

Functional Morphology of the Respiratory System of *Parhyale hawaiiensis* Dana (Crustacea: Amphipoda)

O DIVAKARAN and N KRISHNA PILLAI

Department of Aquatic Biology and Fisheries, University of Kerala, Trivandrum 695007

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The respiratory organs of *Parhyale hawaiiensis* are five pairs of branchiae, the sternal part of the peraeonic and pleonic segments, the coxal plates of peraeonic segments 1-4 and the hinder expanded portion of the basipodites of the last three peraeopods. *P. hawaiiensis* can survive and take up oxygen at a control rate even in the absence of the branchiae. Amputated branchiae acquire normality in one or two moults. The oxygen consumption rate of mature *P. hawaiiensis* ranged between 2.08 and 4.01 ml O₂/g dry wt./hr. Oxygen-uptake declined while lethal oxygen concentration increased with the increase in body size. The rate of respiration decreased both in higher and lower salinities—the optimum being around 33‰.

Key Words: Respiratory system, Respiratory rate, Amputation of branchiae, Regeneration of branchiae, Lethal oxygen concentration, Optimum salinity, *P. hawaiensis*

Introduction

Information on the respiratory organs of Crustacea is scanty. Stray attempts hitherto made on this aspect are those by Ellenby (1951), Kinne (1952), Rao (1958), Edney (1960), Wolvekamp and Waterman (1960), Subrahmanyam (1962), Mcluskay (1968), Sameoto (1969), Fish and Preece (1970) and Jawed (1973). Most of these studies provide only fragmentary information. Hence a comprehensive study on the structural and functional aspects of the respiratory organs of a littoral and semi-terrestrial amphipod, *Parhyale hawaiiensis*, was carried out.

Material and Methods

The structure of the respiratory system was studied by gross dissections, serial sections and in vivo application of India ink. In live specimens, the circulation of the blood in various respiratory organs was studied by observing the movements of the blood cells. The disposition of the blood vessels and sinuses was studied by injecting India ink. For this adult specimens were narcotised at 0°C for 8 to 10 minutes and the ink was injected into the pericardial sinus. The specimens thus

treated were fixed in aqueous Bouin for a day. This helped the tissue to stiffen and the ink to harden. These specimens were later washed in 5% formalin, dehydrated and mounted in methylbenzoate.

To study the function of the respiratory structures, other than the branchiae, adult individuals of *P. hawaiiensis* were narcotised by keeping them at 0°C for 5 min and their branchiae removed from one pair to the entire lot—in different specimens. Care was taken to cause least injury to the organisms during amputation. These specimens were reared in separate glass jars to study their rate of survival. The degree of regeneration of the branchiae during the succeeding moults was also observed.

To assess any significant effect of the amputation of all the branchiae on the efficiency in the intake of oxygen, another experiment was carried out. Fifteen specimens ranging from 7–8 mm in length were taken. The oxygen uptake rate of 10 animals was estimated and their gills removed thereafter. The respiratory rates of the same individuals 1 hr and 24 hr after amputation were estimated. A few experimental animals which died were replaced with similar ones.

To find the relation between body length and rate of oxygen consumption and the lethal oxygen concentration a third set of experiments was carried out. Eight groups of *P. hawaiiensis* with 1 mm difference in body length were selected and the respiratory rate of each group was estimated and the lethal oxygen concentration for each group was determined.

Since *P. hawaiiensis* is a nearshore inhabitant and is exposed to wide salinity fluctuations, the effect of salinity on the rate of respiration was studied. For this *P. hawaiiensis*, 7–8 mm long, were got acclimatised to salinities 15, 20, 25, 33, 35, 40 and 45‰. All the animals were fully established 24 hr before oxygen estimation test were carried out. Some

specimens were exposed to moist air on wet surface and observed for their survival rate.

Observations

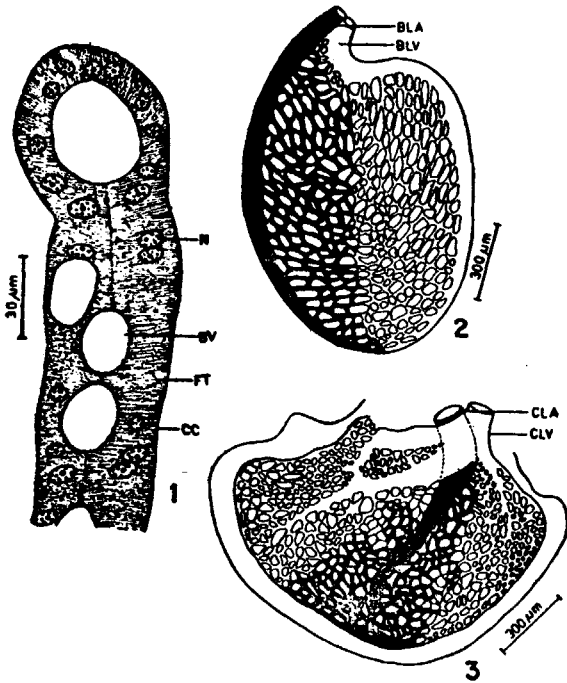
As in other crustaceans in amphipods also gills or branchiae are the main respiratory organs. The branchiae exhibit some variations among amphipods. Their number varies from two to five pairs (Calman 1909). In some cases the surface area is increased by the development of foldings (Mills 1967) or they have pinnately arranged branchial lobes (Calman 1909). In *P. hawaiiensis* the branchiae are five pairs of simple lamellae arising from the inner side of the coxal segments of thoracic appendages two to six. Some of the coxal plates and basipodites function as accessory respiratory structures.

The branchiae of *P. hawaiiensis* are covered by very thin cuticle devoid of any kind of out-growths. The core of the plates is formed by fibrous tissues originating from the two sides and meeting in the middle at right angle to the long axis of the plates (figure 1). These fibres fill the interior of the gill except where there are blood vessels and sinuses. Scattered among the fibrous matrix are large nuclei each with a prominent nucleolus. Blood vessels are mostly in the centre but occasionally they may be peripheral. The branchiae have an extensive network of blood spaces (figure 2) whose limiting membrane is adherent to the surrounding fibrous tissue. Hence they can be considered as sinuses rather than vessels.

The coxal plates of peraeonic segments one to four, which are comparatively large, have a thin inner cuticular lining which apparently acts as a respiratory surface. This is evident from the fact that, like the branchiae, the coxal plates also have an extensive network of blood spaces (figure 3) through which there is a continuous flow of blood.

The sternal part of the peraeonic and pleonic segments and the hinder expanded

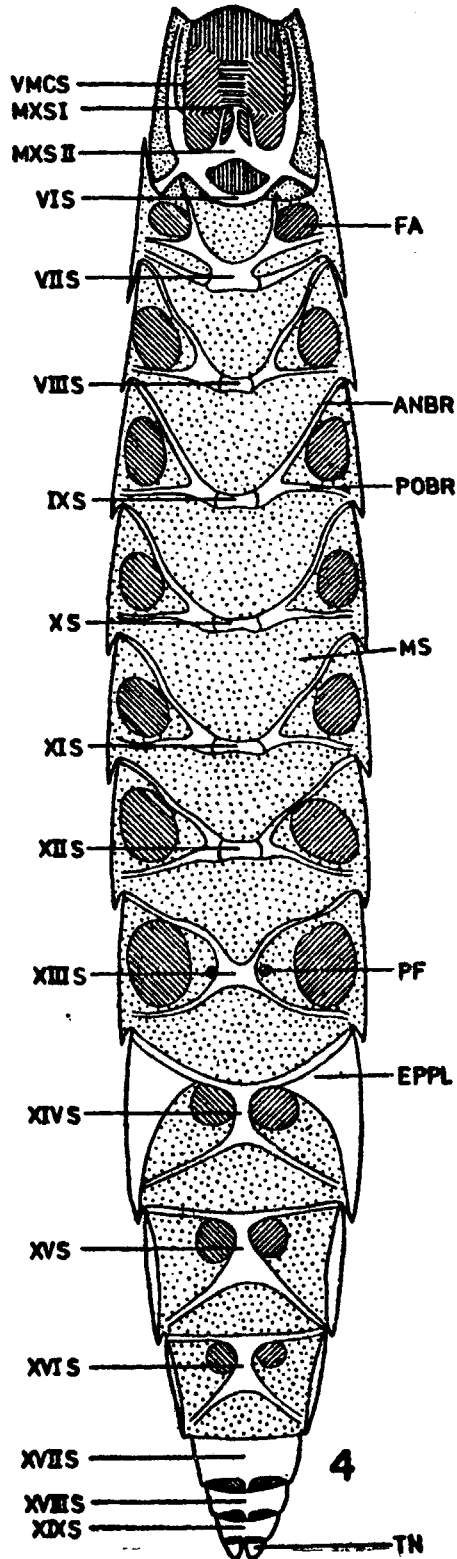
portion of the basipodites of the last three pereopods also appear to have a respiratory function, The sternites of these segments are very delicate and membranous (figure 4) in nature and are bathed by a rich supply of blood through the sternal sinus. Similarly the basipodites of the last three pereopods are very much expanded and highly vascularised. The cuticle of their inner walls is very thin, apparently permitting gaseous exchange.



Figures 1-3 T. S. of a branchia; Blood circulation in the third branchia; Blood circulation in the fourth coxal plate

BV, blood vessel; BLA, branchial artery; BLV, branchial vein; cc, cuticular covering; CLA, coxal artery; CLV, coxal vein; FT, fibrous tissue; N, nucleus

Figure 4 Ventral view of a *P. hawaiiensis* (appendages removed) showing the membranous body wall indicated by large stipples. ANBR, ante coxal sternotergal branchium; EPPL, epimeron of pleon segment I; FA, foramen to appendage; MS, membranous portion of sternite; MXSI; MXSII; maxillary sternites, PF, penial foramen; POBR, postcoxal sternotergal branchium; TN, telson; VMCS, ventral margin of the cephalic shield; VI, S—XIX S sternites



Even in the absence of branchiae *P. hawaiiensis* can survive and carry on normal respiration (table 1). Immediately after amputation there is a definite reduction in the oxygen uptake rate but the rate returns to control level in 24 hr (table 2). Thus, when normal branchial respiration is blocked the accessory respiratory structures can fully meet the respiratory needs. The recorded death of a few experimental animals was probably caused by injury during amputation. Amputated branchiae get regenerated during the succeeding moults. Development of the miniature branchiae reach full growth during the next moult.

Discussion

Wolvekamp and Waterman (1960) cited that *Talitrus saltator* can live for some time with its gill respiration partially or completely blocked but could not live as long as the con-

trols. *Birgus* and *Coenobita* can live for months in air after removal of gills (Edney 1960). As cited by Wolvekamp and Waterman (1960) 3–5% of the respiratory activity is effected through integument in aquatic crustaceans. With regard to *P. hawaiiensis* actual percentage of the respiratory activity of the accessory structures could not be estimated without blocking gill respiration. But from the experiment described above it is evident that the accessory structures can, when necessary, increase their efficiency to fully compensate the loss of gills, i.e., 100% respiration by accessory structures is possible when necessary.

The rate of metabolism of aquatic invertebrates is dependent on several factors like temperature, salinity, amount of dissolved oxygen and also the size of the animal and its physiological conditions. Since the experimental techniques and the units for the oxygen consumption rates reported in literature vary, it is difficult to compare the oxygen consumption rate of even related species.

The oxygen consumption rate of mature *P. hawaiiensis* under laboratory conditions—temperature (29°C), salinity (33‰) and dissolved oxygen content (4.74 ml O₂/l)—varied between 2.08–4.01 ml O₂/g dry wt/hr which is within the range of variation shown by crustaceans in general, namely 20 to 1450 μl O₂/g wet wt/hr (Wolvekamp & Waterman 1960). Sameoto (1969) found significant difference in closely related species of *Haustorius*, *H. canadensis* (204±39), *Acanthohaustorius millisi* (430±12), *Parahaustorius longimerus* (459±176), *Neohaustorius biarticulatus* (776±37) and *Protohaustorius deichmannae* (786±184) μl O₂/g dry wt/hr.

Oxygen-uptake in *P. hawaiiensis* declined with increase in body length (table 3). This is also true of other crustaceans such as *Ligiaoceanica* (Ellenby 1951), *Metapenaeus monoceros* (Rao 1958), *Bathyporeia pilosa*, *B. pelagica* (Fish & Preece 1970), *Sphaeroma*

Table 1 Survival rate after amputation of branchiae

| No. of gills amputated | No. of specimens | No. of survivors |
|------------------------|------------------|------------------|
| 2 | 15 | 15 |
| 4 | 15 | 15 |
| 6 | 20 | 18 |
| 8 | 20 | 14 |
| 10 | 50 | 32 |

Table 2 Efficiency of accessory respiratory organs

| Condition | Oxygen consumption as ml O ₂ /g dry wt/hr ± S.E. |
|-----------------------------|---|
| Before gill amputation | 2.40 ± 0.1292 |
| 1 hr after gill amputation | 2.05 ± 0.0958 |
| 24 hr after gill amputation | 2.20 ± 0.1292 |

terebrans (Cheriyān 1973), *Archaeomysis grebnitzkii* and *Neomysis awatschensis* (Jawed 1973).

Table 3 Oxygen consumption and lethal oxygen concentration for *P. hawaiiensis* in relation to body size at temperature 29°C, salinity 33‰ and dissolved oxygen content 4.74 ml/l

| Length in mm | Oxygen consumption as ml O ₂ /g dry wt/hr ± SE | Mean 50‰ lethal O ₂ concentration as ml O ₂ /l—SE |
|--------------|---|---|
| 2-3 | 6.17 ± 0.1109 | 0.357 ± 0.0112 |
| 3-4 | 4.67 ± 0.0855 | 0.362 ± 0.0087 |
| 4-5 | 4.32 ± 0.0946 | 0.372 ± 0.0100 |
| 5-6 | 4.00 ± 0.0913 | 0.449 ± 0.0071 |
| 6-7 | 3.55 ± 0.1555 | 0.543 ± 0.0050 |
| 7-8 | 3.25 ± 0.0646 | 0.638 ± 0.0025 |
| 8-9 | 2.62 ± 0.0778 | 0.706 ± 0.0235 |
| 9-10 | 2.18 ± 0.0592 | 0.732 ± 0.0029 |

The lethal oxygen concentrations varied between 0.360 and 0.729 ml/l between juveniles and adults. Identical value but of a higher level was found in *Penaeus indicus* (Subrahmanyam 1962). In this species the lethal level increases with increase in weight. The values vary from 1.49 to 3.8 ml/l for specimens weighing from 0.6 to 10.0 g. The lethal level for the 5 haustorids mentioned above is identical to that for *P. hawaiiensis*. But Cheriyān (1973) found that the lethal level of

oxygen concentration for *Sphaeromat erebrans* was 0.6 ml/l in normal salinity and did not change with the increase in weight of the animal.

The rate of respiration of *P. hawaiiensis* decreased in higher and lower salinities and the optimum was around 33‰ (table 4). Within the range of 15-45‰ salinity appeared to have only negligible effect, if at all there was any, on lethal oxygen concentration

Table 4 Oxygen consumption of *P. hawaiiensis* in relation to the salinity of the media

| Salinity of the media (‰) | Oxygen consumption as ml/g dry wt/hr ± S.E. |
|---------------------------|---|
| 15 | 2.05 ± 0.0467 |
| 20 | 2.08 ± 0.0312 |
| 25 | 2.46 ± 0.0838 |
| 33 | 3.04 ± 0.0840 |
| 35 | 2.72 ± 0.1047 |
| 40 | 1.71 ± 0.0630 |
| 45 | 1.29 ± 0.0517 |

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References

- Calman W T 1909 *A Treatise on Zoology* ed. Sir Ray Lankester. Part 7 Appendiculata, Third Fascicle, Crustacea: (London: A & C Black)
- Cheriyān P V 1973 Studies on *Sphaeroma terebrans* Bate (Crustacea: Isopoda) of the port of Cochin; *Forma et Functio* 6 1-68
- Edney E B 1960 Terrestrial adaptations; in *The Physiology of Crustacea* Vol. I pp 367-393 ed. T H Waterman (New York: Academic Press)
- Ellenby C 1951 Body size in relation to oxygen consumption and pleopod beat in *Ligia oceanica* L.; *J. expl. Biol.* 28 492-507
- Fish J D and G S Preece 1970 The ecophysiological complex of *Bathyporeia pilosa* and *B. pelagica* (Crustacea: Amphipoda). I. Respiration rates; *Mar. Biol.* 5 22-28
- Jawed M 1973 Effects of environmental factors and body size on rates of oxygen consumption in

- Archaeomysis grebnitzkii* and *Neomysis awatschensis* (Crustacea: Mysidae); *Mar. Biol.* **21** 173-179
- Kinne O 1952 Zur Biologie und physiologie von *Gammarus duebeni* Lillj. V. Untersuchungen über Blutkonzentration, Herzfrequenz und Atmung; *Kiel. Meeresforsch.* **9** 134-150
- Mclusky D S 1968 The oxygen consumption of *Corophium volutator* in relation to salinity; *Comp. Biochem. Physiol.* **29** 743-753
- Mills E L 1967 The biology of an ampeliscid amphipod crustacean sibling species pair; *J. Fish. Res. Bd. Can.* **24** 305-355
- Rao K P 1958 Oxygen consumption as a function of size and salinity in *Metapenaeus monoceros* Fab. from marine and brackishwater environments; *J. Exptl. Biol.* **35** 307-323
- Sameoto D D 1969 Physiological tolerances and behaviour responses of five species of Haustoriidae (Amphipoda: Crustacea) to five environmental factors; *J. Fish. Res. Bd. Can.* **26** 2283-2298
- Subrahmanyam C B 1962 Oxygen consumption in relation to body weight and oxygen tension in the prawn *Penaeus indicus* Milne-Edwards; *Proc. Indian Acad. Sci.* **55** 152-161
- Wolvekamp H P and T H Waterman 1960 Respiration; in *The Physiology of Crustacea* Vol. 1. 35-100 ed. T H Waterman (New York: Academic Press)