

Ultrastructural Changes in the Cuticle of *Palaemon serrifer* (Palaemonidae, Crustacea) during the Intermolt Cycle

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Ultrastructural changes in the cuticle of *Palaemon serrifer* associated with the intermolt cycle were examined and quantified as changes in the cuticular thickness. The cuticular thickness in each zoea stage increased with time elapsed after molting. The cuticle in the premolt stage was about 1.5 and 3 times thicker than that in the postmolt and intermolt stage, respectively. The cuticle in the premolt stage, including the molting space, was more than 5 times as thick as in the postmolt stage. In addition, newly hatched larvae were individually reared in the laboratory and body length for each specimen was measured frequently until the end of zoea VI. An average increase in body length between one zoea stage and the next is about 10% of the length of the previous stage. Within individual zoea stages, the premolt stage had a body length some 0.3% longer than that of the postmolt stage, indicating a growth rate of about 0.03 mm/d.

Key words: *Palaemon serrifer*, cuticular structure and thickness, intermolt cycle, intermolt growth

Introduction

The intermolt cycle in crustaceans is a period of morphological, physiological and biochemical changes allowing development of the body structure after molting and in preparation for the next ecdysis (Drach, 1939, 1944; Passano, 1960; Scheer, 1960; Kurup, 1964; Lasker, 1964, 1966; Jennings and Halverson, 1971; Aiken, 1973; Reaka, 1975; Moss, 1994; Roff et al., 1994). Two notable morphological events occur during the intermolt cycle. First, the development of new setae is easily observed externally because of changes in their structure, and has been used to define the different stages of the intermolt cycle in a variety of crustaceans (Drach, 1939, 1944; Kurup, 1964; Stevenson et al., 1968; Aiken, 1973; Davis et al., 1973). Second, the formation of the old- and new exoskeleton occurs internally but requires histological sections to describe it.

The crustacean cuticle consists of the epicuticle, exocuticle, endocuticle and epidermis. The intermolt cycle is mainly divided into the postmolt,

intermolt and premolt stage. The endocuticle is absent in the postmolt stage and its formation is completed during the intermolt cycle (Drach, 1939, 1944; Kurup, 1964; Stevenson et al., 1968; Davis et al., 1973; Roff et al., 1994). This is the structural completion of the old exoskeleton. In the premolt stage, considerable amounts of the endocuticle of the old exoskeleton are reabsorbed and the new exoskeleton is developed underneath to replace the old-exoskeleton, cast at ecdysis. These sequential changes in the cuticular structure have been studied as a part of setogenesis, mainly using body appendages (Drach, 1939, 1944; Kurup, 1964; Stevenson et al., 1968; Aiken, 1973; Davis et al., 1973).

Davis et al. (1973) measured the thickness of the cuticle in the intermolt stage of the caudal rami of *Balanus amphitrite* and found that the endocuticle, as a percentage of the total cuticular thickness, varied during its phases of formation, steadiness and reabsorption. Although they did not provide quantitative data on the changes in the cuticular thickness throughout the intermolt cycle, they suggested that the thickness could vary depending on the different stages of the intermolt cycle.

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However, changes in the cuticular thickness associated with the intermolt cycle have not yet been adequately quantified.

Mauchline (1973) classified the larvae of adult female mysids, carrying them in the marsupium, into egg-like embryos, eyeless larvae and eyed larvae, and found that the body length of females with eyed larvae was about 7% greater than that with egg-like embryos. He suggested this increase in body length as evidence of the intermolt growth since females do not molt while carrying the young. In Mauchline's study, however, daily growth rate could not be quantified during the intermolt period because specimens were obtained from natural populations with variation in body size between individuals of the species. Lasker (1966) reared juveniles of *Euphausia pacifica* for over 50 d in the laboratory, and reported that uropod growth rates were 0.034~0.048 mm/day. In his study, measurements were made at about 5 or 10 day intervals without consideration of the frequency of molting during his observational period, causing difficulty in determining the growth rate.

In the present study, zoea I to VI stages of *Palaemon serrifer* were examined to show the relationship between changes in the structure and thickness of the cuticle associated with the intermolt cycle. Ultrastructural changes in the cuticle were examined using Transmission Electron Microscopy (TEM) and quantified as changes in the thickness of the cuticle. In addition, newly hatched larvae were individually reared in the laboratory and body length for each specimen was measured frequently until the end of zoea VI, in order to examine the intermolt growth.

Materials and methods

Sampling and rearing

Ovigerous females of *Palaemon serrifer* were collected in July and August, 1996 by handnetting from intertidal rock pools at Haewundae, Pusan. The females were kept individually in the laboratory until their eggs were hatched. Newly hatched larvae were individually reared in 2 ℓ bottles at $25 \pm 1^\circ\text{C}$ and fed *Artemia* nauplii daily after changing filtered sea water.

Different stages of the intermolt cycle

In zoea I to VI stages of *P. serrifer*, different stages of the intermolt cycle were identified by the morphology of setogenesis in which new setae are

formed in the somatic tissue of the appendages to replace existing setae lost with the old exoskeleton at ecdysis. The intermolt cycle was divided into the postmolt, intermolt and premolt stage as described by Drach (1939, 1944), Scheer (1960), Kurup (1964), Stevenson et al. (1968) and Reaka (1975).

Transmission Electron Microscope

Specimens at different stages of the intermolt cycle were fixed separately, preserved in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) and then postfixed in buffered 1% osmium tetroxide for approximately 4 h before being dehydrated in a series of ethanol solutions and then embedded in Epon 812. Sections were cut on a LKB ultramicrotome, stained with ethanoic uranyl acetate and lead citrate and then examined using a Transmission Electron Microscope (Jeol 1200 EX-II) at 80 kV. Since the structure and thickness of the cuticle varied with different parts of the body, all sections were made around the middle area of the paired dorsal longitudinal muscles of the second abdominal segment.

Measurements of body length

Newly hatched larvae, zoea I, were mass cultured in the laboratory. Of these, 20 specimens were individually reared and body length for each specimen was measured frequently until the end of zoea VI. The live specimens were handled with a pipette, placed on a glass depression slide with a drop of sea water, and then pushed gently out of the depression onto the flat part of the slide using a dissecting needle. The specimen was spread flat dorso-ventrally on the slide and body length was measured using a calibrated ocular micrometer in a compound microscope. In dorsal view, body length was defined as from the base of the rostrum to the posterior end of the telson excluding the distal setae. The specimens were examined at 2, 4 or 6 h intervals depending on the intermolt cycle. Measurements of body length for each specimen were made twice during the intermolt cycle within 4 h after molting and within 8 hrs before the next molting which corresponds with the postmolt and premolt stage, respectively. Hatching and molting always occur at midnight.

Results

Ultrastructure of the cuticle

The intermolt cycle in *Palaemon serrifer* was

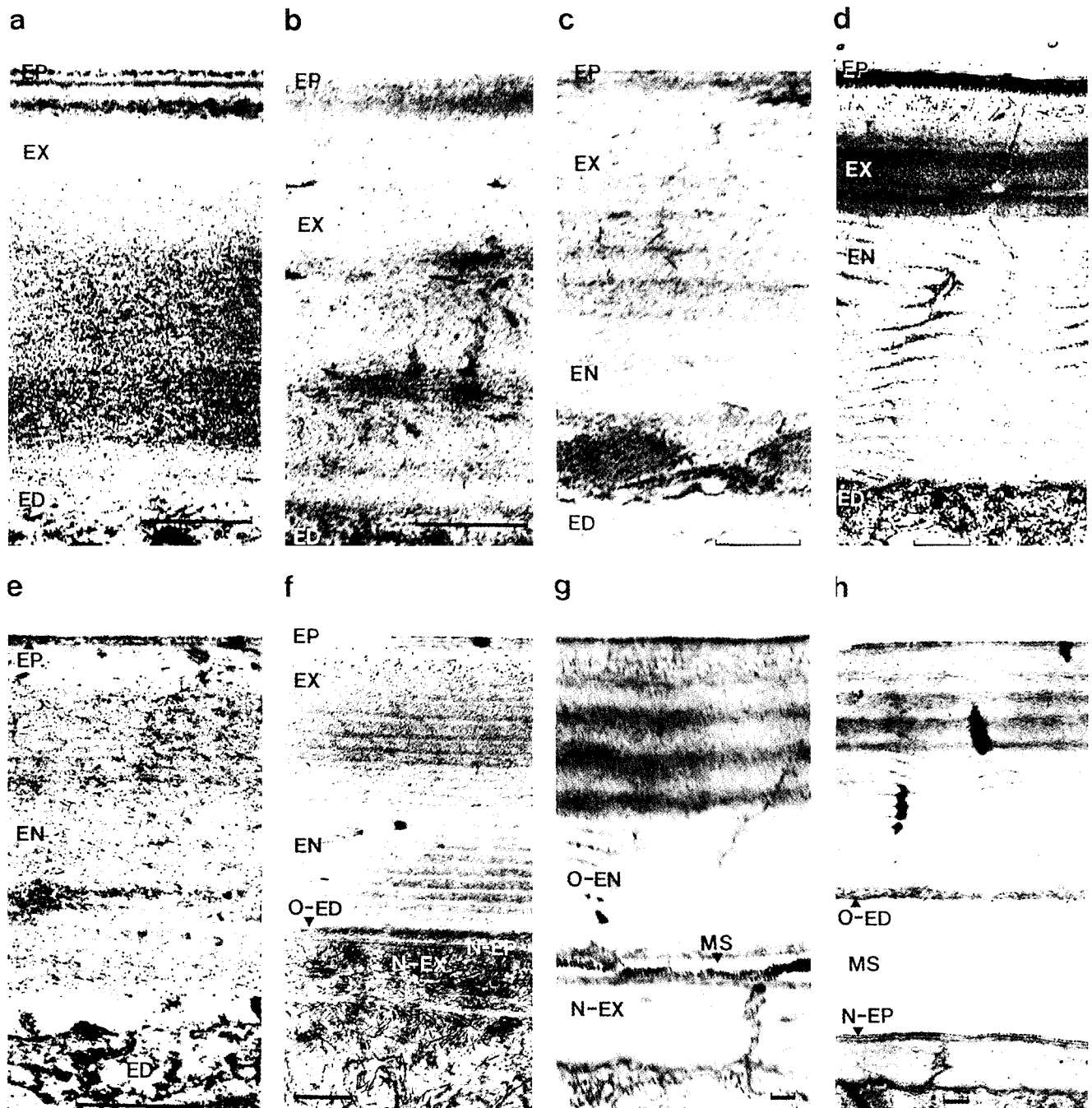


Fig. 1. Transverse TEM sections of the cuticle of *Palaemon serrifer*. EP, epicuticle; EX, exocuticle; EN, endocuticle; ED, epidermis; MS, molting space; O, old exoskeleton; N, new exoskeleton. (a), (b) zoea I in the postmolt stage. (c) zoea I in the intermolt stage. (d) zoea V in the intermolt stage. (e) zoea III in the premolt stage. (f) zoea V in the premolt stage. (g), (h) zoea VI in the premolt stage, with the molting space. Scale bars=0.2 μ m.

divided into the postmolt, intermolt and premolt stages based on the morphology of setogenesis. The cuticular structure varies at different stages of the intermolt cycle (Fig. 1). Structural changes in the cuticle associated with the intermolt cycle were similar throughout the zoeal stages.

In the postmolt stage the endocuticle was absent and stratification of the exocuticle all the way across the cuticle was not clearly seen (Figs. 1a, b). Three lamellar structures are seen in Fig. 1b where the specimen was preserved at 30 minutes after hatching.

The endocuticle was not fully formed in the early intermolt stage (Fig. 1c) and became complete as time went on (Fig. 1d). The exocuticle was more electron opaque than the endocuticle.

A dark layer, which is formed in the intermolt stage, appeared in the middle of the endocuticle of the old exoskeleton and thus the endocuticle could be divided into two (Fig. 1e). The lower part of the endocuticle was reabsorbed and the epicuticle of the new exoskeleton began to be formed (Fig. 1f). This occurred in the early premolt stage. Underneath the epicuticle of the new exoskeleton, the exocuticle developed, forming the new exoskeleton. Finally, the molting space appeared between the old- and new exoskeleton. This occurred in the late premolt stage (Fig. 1g, h).

Changes in the cuticular thickness

Thickness of the cuticle in each zoea stage varied with different stages of the intermolt cycle (Tables 1, 2). The cuticle in the intermolt and premolt stage was always greater in thickness than that in the postmolt stage. Increases in the thickness of the cuticle, from the postmolt to the intermolt stage, is due to the structural completion of the endocuticle which is absent in the postmolt stage (Figs. 1a, b, Table 2). Similarly, the cuticle of zoea V in the premolt stage was about twice as thick as in the intermolt stage because of the development of the new exoskeleton (Fig. 1f-h, Table 2). The thickness of the new exoskeleton being development was $1.260 \pm 0.344 \mu\text{m}$ in zoea V and $0.852 \pm 0.271 \mu\text{m}$ in zoea VI. In the late premolt stage, the size of the molting space between the old- and new exoskeleton can vary and its thickness being as great as 1.5 times the thickness of the old- and new exoskeleton together. However, the thickness of the molting space varied to a great extent, even within a TEM grid of 3 mm diameter of the section, and measurements of its thickness

were not included in the cuticular thickness of zoea V and VI in Table 1.

Body length

Body lengths for zoea I to VI stages of *P. serrifer* are in Table 3. Increase in body length, from one zoea stage to the next, was about 10 % of the length of the previous stage. Body length, stage duration and the student's *t*-test value by the SAS computer program are given in Table 4. Differences between body lengths of the postmolt and premolt stages of all zoea stages, except for zoea IV and V, were statistically significant. In zoea IV and V, however, there was a difference in body length between the postmolt and premolt stages. These data also indicate that an average daily increase in body length during the intermolt cycle was 0.033 mm for zoea I to VI stages. Body length in the premolt stage was about 3 % greater than that in the postmolt stage.

Table 1. Comparison of the thickness of the cuticle of *Palaemon serrifer* at different stages of the intermolt cycle in zoea I to VI. "n" indicates sample size

Stage	Intermolt cycle	Mean \pm SD (μm)
Zoea I	Postmolt	0.786 ± 0.105 , n=3
	Intermolt	1.164 ± 0.422 , n=2
	pre-molt*	1.067 ± 0.111 , n=3*
Zoea II	Intermolt	1.061 ± 0.184 , n=3
Zoea III	intermolt**	0.610 ± 0 , n=1**, 1.105 ± 0.107 , n=3
Zoea IV	Postmolt	0.519 ± 0.068 , n=3
Zoea V	Intermolt	1.471 ± 0.180 , n=3
	Premolt	2.979 ± 0.431 , n=3
		1.721 ± 0.573 for old-exoskeleton 1.260 ± 0.344 for new-exoskeleton
Zoea VI	Postmolt	0.920 ± 0.044 , n=2
	late premolt	3.234 ± 0.513 , n=3
		2.352 ± 0.342 for old-exoskeleton 0.852 ± 0.471 for new-exoskeleton

* The new exoskeleton is not fully formed.

** The endocuticle is not fully formed.

Table 2. Comparison of the thickness (μm), mean SD, of the different layers of the cuticle of *Palaemon serrifer* at different stages of the intermolt cycle. "n" indicates sample size

Stage	n	Epicuticle	Exocuticle	Endocuticle	New exoskeleton
Zoea I					
postmolt	3	0.029 ± 0.006	0.745 ± 0.117	Absent	absent
intermolt	3	0.022 ± 0	0.710 ± 0.273	0.462 ± 0.195	absent
pre-molt	2	0.022 ± 0	0.605 ± 0.015	0.347 ± 0.038	0.066 ± 0.015
Zoea V					
intermolt	3	0.055 ± 0.001	0.509 ± 0.058	0.895 ± 0.467	absent
pre-molt	3	0.050 ± 0.006	0.971 ± 0.261	0.715 ± 0.382	1.260 ± 0.344
Zoea VI					
postmolt	2	0.041 ± 0.012	0.943 ± 0.166	Absent	absent
pre-molt	3	0.041 ± 0.016	1.246 ± 0.330	1.092 ± 0.394	0.854 ± 0.2719

Table 4. Body length of the zoea I to VI *Palaemon serrifer*. Length of the postmolt stage was measured within 4 hours after molting and that of the premolt stage within 8 hours before the next molting. Measurements of length were made using the same 15 specimens reared in the laboratory. The value for the student t-test for body length between the postmolt and premolt stage and the probability (P) in parentheses

Stage	Total length Postmolt stage (mm)	Total lengthpremol stage (mm)	Mean stage duration (day)	Premoltpostmolt (mm)	t-test valuepostmolt vs premolt
Zoea I	2.234 ± 0.018	—	1	—	—
Zoea II	2.410 ± 0.032	2.476 ± 0.035	2	0.065 ± 0.017	5.2915 (P=0.0000)
Zoea III	2.784 ± 2.885	2.885 ± 0.065	3	0.100 ± 0.035	4.8492 (P=0.0000)
Zoea IV	3.197 ± 0.135	3.287 ± 3.287	3	0.089 ± 0.023	1.7552 (P=0.0902)
Zoea V	3.606 ± 0.113	3.704 ± 0.125	3	0.098 ± 0.040	2.4642 (P=0.0189)
Zoea VI	4.003 ± 0.130	4.175 ± 0.131	4	0.172 ± 0.078	3.6775 (P=0.0008)

Discussion

The structure and thickness of the cuticle varied not only with different stages of the intermolt cycle but also with different anatomical locations on the specimen (Fig. 1, Tables 1, 2). Furthermore, the cuticular structure associated with the intermolt cycle did not always progress uniformly at the same rate of change, and differences in the thickness of the cuticle were found even within a TEM grid of 3 mm in diameter of the section, particularly in the premolt stage. In the present study, all sections were made around the middle area of the paired longitudinal muscles of the first abdominal segment, but it appears difficult to compare exactly the same part of the body throughout all samples.

The most distinctive event in the cuticle during the intermolt cycle is the structural completion of the old- and new exoskeleton which also affects the thickness of the cuticle (Fig. 1, Table 2). The thickness of the cuticle in each zoea stage increased gradually depending on the time elapsed after molting. The cuticular thickness of zoea I in the intermolt stage is, however, smaller than that in the premolt stage (Table 1). This might be explained in two ways: 1) variations from different points of the TEM section, different anatomical locations and different individuals; 2) the absorption of the

endocuticle of the old exoskeleton taking place before the formation of the new exoskeleton. Considering that at least 2 specimens were examined for each stage of the intermolt cycle in the present study and up to 75% of the old exoskeleton can be reabsorbed (Skinner and Kumari, 1992; see also Figs. 1e-h), the second explanation is more probable.

Body length increased from one zoea stage to the next, but the thickness of the cuticle did not follow the same pattern of increase in body length (Tables 1-3). The cuticular thickness of zoea VI in the postmolt stage was smaller than that of zoea I in the intermolt stage. In the postmolt stage the cuticle of zoea I was thicker than that of zoea IV. In the present study, however, the thickness of the cuticle of a single specimen with 10 molts (not presented in Table 1) is 1.584 µm in the postmolt stage and 3.681 µm in the intermolt stage, which are apparently greater than that in the comparable intermolt phase of the earlier zoea stages. Increase in the cuticular thickness does not occur stage by stage or molting by molting, but rather the cuticle increases in thickness progressively throughout its life span, possibly related to the stage duration. The mean stage duration of zoea II to VI stages was 3 days and that of post-larva stages more than 5 d.

Body length increased with the frequency of molting as well as increasing during the intermolt cycle (Tables 3, 4). The average increase in body length from one zoea stage to the next was about 10% of length of the previous stage. Body length in the premolt stage was about 3% greater than that in the postmolt stage. There are various possible explanations for this intermolt growth such as active water uptake after molting, assimilation of organic materials and increase in the amount of the somatic tissue (Scheer, 1960; Lasker, 1966; Jennings and

Table 3. Body length of *Palaemon serrifer*. "n" indicates sample size

Stage	n	Carapace length (mm)	Total length (mm)
Zoea I	10	0.6977 ± 0.0280	2.2408 ± 0.1613
Zoea II	29	0.7781 ± 0.0171	2.4591 ± 0.0401
Zoea III	38	0.8458 ± 0.0247	2.7868 ± 0.0831
Zoea IV	14	0.9285 ± 0.0422	3.0850 ± 0.0694
Zoea V	33	0.9894 ± 0.0259	3.3343 ± 0.0857
Zoea VI	18	1.1281 ± 0.0444	3.7947 ± 0.0888

Halverson, 1971; Moss, 1994; Roff et al., 1994). Some parts of these physiological phenomena should be attributed to structural changes in the cuticle during the intermolt cycle. The thickness of the cuticle in the postmolt stage increased up to more than 3 times in the premolt stage, possibly 5 or 7 times if the thickness of the molting space is included (Tables 1, 2). Therefore, increase in body length during the intermolt cycle could be related to changes in the cuticular thickness in combination with its structural changes.

Lasker (1966) reported that in laboratory experiments on juveniles of *Euphausia pacifica* a growth rate of body length was 0.048 mm/d. In his study, growth in length as determined from measurements of molted uropods is an estimated length where the frequencies of molting taking place during the experimental period were not considered. Mauchline (1973) examined adult females of 13 species of mysids carrying young in the marsupium and classified their young into egg-like embryos, eyeless larvae and eyed larvae. The average increase in body length of the females was about 3% between the egg-like embryo stage and the eyeless larval stage (2~15 d) and 7% during the entire period of larval development (18~21 d). Since mysid females do not molt while carrying young, this increase in body length can be represented by the intermolt growth. In Mauchline's study, however, specimens were taken from natural populations containing variation in body length between individuals of the species. Comparable changes in the thickness of the cuticle of mysids will occur and contribute to apparent increase in body length.

Lasker (1964) observed that in *Euphausia pacifica* dry exuviae weights were about 10 % of dry weight of the whole animal. The abdomen of *Pagurus bernhardus* increased in volume by about 15 % in dilute sea water, but it had a soft abdomen unlike mysids, euphausiids, and shrimps (Davenport 1972). These previous studies show the theoretical base of the intermolt growth and this study gives evidence of intermolt growth. In the present study, an average daily growth of body length of *Palaemon serrifer* was about 0.03 mm throughout zoea stages and body length in the premolt stage was 3 % greater than the body length of the postmolt stage (Table 4). Increase in body length presented here was obtained from measurements of the same individual.

Only ultrastructural changes in the cuticle and increase in body length associated with the intermolt cycle were examined here. There remain further studies on the biochemistry and physiology of the crustacean intermolt cycle, especially in order to evaluate the intermolt growth such as the body weight in relation to the intermolt cycle which would be of great use in estimating the secondary production because the majority of metazoan secondary production is represented by crustaceans in aquatic environments.

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