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STUDIES ON THE SHRIMP *CARIDINA LAEVIS* (HELLER)

III. The Respiratory System

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THIS paper, the third of the series on *Caridina laevis*, deals with the respiratory system ; the accounts on the digestive system and the reproductive system have been published (Pillai, 1960*a* and 1960*b*).

The structure and histology of the gills of Crustacea have been studied only in a few forms. The dissimilarity in structure described by different workers in the various forms is very great (Allen 1893, Pearson 1908, Patwardhan 1937, Webb 1940, Smyth 1942 and Pike 1947). It may be remarked that this disparity is at least partly contributed by the difficulty in getting the material fixed properly.

METHODS

Many workers have commented on the difficulty of obtaining good fixation of the branchiae. In the present study, the branchiae were removed from the body before fixing. The pleural membrane with the pleurobranchs was removed quickly and dropped in warm fixing fluids. The entire animal was also sectioned with a view to studying the branchial circulation. Fixatives employed were strong Flemming, Champy's, Helly's and Gilson's fluids. Sections were stained in iron haematoxylin, Heidenhain's Azan and Delafield's haematoxylin. Some fixed in formalin-Zenker were stained in methyl-green-pyronin (Cowdry, 1959). Although the cytological details were poor in the latter, the distribution of blood sinus inside the gill lamella was very clear. The blood sinus appears empty and non-stained in contrast to the brilliant colour of pyronin. The branchial circulation was studied in live animals by fastening them to glass slides on their sides by the help of thin rubber bands and the gills *in situ* were examined under the low power of the stereoscopic binocular microscope in strong transmitted light. Because of the transparency, the course of circulation is clear and this study is facilitated by the refractivity of the corpuscles which show clearly the direction of the flow of blood.

The Branchial Cavity

The line of attachment of the branchiostegite to the carapace dips above the second maxilliped (fig. 1). Consequently the branchial cavity also tapers in front and is demarcated in front by the epimeron of the mandibular segment. This cavity which encloses the branchiae is open below, behind and in front. The lower rim of the branchiostegal membrane is in contact with the coxae of the walking legs. The epipodites are all placed in one line in an antero-posterior direction on

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the respective coxal segments. They bridge the adjacent coxae and water enters the branchial cavity through a series of slits between the coxae. The feathery bristles of the long exopodite of the first pleopod, which is apposed along these slits extend anteriorly upto the base of the third maxilliped in the male and upto the coxa of the first walking leg in the female. These act as sieves preventing the ingress of debris into the branchial cavity with the respiratory current. Behind, the branchial cavity is in communication with the ventral space enclosed by the pleura of the abdominal segments. In front of the branchial cavity and enclosed by the anterior part of the branchiostegite is a space which may be named as the prebranchial cavity, situated on the outer side of the mandible. This diverges anteriorly leading to the outside.

The Respiratory Current

The maintenance of the respiratory current of water is brought about by the working of the flattened scaphognathite of the second maxilla. Its point of attachment to the body is almost directly above the first gill and tapers behind ending in a truncated apex above the branchia of the segment bearing the first walking leg. This end bears a tuft of long and stiff bristles. The flattened end extends into the prebranchial cavity. The branchial cavity leads to the prebranchial cavity which is triangular in cross section (fig. 2). Towards its inner side are the gnathobases of the various mouthparts and laterally is the branchiostegite. The wide gape below is closed by the flattened exopod of the first maxilliped. It has no movement of its own as is generally supposed to assist the scaphognathite but closes the lower opening of the prebranchial cavity so that water is sucked from the branchial cavity from behind and not from below.

Analysis of the movements of the scaphognathite shows that the maximum amplitude is attained by the anterior fringed region because its pivot is behind the middle. The anterior part is flattened and pliable. The posterior part is rigid and flattened vertically and the movement is in a see-saw fashion around the pivot. The posterior part moves over the gills without disturbing the water inside the branchial cavity. But when the anterior part in its turn moves up or down, it bales out a quantity of water from the prebranchial cavity forwards. Consequently, with every beat of this region, more water is drawn from the branchial cavity. The water is expelled in jerks, in unison with the upward or downward movement of the scaphognathite.

The course of the current of water was studied by putting crystals of stain around the branchial cavity of live specimens observed under the binocular microscope. The inhalent current of water enters the branchial cavity from below and behind. The lower ends of the gills are blunt and these are placed in such a way that a current of water entering the branchial cavity in between the coxae, strikes the gill at its lower apex, divides into two and flows on either side. These currents pass upwards and forwards between the slanting gills and collect as one at the upper anterior part of the branchial cavity from where leads the prebranchial cavity with its efficient device for baling out water. Water from behind viz. from the space below the abdomen also enters the branchial cavity. By the vigorous movements of the exopods of the second and third maxillipeds, the exhalent water is driven away towards the anterior side.

The gills are typical phyllobranchs with two rows of flattened gill lamellae attached to an elongated axis. The attachment of each gill to the body is below

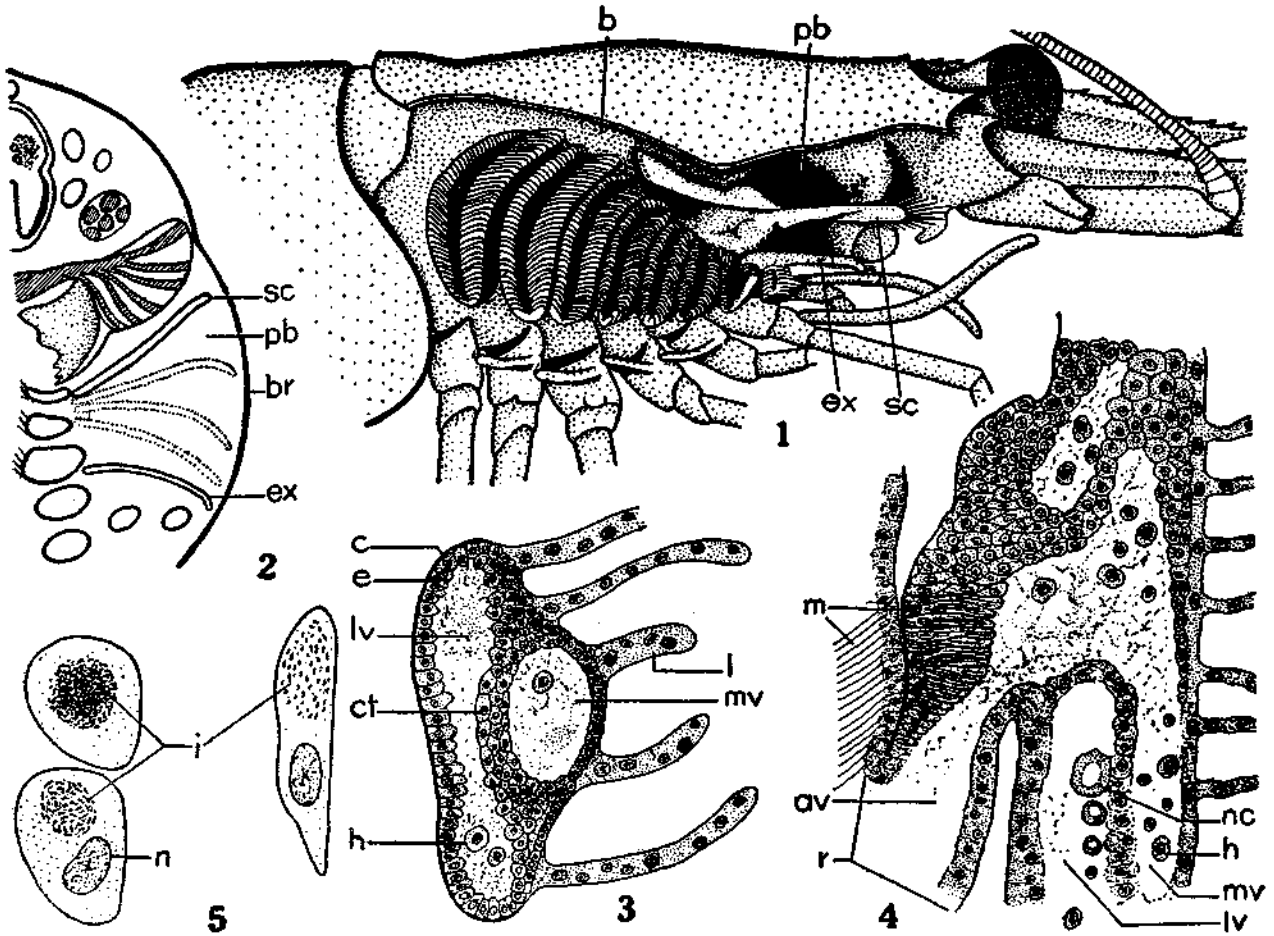


FIG. 1. Lateral view of branchiae after cutting the branchiostegite through its line of attachment. b. branchial cavity, ex. exopodite of first maxilliped, pb. prebranchial cavity, sc. scaphognathite.

FIG. 2. Diagrammatic cross section through prebranchial cavity. br. branchiostegite. Other letterings as in figure 1.

FIG. 3. Cross section through the branchial axis. c. cuticle, ct. connective tissue, e. epithelium, h. haemocyte, l. gil almella, lv. lateral branchial vein, mv. median branchial vein.

FIG. 4. Longitudinal section through the branchial axis. av. afferent branchial vein, m. muscle fibre, nc. nephrocyte, r. branchial root.

FIG. 5. Three nephrocytes blocked by Indian ink. i. Indian ink particles, n. nucleus.

its middle. Like the branchial cavity the branchiae also diminish in size cephalad to get themselves accommodated inside. The branchial formula is given in the table.

TABLE

The Branchial Formula of *Caridina laevis*

	Appendages		Pleurobranch	Arthrobranch	Podobranch	Mastigobranch
1.	Maxilliped	1	0	0	0	1
2.	do.	2	0	0	1	0
3.	do.	3	1	1	0	1
4.	Pereiopod	1	1	1	0	1
5.	do.	2	1	0	0	1
6.	do.	3	1	0	0	1
7.	do.	4	1	0	0	1
8.	do.	5	1	0	0	0
			6	2	1	6=15

(The podobranch of the second maxilliped appears to represent the branchia and the epipod together, though not confirmed by embryological studies.)

The Structure of the Branchiae

The branchial axis is roughly triangular in cross section (fig. 3) and bears two rows of extremely thin gill lamellae. It has an outermost layer of thin cuticle. Inside the gill axis are three blood channels, two at the base of the triangle, the lateral branchial veins and one at its apex, the median branchial vein, separated by connective tissue cells. The blood spaces are without walls of their own and are bounded either by connective tissue or by the epidermis of the branchial axis. The boundary of the median branchial vein is more or less even whereas those of the lateral branchial veins are not. The lateral branchial veins are joined together by short connections throughout their length. Such a connection at the gill root is the largest of the series and the efferent branchial channel (branchiocardiac vein) which takes the oxygenated blood to the pericardium originates from this. Situated above and slightly lateral to the origin of the efferent branchial channel is the afferent branchial blood channel (fig. 4) which joins the median branchial vein bringing blood from the ventral thoracic sinus to the gills. The upper region of the wall of the gill root, with the cuticle and the epidermis dips into the afferent branchial channel at its origin and this tongue-like depression with the muscles attached to it, functions as a sort of regulator of the flow of blood from the ventral sinus into the gill (fig. 4). It is because of the working of the heart and the peculiar arrangement of the ostia that the blood is drawn from the pericardium to be conveyed to the various arteries. As the pericardium is directly connected with the gills by way of blood vessels viz. the branchiocardiac veins, it is this pull that is responsible for the propulsion of blood through the branchiae. Naturally the amount of blood flow through the gills may be determined by the rate of heart beat and since there does not seem to be any relation between heart beat and oxygen pressure of the water (Vide infra), it is likely that the regulation of the flow of blood is controlled by this tongue-like depression.

Structure of the Gill Lamella

The lamella (fig. 5) is invested alround by a thin layer of cuticle, much thinner than the general cuticular layer of the body. The cuticle in the margin of the gill

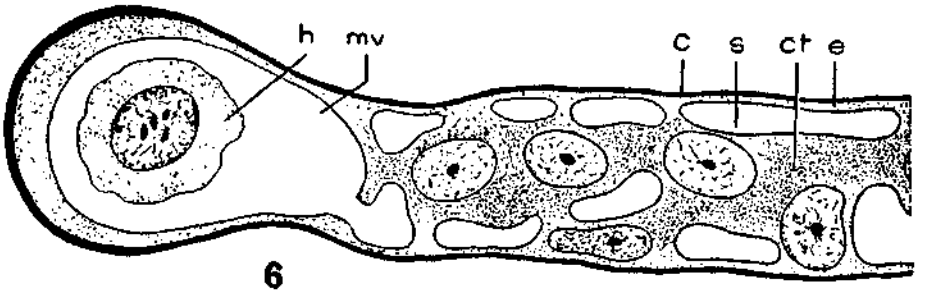
lamella is slightly thickened and forms a reinforcement, giving it rigidity. Below this is a thin epithelium. This represents the epidermal layer which has been reduced to the nature of a membrane with sparse nuclei. The lamella by itself measures only 19μ in cross section and naturally the epidermis, 4 to 14 μ thick elsewhere has to undergo extreme thinning to line the upper and lower sides of the lamella. Connecting the lateral branchial veins with the median branchial vein is the marginal vein, situated below the thickened cuticular rim. The two layers of the epithelium enclose a thin sheet of cells, similar to the connective tissue in the gill axis, with large round nuclei. The cell boundaries of these cells are quite indistinct. Whether these are extensions of the epidermal cells or connective tissue extensions of the gill axis is not clear. The two surfaces of this middle layer have, between themselves and the epidermal layers, a branching system of thin and flattened blood spaces, the marginal sinuses. These have their connection to the marginal vein all along the marginal vein. Though too thin to admit a corpuscle, occasionally the sinuses get oval in section, having a corpuscle inside. The sinus sometimes has been seen to extend deeper into the middle sheath.

Only very little work has been done on the histology of the gills in Crustacea. In the available literature, the structure of the branchiae differs considerably among themselves. But for the general arrangement of the blood vessels, no comparison could be made between the branchiae of *Palaemonetes* (Allen 1813) and the present form. The two parallel limbs of the marginal vein of each lamella are not continuous in *Palaemonetes* but are connected together by a series of transverse sinuses. The 'transverse cells' and the 'clear cells' of the lamella are not represented as such in *Caridina*. The middle sheath of cells of the lamella of the present form might probably correspond to the transverse cells. The 'pillar-cell junctions' described by Smyth (1942) in *Carcinus* and Pike (1947) in *Galathea* and the canals through the cuticle also have not been observed. It is obvious that, with the above two forms, *Caridina* differs considerably with regard to the structure of the branchiae. The glands similar to those described in *Palaemonetes* have also not been observed inside the branchial axis in *Caridina*.

In *Palaemon*, according to Patwardhan (1937) the branchial circulation is as follows. The lateral branchial veins receive the venous blood through the afferent branchial channel from the ventral thoracic sinus. This is conveyed through the marginal channels of the gill lamellae and collects in the median branchial vein. The efferent branchial channel takes the blood from the median branchial vein to the pericardium. But the study of the course of blood circulation in live specimens of *Caridina laevis* does not agree with this. Here the median branchial vein receive blood from the ventral thoracic sinus and this is sent to the two apices of the gill axis. The blood passes into the marginal vein of the lamella and finally enters the lateral branchial veins. Through the efferent branchial channel it reaches the pericardium. In *Palaemon* direct observation of the course of circulation in live animals is hardly possible because of the toughness of the cuticle and therefore it may be possible that the course has been interpreted wrongly.

Nephrocytes

Some of the cells on the wall of the lateral branchial veins are large (15-18 μ) and have fairly large vacuoles which push the spherical nucleus to the periphery. The vacuoles in unstained preparations contain yellow concretions. These cells are of the nature of nephrocytes and have been found to be blocked by Indian ink, as the amoebocytes in *Rhodnius* (Wigglesworth 1955). 0.5 cc. of filtered stick ink



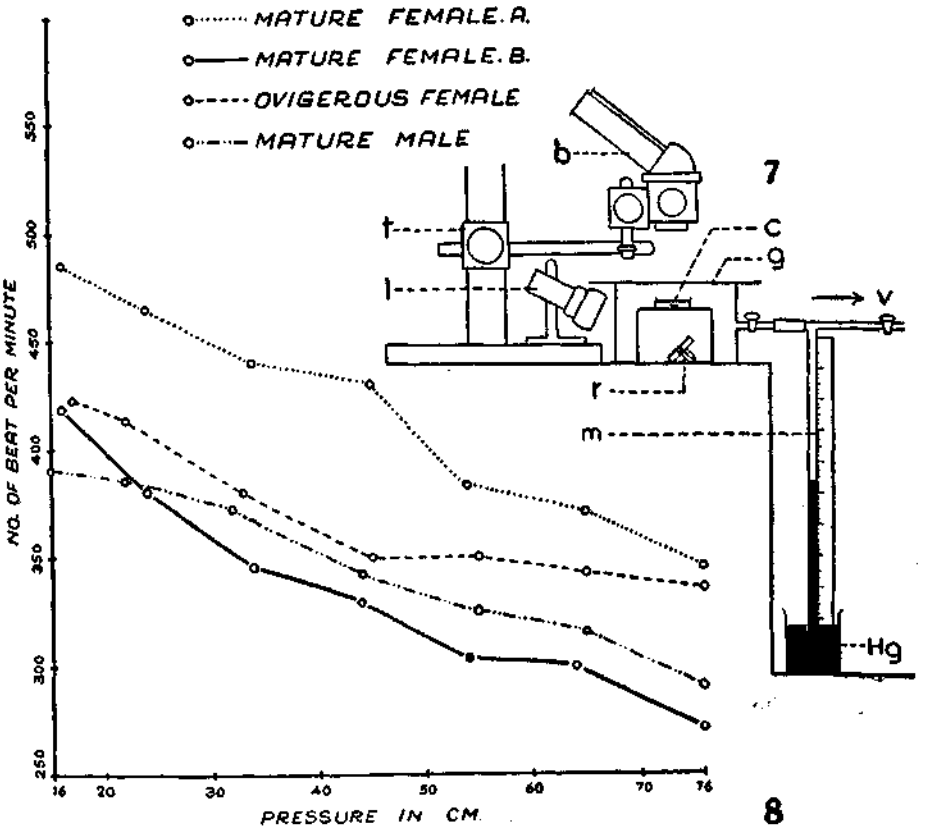


FIG. 6. Transverse section through gill lamella. mv. marginal vein. Other letters as in figure 3.

FIG. 7. An illustration of the experimental device used for the study of respiratory regulation. b. binocular microscope, c. animal in petri dish, g. glass plate, Hg. mercury, l. electric lamp, m. manometer, r. reflector, t. stand, v. vacuum pump.

FIG. 8. Graph showing the inverse proportion between the oxygen content of the wate (plotted in pressure) and the number of scaphognathite beats.

boiled in Ringer solution was injected into the animal through a fine glass capillary. Injected animals were fixed later, sectioned in paraffin and examined unstained. In the blocked nephrocytes (fig. 6) the ink appears unorganised in the cytoplasm. Later, these seem to be assembled inside vacuoles of different sizes and shapes.

Nephrocytes more or less of the same nature have been described by Kowalewsky (1889), Allen (1893), Pearson (1908) and Pike (1947). How these accumulated substances are finally eliminated is not clear. In *Galathea*, Pike (1947) observed the contents as leaving the cell at period of ecdysis into the haemolymph. But the actual fate seems to be obscure. Smaller vacuoles containing ink have been observed in nephrocytes that have ceased to be attached to the wall of the lateral branchial vein. The probable fate of these in *Caridina* is as follows. When the vacuoles get filled, the nephrocytes expand and are then detached. The nuclei undergo disintegration as shown by the development of refrangible bodies inside. The boundary of the cell and its vacuole now gets ill-defined. It is possible that the contents of the vacuole now diffuse into the cytoplasm of the nephrocyte judging from the more or less uniform nature of ink distribution. Fragments of the cells are carried about and brought to the blood sinuses of the antennal gland. Unstained sections of the antennal gland fixed six days after administering Indian ink, show that the blood sinus lying immediately between the lower wall of the bladder and the upper wall of the end sac harbour a large number of nephrocytes containing ink in various stages of disintegration. It appears that the disintegrating nephrocytes get accumulated here, in order to be ultimately eliminated by way of the end sac epithelium.

Respiratory Regulation

Based on Henry's law (Mee 1937) that the amount of a partly soluble gas dissolved in a liquid is directly proportional to the pressure to which it is subjected at a constant temperature ($m/p=K$), various air saturations were created by controlling the atmospheric pressure. Animals collected from different environments were observed under various oxygen pressures in water and their scaphognathite beats were recorded for a definite interval of time.

The experimental device, illustrated in figure 7 consists of the following: A vacuum trough connected through a manometer to a vacuum pump ; an electric lamp, a reflector and a stereoscopic binocular microscope mounted on a movable stand. The experimental animal, contained in a small volume of water in a petri dish is placed on the top of a smaller inverted glass trough inside the vacuum trough with the reflector beneath. A small piece of loosely spread sanitary cotton is put in the water for the animal to cling to and facilitate continuous observation. The vacuum trough is covered over by a thick sheet of glass smeared with vaseline. The beam of light from the lamp is reflected directly on to the animal and the microscope is brought to position. The number of scaphognathite beats is counted by the help of a counter and computed to per minute. The readings are taken in normal and in various reduced atmospheric pressures. For every changed pressure, time is given for the animal to get acclimatised and an average of ten to fifteen readings are taken. Because of a definite rhythm and uniformity in the play of the appendage, it is not difficult to make a more or less accurate count of beats with a certain amount of practice. The result is plotted in the graph (fig. 8).

The respiratory regulation has been studied under different air saturations in many Invertebrates. Walshe-Maetz (1952) has shown graphically the amount of variation in the number of pleopod beats of various Crustacea in fresh water, brackish

water and marine and terrestrial species and that the fresh water and brackish water Crustacea possess the maximum capacity for respiratory regulation than their semi-terrestrial allies.

It is evident from the graph that mature female specimens more or less at the same stage of development of the ovary, collected from two different environments, show different rates of scaphognathite beat for any particular pressure. This can be explained as follows: The one that lives in a small body of water (mature female A) with very little vegetation and plenty of other animals will have a lesser amount of oxygen content at its disposal, hence the increased frequency of beat, while the water in a large pool with plenty of water weeds has a greater air content. Hence there is a decreased frequency of beat (mature female B). But in both, with declining pressures and consequently with declining oxygen content, the scaphognathite beat mounts almost uniformly as represented by the almost parallel slopes. The respiratory control in other fresh water Crustacea (Walshe-Maetz 1952), shown by the steepness of the slope of the curve with declining oxygen content seems striking in contrast with *Caridina* where the slope of the curve is much less steep. The frequency of the working of the flagellum of the second maxilliped which assists the scaphognathite in the maintenance of a respiratory current of water becomes greater with decreased oxygen content. It has not been possible to correlate the heart beat with the oxygen pressure as it does not show any definite pattern.

Summary

The morphology and anatomy of the branchiae have been described. The mechanism and course of the respiratory current have been studied which has revealed that the flattened exopod of the first maxilliped has no function other than closing the prebranchial cavity from below. The branchial circulation, studied in live animals has been shown to be quite different from that postulated by Patwardhan (1937) in *Palaemon*. An attempt has been made to study the functional role of nephrocytes by blocking them with Indian ink. An apparatus for the study of the relation between oxygen pressure of the water and the number of scaphognathite beat has been devised. For any particular pressure, variation in the frequency of beat has been observed in different animals and even between two animals of the same sex at the same stage of development, collected from two different environments and the reasons therefor suggested.

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