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Chromatophore distribution patterns in the first and second zoeae of atyid shrimps (Decapoda: Caridea: Atyidae): a new technique for larval identification

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***Abstract.* — The abdominal chromatophores of the first and second zoeae were examined in five amphidromous atyid shrimps, which are widely distributing in southwest Japan: *Caridina serratirostris* De Man, *C. typus* H. Milne Edwards, *C. leucosticta* Stimpson, *C. japonica* De Man, and *Paratya compressa compressa* (De Haan) (small egg type). Chromatophore distribution pattern of larvae was found to be an effective character to identify respective species.**

Introduction

In Japan 43 species of freshwater caridean shrimps belonging to the families, Atyidae and Palaemonidae, have been recorded. They are divided into two groups, landlocked and amphidromous species, according to the life cycles (Shokita, 1979). The following five species, *Caridina serratirostris* De Man, 1892, *C. typus* H. Milne Edwards, 1837, *C. leucosticta* Stimpson, 1860, *C. japonica* De Man, 1892, and *Paratya compressa compressa* (De Haan, 1844) (small egg type), are all amphidromous species which are widely distributed in rivers flowing into sea affected by the Kuroshio Current in the southwestern part of Japan (Kamita, 1970; Shokita, 1979; Suzuki *et. al.* , 1993). In this region, *C. japonica* is the only species of which the life cycle has been well described. The planktonic zoeae of *C. japonica* hatch from the ovigerous female upstream and float down into the estuary and/or the sea, where they are estimated to undergo nine zoeal stages, and then migrate back to the upstream habitats after metamorphosing into juveniles (Hayashi & Hamano, 1984; Hamano & Hayashi, 1992). However, even for *C. japonica* there is no information on the ecology of larvae in the natural habitat. The primary reason is that it has been impossible to identify individual species of the planktonic larvae of the family, even if they are collected in the sea or estuary.

We closely studied the location and the shape of chromatophores of the first and second zoeae of the five species and found that the differences in chromatophore patterns are effective for the identification of the early zoeal stages.

Materials and Methods

Adults of *Caridina serratirostris*, *C. typus*, and *C. leucosticta* were captured at the Shitsu River, Nagasaki Prefecture, on 21 August 1994 and *C. japonica* and *Paratya compressa compressa* were collected from the Izari River, Tokushima Prefecture, on 4 June 1994. Each species was reared in a separate large tank filled with freshwater at 20-25 °C with aeration and a photoperiod of 14L (600 lx): 10D (2 lx). They were fed daily on an artificial diet for fish (Love Larva No. 6, Maruha Co., Ltd., Tokyo, Japan). When females laid eggs, they were moved into 300 or 1000 ml beakers filled with freshwater

under the conditions described above, and reared individually until the eggs hatched.

The newly hatched first stage zoeae were reared individually. Within 24 hours after hatching, zoeae were transferred into individual 50 ml beakers filled with 75 % (v/v) salt water (salinity ca. 25.4 ‰), excluding *C. leucosticta* (50 % salt water, ca. 16.9 ‰). Zoeae of four species were successfully fed with *Tetraselmis tetrathele* at 10^5 cell/ml in density, based on Y. Nakahara (unpublished data), except for *P. c. compressa* . Rearing of zoeae of *P. c. compressa* failed using *T. tetrathele*, but was successful on the artificial diet for fish culture mentioned above mixed with rice bran and seaweeds powder (RIVIC BW, Riken Vitamin Co., Ltd., Tokyo, Japan) for fish farming, which was improved from Hayashi & Hamano's (1984) method. There was no aeration. Otherwise the conditions were the same as those for adults. Every day zoeae were transferred by pipette to another set of beakers provided with fresh media and food.

After chilling the zoeae together with the rearing water in a refrigerator for 24 hrs, they were fixed and preserved with 3 % (v/v) formalin diluted in the rearing water: freshwater for the first zoeae and 50 or 75 % salt water for the second zoeae. All specimens were stored in the refrigerator at 1 °C and total darkness. Within two days after hatching, 10 specimens of the first and the second zoeae of the respective species were sampled at random for microscopical observations. However only 7 specimens of *P. c. compressa* survived to the second zoea and observations were carried out on these 7 specimens. Carapace length (CL), was measured from the posterior margin of the orbit (posterior margin of the sessile eyes in the first zoea) to the posterior end of the carapace using an ocular micrometer. Body length (BL) is expressed as CL + length from the posterior end of carapace to the end of the telson. CL and BL are shown as the "mean (min–max range)". All the chromatophores that occurred on the first to fifth abdominal somites and pleotelson were closely examined. Chromatophores of the head and thoracic regions covered by the carapace were excluded in this study, because they were often obscured by yolk. The chromatophores that occur only in some specimens are given as the "Number of individuals with the chromatophore/ Number of individuals observed" in parentheses, whilst the chromatophores present in all specimens are shown without parentheses.

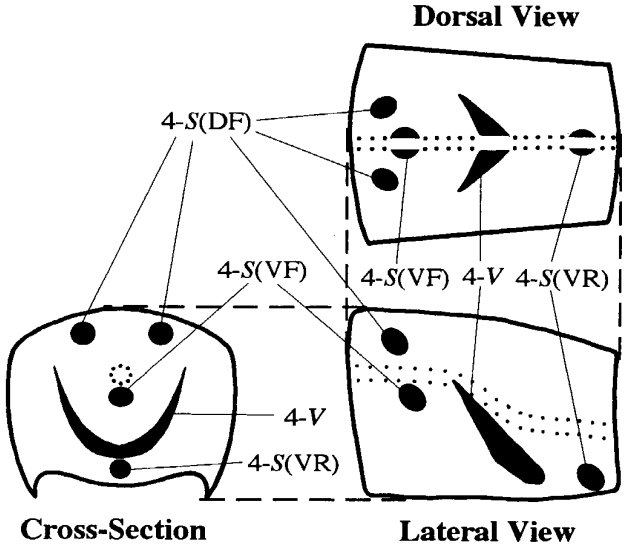

Fig. 1. The shape and location of chromatophores on the fourth abdominal somite in dorsal, lateral and cross-sectional views. Dotted curves indicate the alimentary canal. "4-*V* ": a set of chromatophores concentrated like a "V" shape on the fourth somite. "4-*S*(D/V, F/R)": spot chromatophores formed in a disk or a globe, situated on the dorsal side (D) or ventral side (V) of the alimentary canal and frontal (F) or rear (R) part of the fourth somite.

Results

Chromatophore patterns of the first to fifth abdominal somites (Figs. 1 and 2)

On the first to fifth abdominal somites, there are two patterns of chromatophores present, a *V*- shaped one and a spot:

V-shape — A set of chromatophores

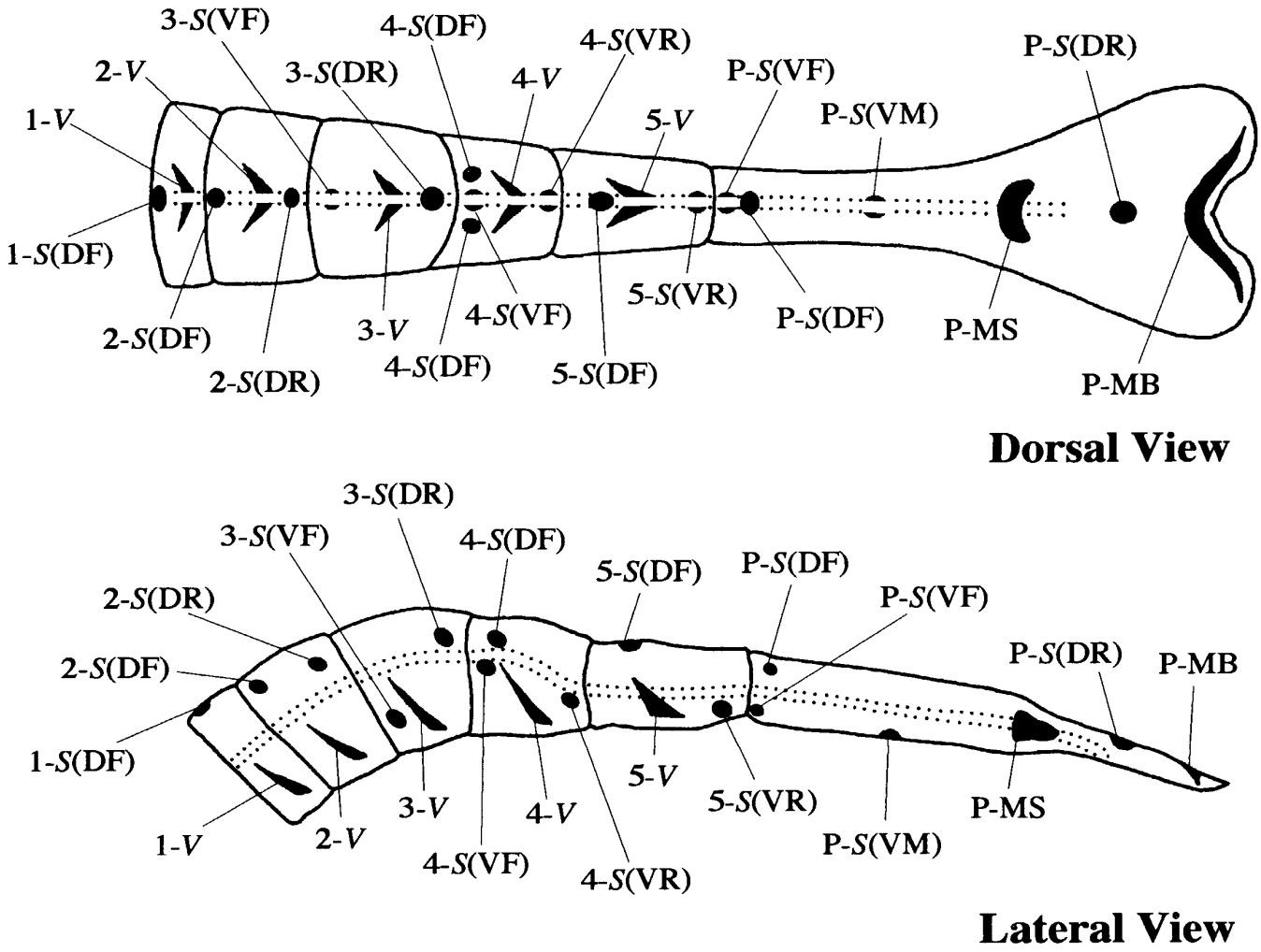

Fig. 2. The dorsal and lateral view of an "idealized" abdomen of the first and second zoeae of atyid shrimps showing the shape and location of each chromatophore. Dotted curves indicate the alimentary canal.

concentrated in a "V" shape. These are situated ventrally of the alimentary canal at the center of the respective somites. e. g., chromatophores of "4-*V*" indicates the *V*-shaped set of chromatophores on the fourth somite (Fig. 1).

Spot — A chromatophore formed in a disk or a globe shape. The location of chromatophores is expressed as the dorsal side (D) or the ventral side (V) of the alimentary canal and also the frontal (F) or the rear (R) part of the somite. A chromatophore of "5-*S*(DF)" means a spot chromatophore near the dorsal surface at the front of the fifth somite. All the spot chromatophores are formed by a single spot except for the 4-*S*(DF) which are a pair of spots situated on either side and above the alimentary canal near the dorsal surface at the front of fourth somite (Fig. 1).

Chromatophore patterns of the pleotelson (Fig. 2)

On the pleotelson, the following patterns are readily distinguishable:

Dorsal Front Spot — A dorsal (D) front (F) spot (*S*) of the pleotelson (P) shown as "P-*S*(DF)".

Ventral Front Spot —A ventral (V) front (F) spot (*S*) of the pleotelson (P) given as "P-*S* (VF)".

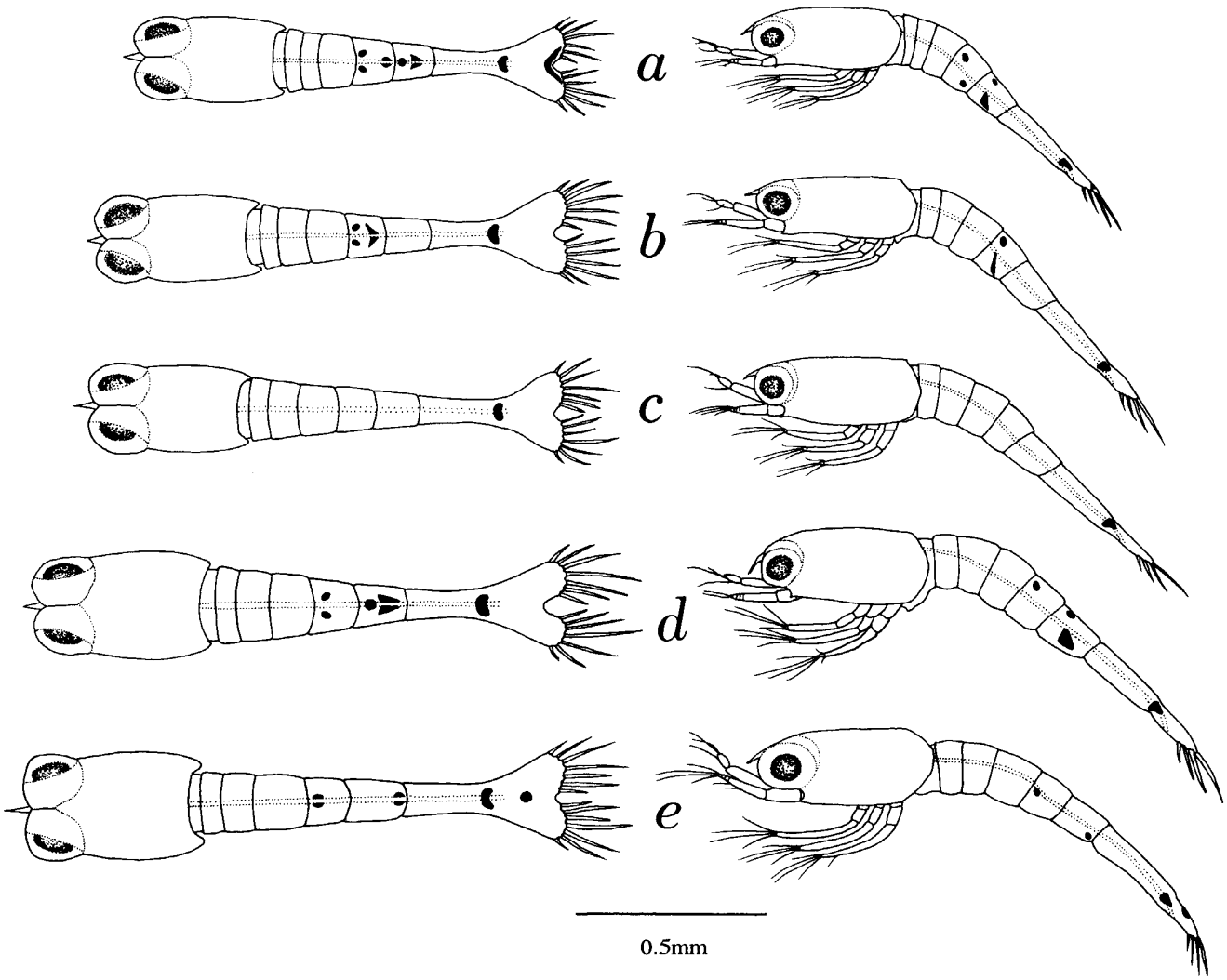

Fig. 3. Typical chromatophore patterns along the abdomen of the first zoeae of five atyid shrimps. Left: dorsal view, right: lateral view. *a*, *C. serratirostris*; *b*, *C. typus*; *c*, *C. leucosticta*; *d*, *C. japonica*; *e*, *P. c. compressa* (small egg type).

Ventral Median Spot — A ventral (V) median (M) spot (*S*) of the pleotelson (P) shown as "P-*S*(VM)".

Medial Set — A set of chromatophores on the caudal portion of the pleotelson. It is situated in front of the anus, and sometimes appears to separate into left and right parts dendritically expanding near the posterior margin of telson. It is indicated as "P-MS".

Dorsal Rear Spot — A dorsal (D) rear (R) spot (*S*) of pleotelson (P) (in the middle of the future telson which separates from the sixth somite at the third zoeal stage) given as "P-*S*(DR)".

Marginal Band — A band of chromatophores along the posterior margin of pleotelson, shown as "P-MB".

First zoea (Fig. 3 and Table 1)

Caridina serratirostris — CL 0.21 (0.13–0.25) mm, BL 0.97 (0.90–1.02) mm. 4-*S*(DF), 4-*S*(VR), 5-*S*(DF), 5-*V*, P-MS, and P-MB are present.

C. typus — CL 0.24 (0.21–0.28) mm, BL 1.08 (1.01–1.18) mm. 4-*S*(DF), 4-*V*, P-MS, 3-*V*(3/10), and 5-*V*(2/10).

C. leucosticta — CL 0.24 (0.22–0.25) mm, BL 1.07(1.01–1.10) mm. Only P-MS occurs in the pleotelson.

Table 1. Appearance and number of individuals showing chromatophores of the first zoeae with the carapace length (CL) and the body length (BL).

	C. serratirostris	*C. typus*	*C. leucosticta*	*C. japonica*	*P. c. compressa*
No. inds. observed	10	10	10	10	10
1-*V*	0	0	0	0	0
2-*V*	0	0	0	0	0
3-*V*	0	3	0	0	0
4-*V*	0	10*	0	3	0
5-*V*	10*	2	0	10*	0
1-*S*(DF)	0	0	0	1	0
2-*S*(DF)	0	0	0	0	1
2-*S*(DR)	0	0	0	0	0
3-*S*(DR)	0	0	0	1	2
3-*S*(VF)	0	0	0	0	0
4-*S*(DF)	10*	10*	0	10*	0
4-*S*(VF)	0	0	0	0	10*
4-*S*(VR)	10*	0	0	0	0
5-*S*(DF)	10*	0	0	10*	1
5-*S*(VR)	0	0	0	0	10*
P-*S*(VF)	0	0	0	0	0
P-*S*(DF)	0	0	0	0	0
P-*S*(VM)	0	0	0	2	0
P-MS	10*	10*	10*	10*	10*
P-*S*(DR)	0	0	0	0	10*
P-MB	10*	0	0	0	0
Mean CL (mm)	0.21	0.24	0.24	0.28	0.27
Range of CL (mm)	0.13–0.25	0.21–0.28	0.22–0.25	0.26–0.31	0.25–0.30
Mean BL (mm)	0.97	1.08	1.07	1.21	1.31
Range of BL (mm)	0.90–1.02	1.01–1.18	1.01–1.10	1.16–1.24	1.26–1.35

* Occurred in all specimens.

C. japonica — CL 0.28 (0.26–0.31) mm, BL 1.21(1.16–1.24) mm. 4-*S*(DF), 5-*S*(DF), 5-*V*, P-MS, 1-*S*(DF) (1/10), 3-*S*(DR) (1/10), 4-*V*(3/10), and P-*S*(VM) (2/10).

Paratya compressa compressa (small egg type) — CL 0.27 (0.25–0.30) mm, BL 1.31 (1.26–1.35) mm. 4-*S*(VF), 5-*S*(VR), P-MS, P-*S*(DR), 2-*S*(DF) (1/10), 3-*S*(DR) (2/10), and 5-*S*(DF) (1/10).

SECOND ZOEA (Fig. 4 and Table 2)

C. serratirostris — CL 0.31 (0.26–0.36) mm, BL 1.14(0.76–1.32) mm. 2-*V*, 3-*V*, 4-*V*, 5-*V*, P-*S*(DF), P-MS, P-MB, and 1-*V*(9/10).

C. typus — CL 0.35 (0.32–0.36) mm, BL 1.34 (1.30–1.37) mm. 2-*V*, 3-*V*, 4-*S*(DF), 4-*V*, 5-*V*, and P-MS.

C. leucosticta — CL 0.37 (0.34-0.45) mm, BL 1.36 (1.30–1.43) mm. 4-*V*, P-MS, and 5-*V*(1/10).

C. japonica — CL 0.35 (0.32–0.37) mm, BL 1.44(1.42–1.46) mm. 4-*S*(DF), 4-*V*, 5-*V*, P-MS, 1-*S*(DF) (5/10), 1-*V*(2/10), 2-*S*(DR) (1/10), 2-*V*(2/10), 3-*S*(DR) (3/10), 3-*V*(3/10), 5-*S*(DF) (5/10), and P-*S*(VM) (4/10). Further, there are many smaller chromatophores found in various parts of all the abdominal somites.

P. c. compressa (small egg type) — CL

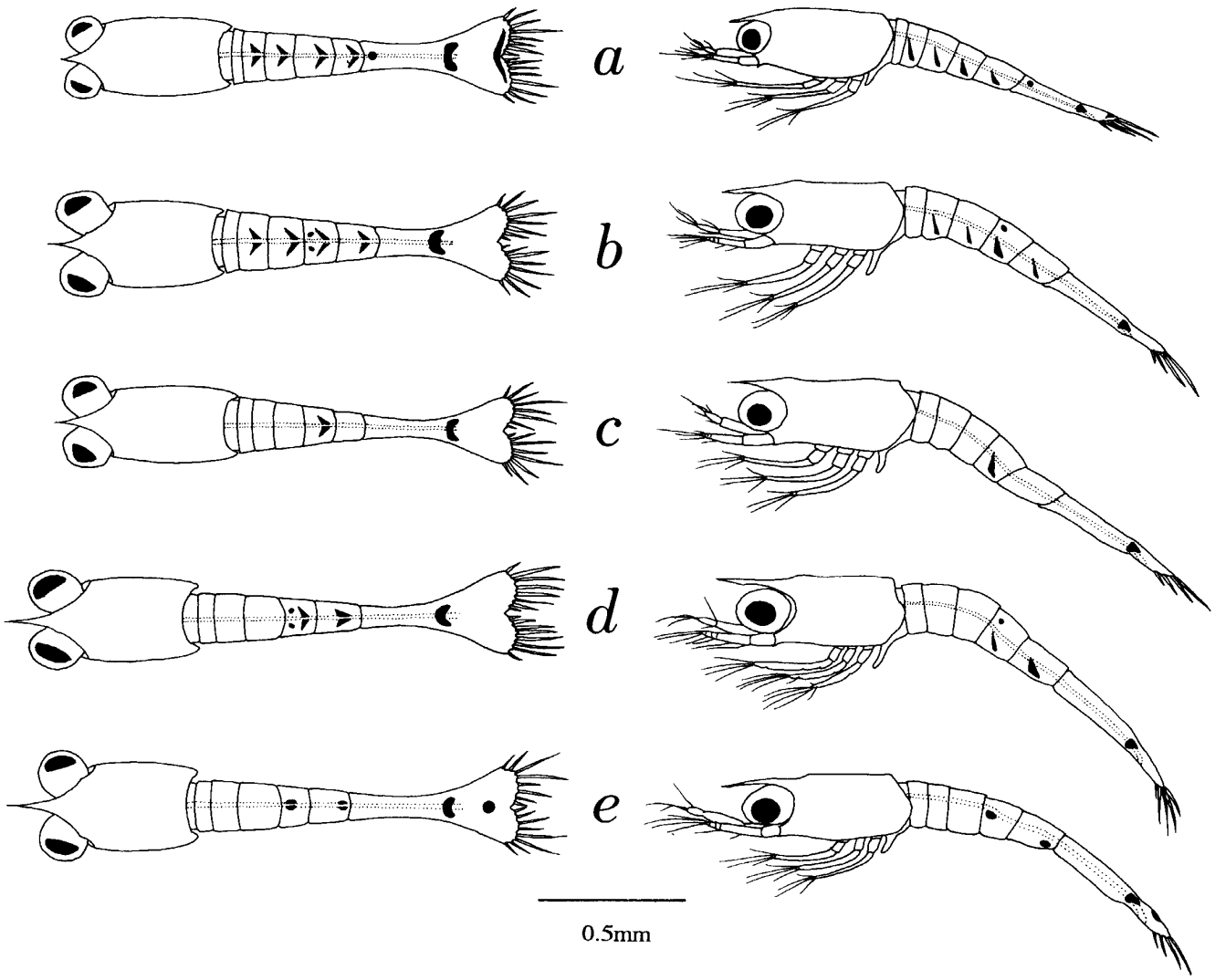

Fig. 4. Typical chromatophore patterns along the abdomen of the second zoeae of five atyid shrimps. Left: dorsal view, right: lateral view. *a*, *C. serratirostris*; *b*, *C. typus*; *c*, *C. leucosticta*; *d*, *C. japonica*; *e*, *P. c. compressa* (small egg type).

0.30 (0.27–0.34) mm, BL 1.38 (1.33–1.44) mm. 4-*S*(VF), 5-*S*(VR), P-MS, P-*S*(DR), 1-*S*(DF) (3/7), 3-*S*(VF) (1/7), and P-*S*(VF) (3/7).

From these results, we propose a key to identify the first and second zoeae of the five atyid shrimps on the basis of the characteristics observed in all specimens:

Key to the first and second zoeae of the five atyid shrimps mentioned above

1. Eyes sessile in carapace; posterior margin of pleotelson bearing 7+7 plumose setae ... 2 (First Zoea)
— Eyes stalked and movable; posterior margin of pleotelson bearing 8+8 plumose setae ... 6 (Second Zoea)
2. P-*S*(DR) present ... First zoea of *P. c. compressa* (small egg type)
— P-*S*(DR) absent ... 3
3. P-MB present ... First zoea of *C. serratirostris*
— P-MB absent ... 4
4. Only P-MS present ... First zoea of *C. leucosticta*
— 4-*S*(DF), together with P-MS, present ... 5
5. 4-*V* present, 5-*V* usually absent ... First zoea of *C. typus*
— 4-*V* absent, 5-*V* present ...

Table 2. Appearance and number of individuals showing chromatophores of the second zoeae with the carapace length (CL) and the body length (BL).

	C. serratirostris	*C. typus*	*C. leucosticta*	*C. japonica*	*P. c. compressa*
No. inds. observed	10	10	10	10	7
1-*V*	9	0	0	2	0
2-*V*	10*	10*	0	2	0
3-*V*	10*	10*	0	3	0
4-*V*	10*	10*	10*	10*	0
5-*V*	10*	10*	1	10*	0
1-*S*(DF)	0	0	0	5	3
2-*S*(DF)	0	0	0	0	0
2-*S*(DR)	0	0	0	1	0
3-*S*(DR)	0	0	0	3	0
3-*S*(VF)	0	0	0	0	1
4-*S*(DF)	0	10*	0	10*	0
4-*S*(VF)	0	0	0	0	7*
4-*S*(VR)	0	0	0	0	0
5-*S*(DF)	0	0	0	5	0
5-*S*(VR)	0	0	0	0	7
P-*S*(VF)	0	0	0	0	3
P-*S*(DF)	10*	0	0	0	0
P-*S*(VM)	0	0	0	4	0
P-MS	10*	10*	10*	10*	7*
P-*S*(DR)	0	0	0	0	7*
P-MB	10*	0	0	0	0
Mean CL (mm)	0.31	0.35	0.37	0.35	0.30
Range of CL (mm)	0.26–0.36	0.32–0.36	0.34–0.45	0.32–0.37	0.27–0.34
Mean BL (mm)	1.14	1.34	1.36	1.44	1.38
Range of BL (mm)	0.76–1.32	1.30–1.37	1.30–1.43	1.42–1.46	1.33–1.44

* Occurred in all specimens.

..... First zoea of *C. japonica*

6. P-*S*(DR) present .....
Second zoea of *P. c. compressa* (small egg type)

— P-*S*(DR) absent 7

7. P-MB present .....
..... Second zoea of *C. serratirostris*

— P-MB absent 8

8. 2-*V* and 3-*V* present .....
..... Second zoea of *C. typus*

— 2-*V* and 3-*V* usually absent 9

9. 5-*V* present .....
..... Second zoea of *C. japonica*

— 5-*V* usually absent .....
..... Second zoea of *C. leucosticta*

Discussion

The object of this study is to gain better knowledge for the identification of wild larvae of atyid shrimps. Discrimination between the first and second zoeal stages is clear from differences of their eyes and the pleotelsonal setae, and this is used in the present key. The uropods are also absent or rudimentary in these two zoeal stages, though they are present in the third and more advanced stages (e.g., Hayashi & Hamano, 1984). Gore (1985, Table 2) summarizes the selected morphological characters, including the eyes and pleotelsonal setae (= his "pro-

cess" of tailfan), which are usually shown in the regular (multi-stage) development of decapod crustaceans. However, it may be difficult to identify correctly the respective zoeae of atyid shrimps by their morphological differences alone. We successfully identified the first zoeae of *Caridina typus* from plankton net samples from shallow sea water near the mouth of the Sugitani River, Nagasaki Prefecture, and those of *C. japonica*, *C. typus*, and *Paratya compressa compressa* collected from the Izari River, comparing them with the chromatophore pattern described here. The chromatophore pattern has been shown to be effective in identifying the postlarvae of penaeid prawns by Motoh & Buri (1981). Thus, chromatophores seem to be very useful to discriminate shrimp larvae.

In the present study only the chromatophores appearing in all the specimens examined are used as the characteristics for the identification of the zoeae, as shown in the key and Figs. 3 and 4. However, we found varying degrees of individual variations in the occurrence of chromatophores among a small number of zoeae hatched from single clutches of these respective species (Tables 1 and 2).

The rate of loss of pigments in a preserved specimen is markedly affected by the method of preservation (Omori & Freminger, 1976; Omori & Ikeda, 1976). We observed the zoeae, fixed and preserved in 3 % formalin at 1°C and kept in darkness, within two days. In all specimens, the chromatophores recorded in the key remained distinct during this period.

The atyid shrimps described here are unable to migrate to the headwater habitat where the river is dammed and has no fishway (Miya & Hamano, 1988). Therefore, Hamano *et al.* (1995) studied the basic requirements of fishways for these freshwater amphidromous shrimps and concluded that an experimental fishway was successful for the upstream migration of shrimps. However, it appears to be difficult for zoeae which are hatched upstream to flow safely down beyond the fishway to the estuary and sea where they develop (Hamano *et al.*, 1995). To maintain the population density of these atyids in the habitat above the dam, Hamano *et al.* suggested that the parental population in tributary streams, down stream of the dam and from a neighboring river, should be protected to maintain the gene pool and juveniles from this source could migrate to the upstream using the fishway. From data presented here we hope to examine the dispersal pattern of larvae into the estuary and sea, the microhabitat of larvae, the daily periodicity of vertical migration, and so on, which will allow a clearer understanding of the ecology and interspecific differences between these species and ultimately how to preserve this fragile ecosystem.

Acknowledgments

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