Calcification and Age Determination in Crustacea—I. Possibility of Age Determination in Crabs on the Basis of Number of Lamellae in Cuticles

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From the ecological point of view, age determination of crustacean organisms is an important subject for analyzing their population dynamics. For example, studies on the age determination of the king crab, *Paralithodes camtschatica*, in the northern waters of the Pacific Ocean have been done by many authors on account of the indispensable research of this important fishery resource.¹⁾⁻¹⁰⁾

However, the age of the king crab determined by all these previous searchers is that which has been estimated on the basis of an analysis of the progression of modes in successive length-frequency distributions. It is well known that fisheries scientists have estimated the age of fish by counting the number of concentric rings or zones of the hard tissues such as scale, centrum of vertebra, otolith, and so on. In crabs, however, for reasons of the fact that all of the hard tissues are molted completely in the process of their growth, it has generally been believed that it is impossible to find any of the hard tissues bearing such age characteristics in their body as found in fish.

During the course of histological observations on the cuticles of the carapace of the shore crab, *Gaetice depressus*, the authors have noticed that different numbers of the lamellae are found to be formed in this structure corresponding to different dimensions of its carapace length. It is probable that the number of lamellae increases with the crab's age even in the newly formed cuticles after their molting to sustain a structural stability of the enlarging carapace. In fact, Neville¹¹⁾ has demonstrated an interesting study that in locusts the lamellae of endocuticle are formed to be consequent upon the daily growth representing their daily age, though he has not given any description about the relationship between an increase of the number of lamellae and their molting.

Although the importance of studies of lamellae in cuticles of crustacean animals has been emphasized by several workers,¹²⁾⁻¹⁷⁾ most of the investigations made in this field have been concentrated upon the chemical nature of cuticle components, omitting consideration of the number of lamellae in relation to the age determination. Accordingly, the present observation was undertaken, as an initial study in this field, to learn the presence or absence of the relationship between the number

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of lamellae and the carapace length in *G. depressus*.

Materials and Methods

In the present study *Gaetice depressus*, one of the most common shore crabs in Hakodate Bay, Hokkaido, Japan, was mainly used. The specimens were 50 males of 6.5–24.5 mm and 50 females of 6.9–22.0 mm in carapace length, all of which were at the intermolt stage C_4 .¹⁸⁾ All these specimens were collected from April to June, 1968. Immediately after their collection, the specimens were fixed in 10% neutral formalin.

Fig. 1. Diagram of the carapace showing the part measured. The square represents the heart zone removed. HZ: Heart zone of the carapace; CL: Carapace length.

As illustrated in Fig. 1, the carapace length is defined as a distance measured from the knot between extreme concavities of the eye orbits to the posterior median edge of the carapace.

Irrespective of body-size of the animals, a shell-piece of about 1.3×2.3 mm was removed from the so-called heart zone of the carapace of each specimen (Fig. 1). Each piece was dehydrated through the transfer of a series of 70–90% ethanol, embedded within a mass of methacrylate, and then ground up to about 50μ in thickness.

For counting the number of lamellae, the endocuticle was selected as a proper object of the present observation owing to the following reason,

as shown in Figs. 2 and 6–10. A laminated pattern of the lamellae was found to be most clearcut in this region as compared with those of both the exocuticle and the membranous layer. Nevertheless, even in the endocuticle of the heart zone obtained from the same individual, there was found a variation of the number of lamellae. The variation seems to occur mainly from a partial difference of the thickness of endocuticle owing to its uneven basal part as is seen in Figs. 6 and 8–10. An example of the data counted in the same section of the endocuticle is presented in Table 1. Under such circumstances, the maximum number of lamellae counted in the section of the heart zone (cf. Fig. 1) in the individual crab is expressed in this paper. Furthermore, the total thickness of endocuticle was measured in comparison with the number of lamellae.

Results

Relationship between the number of lamellae and the carapace length obtained from 50 males and 50 females at the intermolt stage C_4 is illustrated in Fig. 3. It is noted from this figure that (1) in spite of the molting of the crab, the number of lamellae in the newly formed and completed cuticle increases with the increment of

Fig. 2. Diagrammatic representation of a cross-section of the cuticles of the heart zone in the carapace of the crab at the intermolt stage C₄. EpCu: Epicuticle; ExCu: Exocuticle; EnCu: Endocuticle; ML: Membranous layer; PC: Pore canal; La: Lamella.

the carapace length, irrespective of sexes, and that (2) regarding the number of lamellae, 4 groups are distinguishable from one another among the specimens examined, representing a stepwise increase of 7-8 lamellae corresponding to the advancing growth of their carapace.

The same relationship was found to occur among the specimens of 28 males and 26 females collected at a single littoral area on April 20, 1968 (Fig. 4). Namely, this result means to be substantially identical with that of Fig. 3 obtained from the sum totals of 100 specimens collected repeatedly in three different times in the same area. The laminary structures of the lamellae of these 4 groups are shown in Figs. 6-10, according to different dimensions of the carapace length of the crab.

Table 1. Maximum and minimum numbers of the lamellae in the endocuticle in the same section of the heart zone in 4 groups of the shore crab, *Gaetice depressus*.

Carapace length (mm)	Sex	Number of lamellae	
		Maximum	Minimum
8.0	Male	14	8
8.2	Female	15	13
12.2	Male	23	15
12.2	Female	22	16
17.0	Male	27	21
16.5	Female	29	24
21.5	Male	36	28
21.0	Female	37	26

The relationship between the total thickness of endocuticle and the carapace length is presented in Fig. 5. This figure indicates that (1) for all their molting, the total thickness of endocuticle increases with the growth of the carapace not

Fig. 3. Relationship between the number of lamellae and the carapace length obtained from 50 males and 50 females at the intermolt stage C_4 , collected repeatedly in three different times at the same area from April to June, 1968.

Fig. 4. Relationship between the number of lamellae and the carapace length obtained from 28 males and 26 females at the intermolt stage C_4 , collected at a single littoral area on April 20, 1968.

showing any distinct sex difference and that (2) regarding the total thickness of the endocuticle, 4 groups are similarly distinguishable from one another among the

specimens examined though the stepwise increase of the thickness among them is not so distinct as in the case of the number of lamellae. While sorting those groups, a few specimens were found to show an equivocal number of lamellae. In such cases, one may appropriately group those specimens according to the total thickness of their endocuticle. A few data plotted in Figs. 3 and 4 situated close to the extremities of the dotted frames are those which were classified into the particular groups under such considerations.

Fig. 5. Relationship between the total thickness of endocuticle and the carapace length obtained from 50 males and 50 females at the intermolt stage C_4 . The specimens examined are the same as those in Fig. 3.

Discussion

There have been many studies made on the age determination of crabs from both ecological and fisheries' points of view. However, all these studies are based on an analysis of the progression of modes in successive length-frequency distribution. Although that may be possible, even by using this method, it is apparent that a sufficiently large number of specimens are necessary to detect the modes statistically, each of which suggests the age of the crab. Accordingly, it is actually desirable for all biologists to learn the age of the individual crab by some appropriate methods.

It is clear from the present observation that in spite of the molting of the crab the number of lamellae in the newly formed and completed cuticles increases with the increment of the carapace length, irrespective of sexes. In other words, this result implies the possibility that the increasing number of lamellae of the newly formed and completed cuticles at a regular rate may be used as their age characteristics even though they molt away all of the hard tissues. At the same time, the

fact that 4 groups are found among the specimens used, representing a stepwise increase of 7-8 lamellae corresponding to the growth of their carapace, suggests that these groups possibly correspond to 4 age-groups of the crab. The validity of this assumption will be confirmed in the future when using many other species also.

Furthermore, the ratio of the number of lamellae to the total thickness of endocuticle was calculated to show a stepwise decrease in the specimens used, though the tendency was found to be indistinct in the groups of the larger specimens. A more precise analysis of this tendency awaits further study. It has been reported by NEVILLE¹¹⁾ that in locusts and cockroaches one layer composed of several lamellae is formed to be deposited during nighttime while the other layer lacking any lamellate structure is formed during daytime. However, it is still in the dark whether the lamellae are formed being regulated by any biological rhythms. Moreover, it is not explainable at present by what mechanism is induced to occur the formation of the increasing number of lamellae in the newly formed and completed cuticles even after molting.

Summary

Relationship between the number of lamellae in endocuticle and the carapace length in the shore crab, *Gaetice depressus*, was studied in relation to age determination of crustacean animals.

1. In spite of their molting, the number of lamellae in the newly formed and completed endocuticle was found to increase with the increment of the carapace length, irrespective of sexes.

2. Regarding the number of lamellae, 4 groups were distinguishable from one another among the specimens examined, representing a stepwise increase of 7-8 lamellae corresponding to the growth of their carapace.

3. For all their molting, the total thickness of endocuticle increases with the growth of the carapace not showing any distinct sex difference. With this total thickness of the endocuticle, 4 groups were also distinguishable from one another, though its stepwise increase was not so distinct as in the case of the number of lamellae.

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Explanation of Plate I.

Figs. 6-10. Photomicrographs of the ground cross-sections showing the laminary structure of lamellae of the cuticles of the heart zone in 4 groups of the shore crab, *Gaetice depressus*. EpCu: Epicuticle; ExCu Exocuticle; EnCu: Endocuticle; ML: Membranous layer; PC: Pore canal; La: Lamella.

6. Male of 20.5 mm in carapace length. $\times 270$. 7. Detail of the framed area in Fig. 6. $\times 1240$. 8. Male of 16.4 mm in carapace length. $\times 270$. 9. Male of 12.5 mm in carapace length. $\times 270$. 10. Male of 8.0 mm in carapace length. $\times 270$.