THE CONTROL OF RESPIRATORY MOVEMENTS IN CRUSTACEA BY OXYGEN AND CARBON DIOXIDE. II

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(With One Text-figure)

THE investigations made by Fox and Johnson (1934) into the respiratory control of certain Crustacea showed that there was considerable variation in the response to carbon dioxide and oxygen. Thus the rate of limb movements of *Balanus* and *Chirocephalus* was not affected by the gas content of the water, whereas the respiratory rhythm of *Astacus* and *Asellus* was quickened by oxygen lack, and that of *Gammarus* by both oxygen lack and carbon dioxide excess. Even within one genus some degree of variation was found, for whereas the effect of changes of the gas content of the medium on the rate of pleopod beat in *G. pulex* was permanent, in *G. locusta* it was transitory. It was hoped that by extending these investigations to more species of Crustacea, some phylogenetic or ecological significance might be discovered in this variation of response to the respiratory gases.

I. *SQUILLA MANTIS* L.

The respiratory organs of *Squilla* are described fully by Giesbrecht (1910). In the adult the exopodites of the pleopods bear large tufts of branchial filaments which are not developed in the young forms. The movements of the pleopods draw a current of water through the burrow in which the animal lives. The respiratory organs of the larvae are discoid membranous plates or vesicles—the epipodites of the five maxillipedes. These thoracic branchiae persist in the adult but would seem to be too small to be important in respiration.

Bethe (1897) and Matula (1912) among others have described the respiratory movements of the pleopods, but the fact that the thoracic branchiae also move in the adult seems to have escaped notice until now; and the fact that they do move is interesting not because of their respiratory function which is presumably negligible in the adult, but because of their analogy with the scaphognathite of the decapods (epipodite of the second maxilla). When the animal is at rest the five branchiae protruding from the back of the carapace on each side can be seen to flick occasionally; after locomotion the rate of movement is higher. The movements of the epipodites

of one side are not always synchronous with those of the other, and often have a quite different rate. This recalls the asymmetrical condition of the scaphognathite of the crayfish and crab.

The rate of movement of the pleopods varies from individual to individual; it is generally higher in small animals than in large. The rhythm may be continuous or irregular and broken. The amplitude varies with the rate, being greatest when the rate is high. After the animal has been stimulated the rate of beat is high, but it gradually falls off when the animal is quiet and may remain constant for hours in running water; in a small volume of water the rate rises as the oxygen tension falls and carbon dioxide accumulates.

The effects of oxygen and carbon dioxide. Matula (1912) reports that there is no increase in rate of pleopod beat after the animal has been in oxygen-free (boiled) water for 20 min. On the other hand, when the quantity of carbon dioxide is gradually increased the rate rises to a critical point after which the animal is poisoned and the rate falls off. This dissimilarity between the effects of oxygen lack and carbon dioxide excess is so unusual and important that the question was reinvestigated, as it seems possible that Matula did not allow the animals to settle down in aerated water before transferring them to boiled water.

Table I. Squilla E. *Temp.* 23° C.

Time	Beats per minute (average of 3 readings)		
	Pleopods	Right thoracic branchiae	Left thoracic branchiae
Aerated water pH 8·4			
9.0 a.m.	29	—	—
9.30 ,,	14	7	Occasionally
9.40 ,,	19	12	,,
Replaced by aerated water + carbon dioxide (pH 7·1) at 10.8 a.m.			
10.10 a.m.	41	40	40
10.30 ,,	32	18	Occasionally
10·50 ,,	27	14	11
11.15 ,,	29	14	17
Replaced by aerated water at pH 8·4 at 11.24 a.m.			
11.25 a.m.	41	20	12
11·50 ,,	20	7	Occasionally
12.10 p.m.	19	7	,,
1.30 ,,	16	11	,,
Replaced by aerated water + carbon dioxide at (pH 6·7) 1.48 a.m.			
1.55 p.m.	41	46	46
2.15 ,,	43	54	43
2.35 ,,	44	60	43
2·50 ,,	44	60	50
Replaced by aerated water at pH 8·4			
3.0 p.m.	37	43	35
3.15 ,,	30	20	0
3.40 ,,	26	10	0
4.30 ,,	24	13	0
4.45 ,,	28	12	0

Only seven animals were available and on these thirty-two experiments were made. The work was done at Banyuls-sur-mer on the Mediterranean coast of France. If undisturbed the animals are quiet for long periods and the respiratory movements can easily be counted or recorded on a smoked drum with a tapping key in circuit with an electric time-marker. A typical case of the effect of carbon dioxide is given in Table I; the rate of movement of both pleopods and thoracic branchiae is raised when the amount of carbon dioxide in the medium is increased. Similarly oxygen lack has a stimulating effect; thus in one animal the pleopods beat at an average rate of 29 per minute in water containing 5·2 c.c. oxygen per litre, the thoracic branchiae moving occasionally and not synchronously. In water containing 2 c.c. oxygen per litre the pleopods beat at 60 per minute and the thoracic branchiae at 50 per minute (synchronously).

II. *PANDALUS BOREALIS* KRØYER

Experiments on this prawn were made at Bergen, where it is taken at a depth of 100 metres. When the animal is lying still the movements of the scaphognathite can be watched easily through the branchiostegite. Fifty-six experiments were made, involving thirty-nine animals. The rate of beat is affected by both oxygen and carbon dioxide. In a typical case, at 18° C., the average rate in an oxygen tension of 3·4 c.c. per litre was 94 beats per minute, in 0·95 c.c. per litre 200 per minute, and in 10 c.c. per litre 46 per minute. In another case, at 13° C. in pH 8·3 the average rate was 59 beats per minute, in pH 6·9 185 per minute, returning to 60 per minute in pH 8·3. In pH 6·6 the animals became very excited, the scaphognathite beat slowly and irregularly and death soon ensued.

III. *CARCINUS MAENAS* L.

The experiments on the shore crab were done in Birmingham on animals sent from Plymouth. A small piece of the branchiostegite was removed on each side to expose the tip of the scaphognathite. The movements of the scaphognathite are very variable, both among different animals, and in the same animal at different times. Sometimes the rate is quick and regular over long periods, in which case the scaphognathites of right and left sides may beat at the same rate, though not necessarily synchronously. At other times the beat may be slow and irregular, stopping completely at intervals. In this case the scaphognathites of right and left sides beat at quite different rates, indeed one may be still while the other is beating quickly and regularly.

Experiments were made at first with animals in open vessels through which passed a current of water. The animals were lively and continually tried to climb out of the vessel, or remained for long periods with the anterior part of the body exposed to air. In this position the scaphognathite continued to beat drawing a current of water forwards, but more slowly than when submerged. In later experiments the animals were enclosed in rectangular glass jars filled with sea water, the

lids of which were vaselined and clamped down so that the jars could be submerged
in a water bath. The lids were perforated for a bung which carried glass tubes
through which water passed in and out of the jar. In this way the crabs could be
watched all day, and the water supply could be changed without disturbing them.

The response to oxygen and carbon dioxide was as variable as the rate of
scaphognathite beat itself. 138 experiments were made on 40 animals, covering
a range of oxygen tension from 17 c.c. per litre to 0·2 c.c. per litre, and carbon
dioxide tension up to pH 6·3. In a few cases there was an increase in rate in carbon
dioxide excess and in oxygen lack, and decrease in oxygen excess, but the response
varied in different animals under precisely the same experimental conditions, and
also in individuals from day to day. Moreover, in some cases one scaphognathite
beat more quickly in low tensions and slowly in high, while the other showed the
opposite response.

It is clear, then, that some internal factor is much more important in controlling
the rate of scaphognathite beat of crabs in the laboratory, than are carbon dioxide
or oxygen. Perhaps this insensitivity to the respiratory gases is correlated with the
amphibious habits of the animals. The crabs were kept in a sloping tank, the water
at the lower end being deep enough to submerge them completely. The water was
aerated, and frequently renewed. Observations showed that the crabs spent most
of their time either at the shallow end of the tank, where they were only partly
covered, or in the deep end but with the anterior half of the body stretched up into
the air.

IV. *CIROLANA BOREALIS* LILLJEBORG

The work on *Cirolana* was done at Bergen. This isopod is taken at about
100 metres and is found in great numbers on dead fish. Sixty experiments were
made, involving twenty-three animals. In experimental conditions the animals
remained quiet except for occasional bouts of swimming with the pleopods. The
respiratory movements are regular and were counted with a stop watch, readings
being taken at intervals of 15 min. The rate of beat is affected by oxygen (*e.g.* at
14° C., 37 beats per minute in 5·2 c.c. oxygen per litre; 83 per minute in 1·45 c.c.
per litre and apnoea in 21·6 c.c. per litre); and by carbon dioxide (*e.g.* at 14° C.,
pleopods beating occasionally in pH 8·3; 75 beats per minute in pH 7·2; 150 beats
per minute in pH 6·6; occasional beats when returned to pH 8·3).

V. *IDOTEA NEGLECTA* G. O. SARS

Work on this isopod was begun in Birmingham with material sent from Plymouth
and was finished at the Plymouth laboratory. In all, 157 experiments were made,
involving sixty-five animals. The animals are found under rocks and stones and are
able to swim rapidly by means of the pleopods, but if provided with a suitable
foothold they will cling to it, usually in a supine position, and remain stationary for
long periods. Advantage was taken of this habit in the following way. An animal

was placed in a watch-glass and coarse bolting silk was stretched over it and fixed with rubber bands. The watch-glasses were put in a large dish filled with sea water. The animals cling to the silk upside down, and the movements of the pleopods can then easily be watched by transmitted light. The small animals were observed with the binocular, but for the large males this was unnecessary. The respiratory movements were recorded on a drum with a tapping key.

After an animal has been swimming, the respiratory movements are quick and regular. When it has been quiet for some time respiration tends to become intermittent; periods of apnoea, when the valves (uropods) are closed over the gills, alternate with shorter periods of rapid breathing (Fig. 1, T (1) and (2)).

Effect of carbon dioxide. The reaction to an increase of carbon dioxide can best be explained by reference to Fig. 1, which shows records of two animals, P and T. These and eight others were in separate watch glasses in a glass dish through which passed a slow stream of sea-water.

In animal P, a change of pH from 8·2 to 6·8 causes little change in rate of respiratory movement, but the gaps between the periods of beating are lengthened out. After 4 hours the effect of the change has worn off (compare reading 6 in pH 6·8 with 1 and 2 in pH 8·2). When the carbon dioxide content is still further increased (pH 6·4) there is at first complete inhibition (7) followed by continuous breathing (8), in which there is a slight slackening of rate after $2\frac{1}{2}$ hours (9). When the acid medium is replaced by fresh sea water at pH 8·2, (10) the number of beats per minute is the same as that at the beginning of the experiment (1 and 2) though the beats are more evenly spaced.

Animal T is more sensitive than P to pH 6·8, responding first with inhibition (3) then with rapid and regular breathing (4) which slackens already after $1\frac{1}{4}$ hours (5). In reading (6), pH 6·8 the breathing is practically the same as in (1) and (2) in pH 8·2. When the pH is lowered to 6·4 there is a period of inhibition lasting a few minutes (not recorded) followed by rapid, continuous breathing which is kept up for at least $2\frac{1}{2}$ hours (7–9). When the medium is changed again to pH 8·2, the rate returns to normal, (10).

Animal U, in the same experiment, showed the same response to the change from pH 8·2–6·8 as from 6·8–6·4, rapid regular breathing returning to normal after only $\frac{3}{4}$ hour.

It appears then that the effect of high concentrations of carbon dioxide on the respiratory movements is twofold: at first the movements are completely inhibited and then their rate is greatly increased. Intermittent respiration may perhaps be regarded as the result of a compromise between these two effects, the inhibition being periodically broken down; it is found at moderate concentrations of carbon dioxide, and precedes and follows dyspnoea in high concentrations. However, animals breathing intermittently in pH 8·2 do not breathe more regularly in pH 8·9.

The effect of oxygen. The effect of oxygen deficit is to increase the rate of respiratory movements; thus on decreasing the oxygen concentration from 3·6 c.c. per litre to 1·5 c.c. per litre the rate in one animal rose from 23 to 87 per minute;

in 15 c.c. oxygen per litre the pleopods beat only occasionally. The same individual variation is met with in the response to low oxygen tensions as to carbon dioxide excess; in some animals the high rate is kept up for 2–3 hours but in others it falls
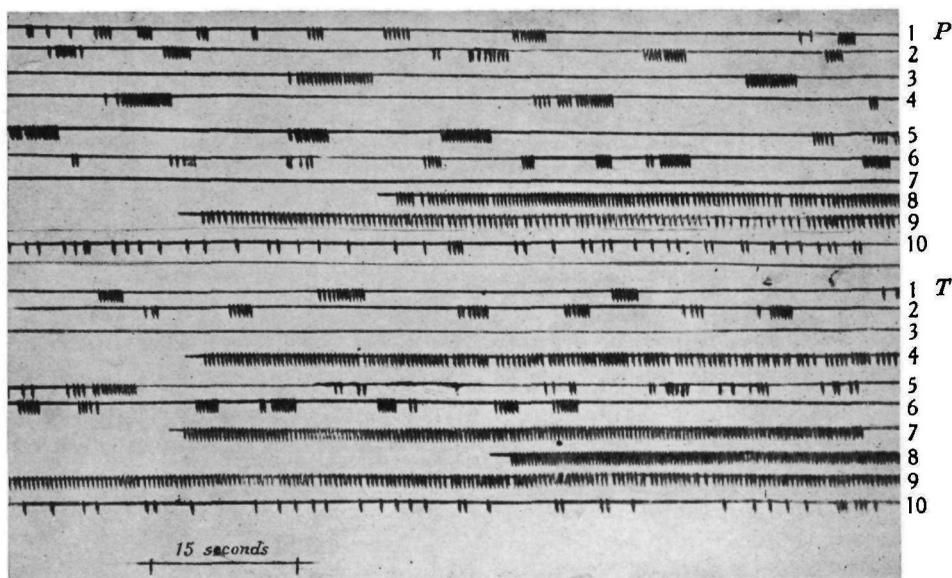

Fig. 1. Respiratory movements of two individuals, P and T, of *Idotea neglecta*, recorded on a drum with a tapping key. Temp. 16° C. Each vertical stroke represents one movement of the pleopods. The records should be read from right to left.

Reading	Time	Medium
1	10 a.m.	pH 8·2
2	10.30 ,,	pH 8·2
	Changed at 11.0 a.m.	
3	11.10 a.m.	pH 6·8
4	11.45 ,,	pH 6·8
5	12.15 p.m.	pH 6·8
6	3.15 ,,	pH 6·8
	Changed at 3.45 p.m.	
7	3.55 p.m.	pH 6·4
8	4.15 ,,	pH 6·4
9	6.15 ,,	pH 6·4
	Changed at 6.30 p.m.	
10	7.0 p.m.	pH 8·2

off; *e.g.* in one example at 18° C. the pleopods were beating at the rate of 30 beats per minute in 4·7 c.c. oxygen per litre and at 140 beats per minute in 2·5 c.c. oxygen per litre; this rate was kept up for 25 min., but had fallen to 94 per minute 40 min. after the change, and to 60 per minute after a further hour.

It is clear that decreasing the oxygen tension also increases the rate of beat as does excess of carbon dioxide but without an initial inhibitory phase. If respiration is of the intermittent type, lowering the oxygen tension will increase the rate by extending the length of the periods of beat—not by spacing out the beats. But the intermittent type is not imposed by oxygen lack on an animal breathing continuously as it is by carbon dioxide excess.

VI. *ANILOCRA PHYSODES* L. AND *NEROCILA BIVITTATA* RISSO

These isopods are found as ectoparasites on various fishes (*Corvina nigra, Crenilabrus pavo, Box boops, Pagellus erythrinus*) taken at a depth of 10–20 metres. They are sluggish animals and lie on their backs when removed from the host, moving the pleopods regularly. The experiments, of which twenty-four were done on *Anilocra* and thirteen on *Nerocila*, were made at Banyuls. Readings were taken with a stopwatch at intervals of 20 minutes, each reading being the average of 3 consecutive counts. Most experiments were done at 10° C. but some on the effect of oxygen excess were done at 22° C. so that any slackening would be more obvious. Both the rate and amplitude of beat are increased with temperature.

Low oxygen concentrations (1·7–0·6 c.c. per litre) do not increase the rate of beat of the pleopods even after 2 hours; in some cases there is at the lowest oxygen tensions a decrease of rate and amplitude. In many cases an increase of the oxygen concentration to 15 c.c. per litre had no effect but in other animals there was a lowering of rate and amplitude. Increasing the carbon dioxide concentration to *p*H 6·5 has no effect, but between *p*H 6·3–6·0 there is in most cases a decrease of rate and amplitude.

The reaction of *Anilocra* and *Nerocila* (Flabellifera) to oxygen and carbon dioxide thus seems to differ markedly from that of *Cirolana* belonging to the same suborder, and resembles that of *Ligia* (Oniscoidea).

VII. DISCUSSION

The results of this, as of the previous work, show that there is great variation in the response of respiratory movements to oxygen and carbon dioxide. Table II summarizes the data. It is clear that the distribution of chemical control does not follow the classificatory system. The insensitivity of *Balanus* and *Chirocephalus* can probably be considered primitive; in these animals the limbs concerned in forming a respiratory current are not specialized for this purpose. The insensitivity of *Carcinus, Ligia, Anilocra* and *Nerocila*, however, and the incompleteness of the control in *Gammarus locusta* and *Idotea*, may be secondary. It is possible that in these forms the loss or weakening of chemical control is associated with the littoral habitat. Thus there is no control in *Carcinus* and *Ligia*, which are semi-terrestrial. *Gammarus locusta* and *Idotea neglecta*, which are littoral forms, have an incomplete control, in that the quickened rate following an increase in carbon dioxide or decrease in oxygen tension is not kept up, but returns to that normal for aerated

water. Contrasting with these forms we find that *Pandalus* and *Cirolana* from deep waters are controlled by oxygen and carbon dioxide. *Nerocila* and *Anilocra* however do not fall into this grouping. These animals resemble *Ligia* in their failure to respond, and contrast strongly with the more closely allied *Cirolana*. Their insensitivity is correlated with a sluggish ectoparasitic habit.

The thoracic branchiae of *Squilla* are interesting in that, although vestigial in the adult, their movements are controlled by the concentration of the respiratory gases. A further point of interest is that these gills (epipodites of the maxillipedes) resemble the scaphognathite of the decapods (epipodite of the second maxilla) in that the organs of right and left side do not necessarily move synchronously, nor at the same rate.

Table II.

Group	Species	Habitat	Carbon dioxide	Oxygen
Class Branchiopoda	*Chirocephalus diaphanus*	Fresh water	—	—
Class Cirrepedia	*Balanus balanoides*	Littoral marine	—	—
Class Malacostraca				
Order Isopoda				
Suborder Asellota	*Asellus aquaticus*	Fresh water	—	Effective
Suborder Oniscoidea	*Ligia oceanica*	Littoral marine	—	—
Suborder Valvifera	*Idotea neglecta*	Littoral marine	Transient effect	Transient effect
Suborder Flabellifera	*Cirolana borealis*	100 m. marine	Effective	Effective
	Anilocra physodes	10–20 m. marine	—	—
	Nerocila bivittata	10–20 m. marine	—	—
Order Amphipoda	*Gammarus pulex*	Fresh water	Effective	Effective
	Gammarus locustra	Littoral marine	Transient effect	Transient effect
Order Decapoda	*Astacus fluviatilis*	Fresh water	—	Effective
	Pandalus borealis	Marine 100 m.	Effective	Effective
	Carcinus maenas	Littoral marine	—	—
Order Stomatopoda	*Squilla mantis*	Marine	Effective	Effective

VIII. SUMMARY

1. The movements of the pleopods and of the epipodites of the maxillipedes of the stomatopod *Squilla mantis* are quickened by lack of oxygen and excess of carbon dioxide.

2. The rate of beat of the scaphognathite of the prawn *Pandalus borealis* is increased by oxygen lack and carbon dioxide excess.

3. The rate of movement of the scaphognathite of the shore crab *Carcinus maenas* is very irregular and is apparently independent of the gas content of the medium.

4. The rate of respiratory movements of the pleopods of the isopod *Cirolana borealis* is increased in low oxygen and high carbon dioxide tensions.

5. Both carbon dioxide excess and oxygen lack increase the rate of pleopod movement of the isopod *Idotea neglecta*, but the effect is not always permanent. Great individual variation is shown both in the threshold of response, and the extent of recovery. Carbon dioxide excess at first inhibits respiratory movements.

6. The isopods *Anilocra physodes* and *Nerocila bivittata* show no significant response to changes in the gas content of the medium.

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