6	6	6	20
<i>P. semisulcatus</i> (24-27°C)	Kungvankij <i>et al.</i> , 1972	18	4	6	6	6	6	24
<i>P. semisulcatus</i> (26-30°C)	Hassan, 1982	14	11	10	12	11	13	-
<i>P. brevivirostris</i> (26-28°C)	Kitani, 1997	13	03:50	03:50	05:05	05:20	05:35	10:50
<i>P. occidentalis</i> (26-28°C)	Kitani, 1996	14:25	04:25	04:20	05:15	05:30	06:45	12:15
<i>P. japonicus</i> (27-29°C)	Hudinaga, 1942	13-14	3	3	10	7	7	7
<i>P. kerathurus</i> (27°C)	Lumare and Gozzo, 1973	-	5	5	6	7	8	19
<i>P. kerathurus</i> (20°C)	Lumare and Gozzo, 1973	-	11	11	11	15	12.5	45.5
<i>P. kerathurus</i> (20-28°C)	Uçal and Hoşşucu, 1987	15	11	10	11	10	18	-
<i>P. kerathurus</i>	present study	12:30	5	5	6	6	5	9

Table 4. Comparison of setal number on 2nd antenna exopod in naupliar stages between *P. kerathurus* and other *Penaeus* sp.

Egg / N1-N6 (Hrs)	References	N1	N2	N3	N4	N5	N6
<i>P. monodon</i>	Kungvankij, 1976	5	6	7	8	8	9
<i>P. semisulcatus</i>	Kungvankij <i>et al.</i> , 1972	5	6	7-8	7-8	8	9
<i>P. semisulcatus</i>	Hassan, 1982	5	6	7-9	9	9-10	-
<i>P. brevirostris</i>	Kitani, 1997	5	5-6	6-7	7-8	8-9	9-12
<i>P. occidentalis</i>	Kitani, 1996	5	5-6	6-7	8-9	9-10	10-12
<i>P. japonicus</i>	Hudinaga, 1942	5	6	7	7-9	9-10	10-12
<i>P. kerathurus</i>	Lumare and Gozzo, 1973	5	6	7	7	8	10
<i>P. kerathurus</i>	Haliki, 1981	5	6	7	8	9	-
<i>P. kerathurus</i>	Uçal and Hoşsucu, 1987	5	6	7-8	9-10	9-10	-
<i>P. kerathurus</i>	present study	5	5-6	6-7	7-9	8-10	10-12

Table 5. Differences of furcal spine formula in naupliar stages between *P. kerathurus* and other *Penaeus* sp.

Egg / N1-N6 (Hrs)	References	N1	N2	N3	N4	N5	N6
<i>P. monodon</i>	Kungvankij, 1976	1+1	1+1	3+3	4+4	5+5	7+7
<i>P. semisulcatus</i>	Kungvankij <i>et al.</i> , 1972	1+1	1+1	3+3	4/5+4/5	5/6+5/6	7+7
<i>P. semisulcatus</i>	Hassan, 1982	1+1	1/2+1/2	3/4+3/4	6+6	7+7	-
<i>P. brevirostris</i>	Kitani, 1997	1+1	1+1	3+3	4+4	6+6	7+7
<i>P. occidentalis</i>	Kitani, 1996	1+1	1+1	3+3	4+4	6+6	7+7
<i>P. japonicus</i>	Hudinaga, 1942	1+1	1+1	3+3	4+4	6+6	7+7
<i>P. kerathurus</i>	Lumare and Gozzo, 1973	1+1	1+1	2+2	4+4	6+6	7+7
<i>P. kerathurus</i>	Haliki, 1981	1+1	1+1	3+3	5/6+5/6	7+7	-
<i>P. kerathurus</i>	Uçal and Hoşsucu, 1987	1+1	1/2+1/2	4/5+4/5	6+6	7+7	-
<i>P. kerathurus</i>	present study	1+1	1+1	2/3+2/3	4+4	6+6	7+7

increased in length without molting, which was induced by taking foods, and the digestive tract was elongated as a result. The non-molt growth was also reported in other species (Hudinaga, 1942; Kungvankij *et al.*, 1972; Kungvankij, 1976; Hassan, 1982; Kitani and Alvarado 1982; Kitani, 1986(a); Kitani, 1986(b); Kitani, 1996; Kitani, 1997) and seems to be common among the *Penaeus* shrimps, assuming that the phenomenon is an efficient mechanism for absorbing nutrition.

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