

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

BY H. MUNRO FOX AND M. L. JOHNSON.

(From the Zoology Department, University of Birmingham.)

(Received 11th March, 1933.)

(With Two Text-figures.)

### CONTENTS.

	PAGE
I. Introduction . . . . .	1
II. <i>Balamus</i> . . . . .	2
III. <i>Cheirocephalus</i> . . . . .	3
IV. <i>Astacus</i> . . . . .	3
V. Isopods . . . . .	4
VI. Amphipods . . . . .	5
VII. Conclusions . . . . .	8
VIII. Summary . . . . .	10
References . . . . .	10

### I. INTRODUCTION.

THE effects of an accumulation of carbon dioxide and of a lack of oxygen on the respiratory movements of mammals have been intensively studied (Winterstein, 1923; Gesell, 1925). In the invertebrates, however, although it is known for a number of species that one or both of these two factors are operative, our knowledge of the whole question is fragmentary.

Both an accumulation of carbon dioxide in the environment and a deficiency in oxygen accelerate the ventilation movements of the stick insect *Dixippus* (Buddenbrock and Rohr, 1922; Stahn, 1928) and those of the "Maikäfer" (Demoll, 1928). In the cockroach *Periplaneta* and other insects the stigmata, which are normally almost closed when the animal is at rest, open in response to small quantities of carbon dioxide; but only a great reduction in oxygen produces this effect on the cockroach's stigmata (Hazelhoff, 1927). Both carbon dioxide excess and oxygen lack accelerate the limb movements of the phyllopod crustacean *Branchipus*, which serve for respiration as well as for swimming and feeding (Babák, 1921). In the pulmonate molluscs *Arion*, *Limax* and *Helix*, an increase in carbon dioxide results in an enlargement of the respiratory aperture, while oxygen lack causes contractions of the lung wall (Dahr, 1924; Ysseling, 1930). In *Octopus* both a rise in carbon dioxide and a fall in oxygen tension increase the respiratory volume up to ten times its normal value, usually with little alteration in the frequency of the respiratory movements (Winterstein, 1925).

A rise in the tension of carbon dioxide in the sea water accelerates the respiratory rhythm of the abdominal appendages in the stomatopod crustacean *Squilla*, but a

fall in oxygen tension is without effect (Matula, 1912). Hazelhoff (1927) reports that the lung book apertures of certain spiders open in response to carbon dioxide, but he does not give the effect of oxygen lack.

Oxygen, but not carbon dioxide, affects respiratory movements in the following cases: (1) The extent to which the posterior end of the fresh-water oligochaete worm *Tubifex* projects above the mud and the frequency of its undulatory movements are inversely proportional to the oxygen content of the water, without being influenced by an accumulation of carbon dioxide (Alsterberg, 1922, 1924; Dausend, 1931). (2) Babák and Foustka (1907) found that ventilation movements of the beetles *Lucanus*, *Oryctes* and *Melolontha* are stimulated by oxygen lack alone (contrast Demoll above). (3) The rhythmic abdominal movements which pump water into and out of the rectum in the nymphs of dragon flies are, according to Babák and Foustka (1907), accelerated by oxygen deficit and inhibited by oxygen excess, while an increase in carbon dioxide causes no acceleration. Stahn (1928), however, also obtained a quickening of the respiratory movements of dragon-fly nymphs in response to a small increase in carbon dioxide tension.

It is clear that in different invertebrates one or other, or both, of the factors carbon dioxide excess and oxygen deficit can stimulate respiratory movements. Moreover, as will be shown below, in yet other cases neither carbon dioxide nor oxygen is effective in accelerating the respiratory rhythm. No reasons, physiological or ecological, are known for these differences, and we therefore thought it desirable to study the conditions prevailing in several members of one large group of animals. The Crustacea were chosen as being suitable for such an investigation, since they include forms from very different habitats—marine, intertidal, terrestrial, and from running and stagnant fresh waters. The results of the first part of this investigation are reported below.

Except in the case of barnacles, the various respiratory movements were recorded on a smoked drum by pressing a tapping key, in circuit with an electric time marker, each time that a movement was seen to take place (see Figs. 1 and 2).

The oxygen content of the water in which animals were studied was changed by siphoning off the greater part of the water and replacing it by water of lower or higher oxygen content. In this way the animals were not unduly disturbed. The water was covered with a layer of liquid paraffin, and samples were analysed by the Winkler method. The carbon dioxide content of the experimental water was altered by adding small quantities of water saturated with the gas: in this way the oxygen content was not appreciably changed. The pH of the water was determined colorimetrically. Precautions were taken to keep a uniform temperature throughout each experiment.

## II. *BALANUS*.

The branchiae of the acorn barnacle *Balanus* are a pair of plicated folds projecting into the mantle cavity. Water is pumped into and out of the mantle cavity by movements of the terga and scuta. The branchiae are closely attached to the terga, and when the latter move, the branchiae are agitated and their folds are drawn

apart, with the result that fresh sea water bathes the whole of the gill surface (Darwin, 1854).

Roaf (1912) found that oxygen lack (3.93 c.c. per litre) had no effect on the rate of respiratory movements of *Balanus balanoides*, and that carbon dioxide excess caused no acceleration of these movements.

In the present investigation, graphical records of the respiratory movements of *B. balanoides* were made on a smoked drum with the aid of a capillary glass lever connected to one of the scuta by a silk thread attached to the shell with dentist's cement. This method enabled the amplitude as well as the frequency to be studied. In none of the movements recorded did the cirri project: thus the movements may be considered as purely respiratory. Great care was taken to avoid interrupting the respiratory rhythm by mechanical or light stimuli.

It was found that neither oxygen lack nor carbon dioxide excess increases the rate or amplitude of respiratory movements. This agrees with Roaf's results. In one experiment the oxygen content of the sea water was successively lowered from 5.3 to 3.5, 2.4 and 0.9 c.c. per litre. No acceleration was caused by the low oxygen concentrations, but in 0.9 c.c. per litre the beats became irregular. When the oxygen content of the water was reduced to 0.25 c.c. per litre the movements stopped and the valves remained slightly gaping. In oxygenated water (11.7 c.c. per litre) there was no change in rate or amplitude of movements as compared with aerated water. The movements were unchanged in rate between pH 9 and 7 in waters made alkaline with sodium hydroxide and acid with carbon dioxide, but the amplitude was lower in the more acid waters, returning to normal when the pH was raised again to 8.

### III. CHEIROCEPHALUS.

The gills of *Cheirocephalus* are the epipodites of the limbs. The limbs beat rhythmically, causing a respiratory current to flow over the gills. This rhythmic movement also serves to sweep food to the mouth, to maintain the position of the animal in the water when stationary, or to move it along.

In contrast to the results of Babák (1921) with *Branchipus* (*vide supra*), we found that neither oxygen lack nor carbon dioxide excess accelerates the limb movements of *Cheirocephalus diaphanus*<sup>1</sup>. Water with a low oxygen content of 2.2 c.c. per litre caused no change in 1½ hours, and water with an abnormally high oxygen content was equally without effect; nor was the rhythm accelerated by gradually increasing the carbon dioxide content of the water up to a concentration which began to slow down the movements.

### IV. ASTACUS.

In the crayfish a current of water is kept moving over the gills from behind forwards by the rhythmic movements of the scaphognathite.

Babák and Foustka (1907) state that the frequency of the scaphognathite beat varies with the oxygen content of the water, but they give no data.

<sup>1</sup> The animals were kindly sent to us from Marlborough by Mr A. G. Lowndes.

The experiments reported here were made with *Astacus fluviatilis*. The animal was fastened on its back with its chelae extended to either side. Under these conditions the tip of the scaphognathite, protruding from the anterior opening of the branchial chamber, can be seen clearly, especially if a small piece of the branchiostegite is removed.

Considerable fluctuations occur in the frequency of the scaphognathite beat. After movements of the body and limbs, whether these are spontaneous or caused by irritating the animal, the rate becomes high and then slackens off. Spontaneous movements of body and limbs take place periodically, at intervals of about a quarter of an hour. The factors influencing the occurrence of these movements are at present unknown, but the oxygen and carbon dioxide tensions in the water appear to be without effect. In a typical case the scaphognathite rate in aerated water was 34 beats per minute ( $12^{\circ}$  C.). When suddenly the animal spontaneously moved its body and limbs, the rate of beat rose to 132. Afterwards the rate began to slow down, and 10 minutes later had returned to its initial value.

Oxygen has a marked effect on the rate of scaphognathite movement. In a typical experiment ( $12^{\circ}$  C.), on lowering the oxygen concentration from 8.3 c.c. to 2.2 c.c. per litre, the rate was accelerated from 34 to 140 per minute. This more rapid rhythm was maintained for 1 hour<sup>1</sup> in the lowered oxygen tension. When the oxygen content of the water was then raised to 10.6 c.c. per litre the scaphognathite ceased to beat except when the animal moved.

Changing the carbon dioxide content of the water was found to have no accelerating effect on the scaphognathite rhythm. The alkali reserve of the water was 0.0025N and its pH was lowered progressively from 8.1 to 6.1 by the addition of carbon dioxide. No increase in scaphognathite rate could be detected whether the change was made slowly or suddenly, and in some cases the rate decreased in the more acid media.

## V. ISOPODS.

The respiratory organs of isopods are the lamelliform abdominal pleopods, which, in aquatic forms, move rhythmically.

(a) *Asellus aquaticus*. Oxygen tension was found to affect the rate of respiratory movements in this fresh-water species. In many animals the pleopods were motionless in fully aerated water but they beat when the oxygen content was reduced. In one experiment, for example, when the oxygen concentration was lowered to 3.36 c.c. per litre there was still no movement, at 1.12 c.c. there was slow and discontinuous movement, the average rate of which was 52 beats per minute, while at 0.84 c.c. a rapid and regular rhythm of 148 per minute was kept up for 3 hours ( $12^{\circ}$  C.). In other cases the pleopods moved in aerated water, but the rate of beat was increased by reducing the oxygen concentration.

Carbon dioxide, on the other hand, has no effect on respiratory movements. A gradual increase in the carbon dioxide content of tap water was without effect on the animals, nor was there any initiation or acceleration of movements as a result

<sup>1</sup> In other experiments, 3 hours.

of changing the  $pH$  of a  $0.0044N$  sodium bicarbonate solution from 8.5 to 6.4 by the addition of carbon dioxide.

(b) *Ligia oceanica*. This animal is chiefly terrestrial, living in rock crevices on the sea coast above high-tide mark, but it is also found in rock pools. The animals used in our experiments came from Plymouth. Tait (1917) reports that *Ligia* can stand immersion in sea water for as long as 83 days. We have watched animals in air (dry or water-saturated), in an atmosphere of nitrogen, and in presence of excess of carbon dioxide, without being able to detect any respiratory movements. As soon, however, as the body, or only the end of the abdomen, is submerged under water the pleopods begin to beat rhythmically.

When the oxygen tension of the water is raised above that in equilibrium with the atmosphere, the rate of pleopod movement becomes slower, with pauses interrupting the previously continuous rhythm, and the amplitude is less than normal. These symptoms are reversible when the animals are replaced in air-saturated water. In other words, diminution in oxygen tension accelerates the respiratory movements. But this acceleration does not continue when the oxygen content of the water is still further reduced. From air-saturated water down to water with an oxygen content of 2 c.c. per litre, the rate of beat is unchanged, and below 2 c.c. per litre the rate slows and the amplitude diminishes. Thus in the life of the animal there is no respiratory control by oxygen deficiency.

Carbon dioxide excess does not accelerate the respiratory movements of *Ligia*. The  $pH$  of the sea water in which the animals were observed was gradually lowered to 6.4 by the addition of carbon dioxide, but the respiratory rhythm in the acid water was either unchanged or very slightly slowed.

#### VI. AMPHIPODS.

The branchiae of amphipods are borne on the thoracic legs, but respiratory movements are performed by the abdominal pleopods, which draw a current of water over the branchiae. The pleopods are also used for swimming: the animals were therefore kept quiet for observation so that the movements recorded were mainly respiratory. Locomotion was restricted by putting a watch glass, notched in three places at its edge, over the animal.

(a) *Gammarus pulex*. This is a fresh-water species. Water from the stream in which the animals were found was used in the experiments. Its alkali reserve was  $0.001N$ .

Lowering the oxygen content of the water was found to accelerate the respiratory rhythm. Fig. 1 A shows this result in a typical experiment. In water containing 6.7 c.c. oxygen per litre the limbs rarely moved. On lowering the oxygen content to 2.8 c.c. the rate of movement gradually rose and remained high  $1\frac{1}{4}$  hours after the change had been made. After this the oxygen was raised to its initial concentration and the rate diminished again. In other experiments the accelerated rhythm in water of low oxygen content was maintained for as long as  $2\frac{1}{4}$  hours.

Excess of carbon dioxide also increased the rate of pleopod beat. Fig. 1 B shows a typical case. Lowering the  $pH$  from 7.6 to 6.7 by the addition of carbon dioxide

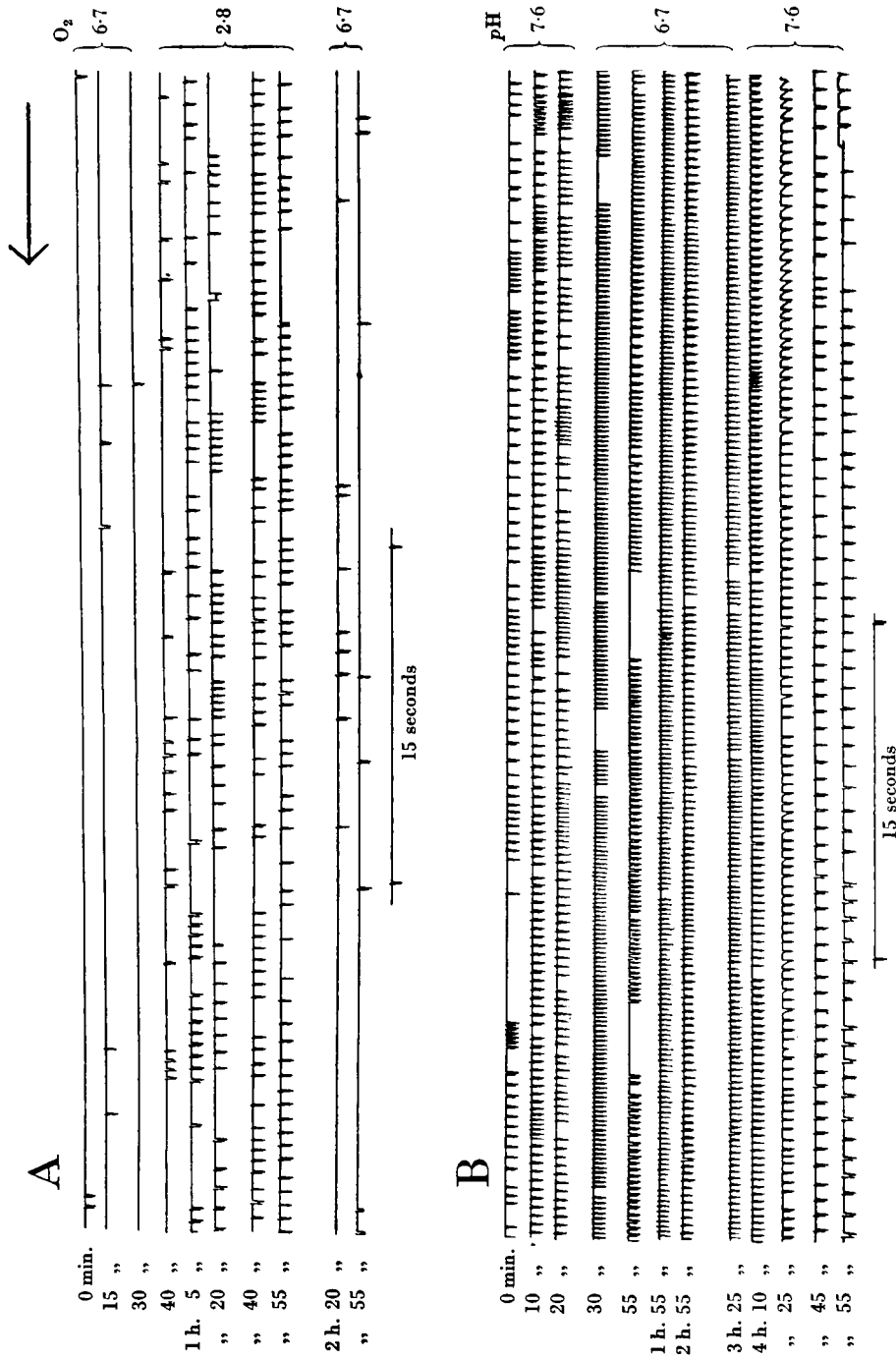


Fig. 1. Records of the respiratory movements of the pleopods of *Gammarus pulex* in fresh water containing various amounts of dissolved oxygen (A) and carbon dioxide (B). Each vertical stroke represents one movement of the pleopods. Times from the beginning of the experiments are given on the left of each record. In A oxygen concentrations (c.c. per litre) and in B pH values are given on the right of the records. Temperature 10°.

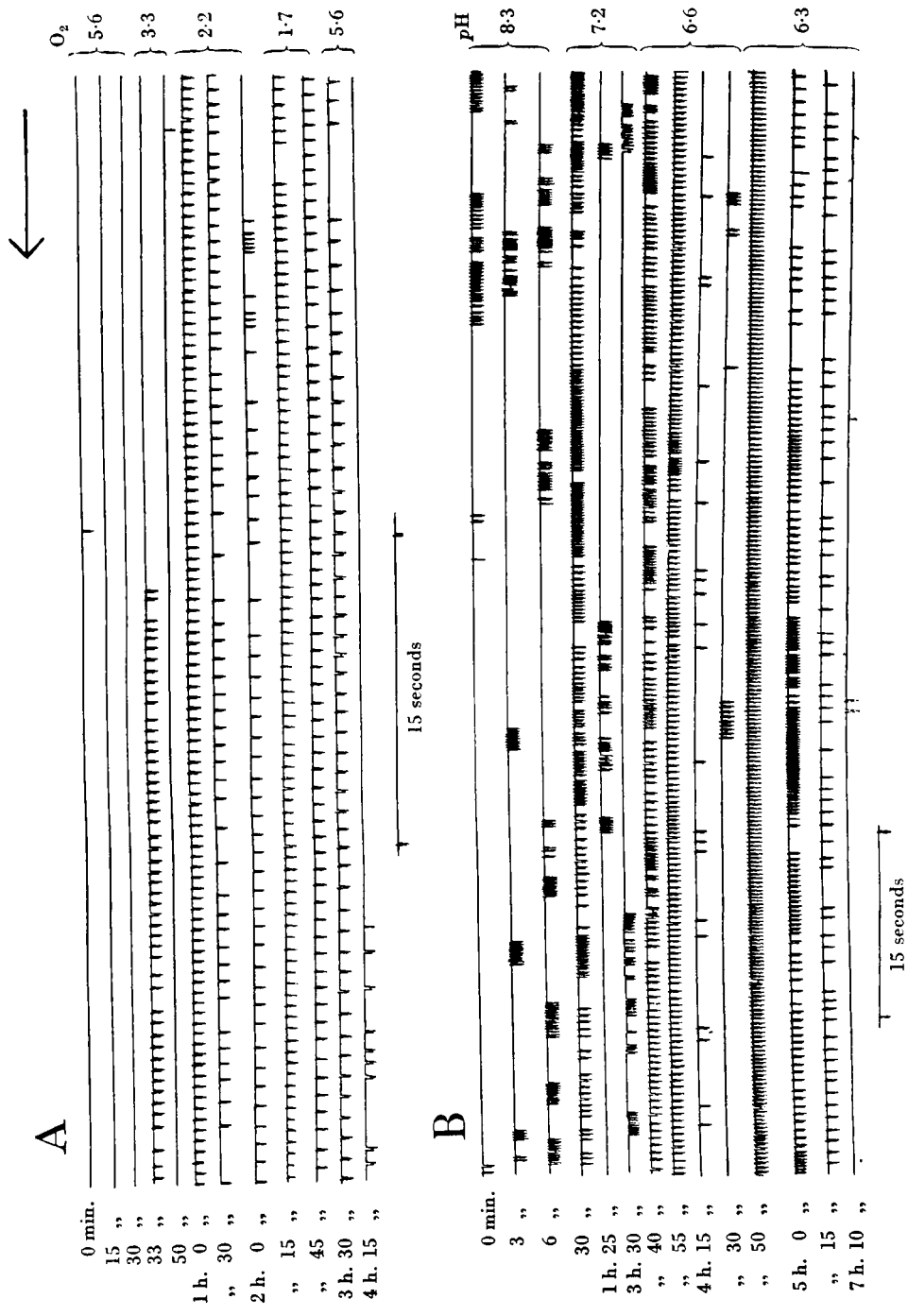


Fig. 2. Records of the respiratory movements of the pleopods of *Gammarus locusta* in sea water containing various amounts of dissolved oxygen (A) and carbon dioxide (B). Times from the beginning of the experiments are given on the left of each record. In A oxygen concentrations (c.c. per litre) and in B pH values are given on the right of the records. Temperatures: A, 9°; B, 6.5°.

increased the rate of respiratory movements, and the accelerated rate was maintained for 2 hr. 55 min. After this the acid water was replaced by water at pH 7.6 and the initial rate was gradually (but not immediately) resumed.

(b) *Gammarus locusta*. This marine species came from Plymouth. It is common under rocks and among weeds between tide marks, and occurs down to 6 fathoms.

The result of lowering the oxygen content of the sea water is that the pleopods beat if previously they are still, or increase their rate if previously they were beating. The accelerated rate, however, is not long maintained. Fig. 2 A records a typical experiment. In water containing 5.6 c.c. oxygen per litre the pleopods were still. On replacing this by water with 3.3 c.c. oxygen per litre a rapid rhythm was induced, but 17 minutes after the change the pleopods rarely moved. On reducing the oxygen concentration successively to 2.2 c.c. and then to 1.7 c.c. per litre the result was the same: each change caused a rapid rate of beat which subsequently slowed down. The response to changed oxygen concentration may not be immediate: this is seen in Fig. 2 A for the changes from 5.6 to 3.3 c.c. and from 1.7 to 5.6 c.c. per litre.

Excess of dissolved oxygen, above the amount in equilibrium with air, inhibits respiratory movements.

Just as the respiratory rhythm is accelerated by lowering the oxygen tension and not by its absolute value, so too, when the carbon dioxide content of the water is raised, each successive increase results in an accelerated rhythm, which subsequently slows down. Fig. 2 B shows a typical case. At pH 8.3 the pleopods moved only when the animal swam. On successively lowering the pH to 7.2, 6.6 and 6.3, each change resulted in an accelerated rhythm which gradually slowed down.

The subsequent slackening of the accelerated pleopod beat of *G. locusta* in water with lowered oxygen or raised carbon dioxide concentration is not a sign of injury, for the animals were perfectly normal when replaced in water saturated with air (Fig. 2 A).

## VII. CONCLUSIONS.

Among the Crustacea studied there is great diversity in the response of the respiratory appendages to oxygen deficiency and carbon dioxide excess. *Cheirocephalus* and *Balanus* are unaffected by the gas content of the water, *Asellus*, *Ligia*, and *Astacus* respond to changes in oxygen concentration alone, while the two species of *Gammarus* are sensitive to both gases. What may be the physiological causes or the ecological significance of these differences, and of the similar differences of respiratory control in other animals quoted in the introduction to this paper, we do not know.

It is not known for the invertebrates whether an environmental increase in carbon dioxide or decrease in oxygen pressure produces its stimulating effect by altering the gas tensions, and perhaps eventually the hydrogen-ion concentration, of the blood, or whether respiratory centres are influenced by nervous reflexes arising from peripheral chemical receptors. Owing to the small size of most of the animals no perfusion experiments have been made, nor have changes in the blood been directly studied, except for the hydrogen-ion concentration in *Octopus* (Winterstein,



1925). Regarding the possibility of reflex respiratory control, there is evidence that *Tubifex* possesses oxygen receptors (Alsterberg, 1924), while Hazelhoff (1927) concludes that carbon dioxide acts locally on the spiracles of *Periplaneta*, and Stahn (1928) believes that there are carbon dioxide receptors on the tracheal walls in *Dixippus*.

Much more experimental work is required before we can know why the environmental factors influencing the rate of respiratory movements vary in different invertebrates, and how these stimuli are transmitted to the respiratory nerve centres. Nevertheless, certain interesting conclusions arise out of the work described above.

In the case of the amphipods, whatever may be the mechanism through which environmental oxygen deficit and carbon dioxide excess react on the animals, it seems likely that it is one and the same mechanism for the two factors, since the effects are so closely similar. This is particularly so in *Gammarus locusta*, where the effect gradually wears off after each change in gas content of the water (see Fig. 2). It is unlikely, moreover, that the mechanism here is a nervous reflex, since there is a considerable latent period before the response appears. This is shown in the change from pH 6.7 to 7.6 in Fig. 1 B, and in the changes from 5.6 to 3.3 and from 1.7 to 5.6 c.c. oxygen per litre in Fig. 2 A<sup>1</sup>. It is possible that both environmental carbon dioxide excess and oxygen lack act on the respiratory centre of amphipods through a rise in hydrogen-ion concentration of the blood, oxygen deficiency causing an accumulation of acid metabolites due to incomplete oxidation. Nevertheless, oxygen deficiency cannot thus produce its effect in all animals, for we found that carbon dioxide is without influence in the crayfish and in isopods, whereas oxygen lack accelerates their respiratory movements. The same is true for *Tubifex* (Alsterberg, 1922, 1924; Dausend, 1931).

The two species of amphipods studied differ in that the respiratory control is considerably more efficient in the fresh-water *Gammarus pulex* than in the marine *G. locusta*. In the two species both oxygen deficiency and carbon dioxide excess accelerate the respiratory rhythm, but whereas in *G. pulex* dyspnoea is permanent at each gas tension, in *G. locusta* it is transient only (cf. Figs. 1 and 2). This means that the respiratory control is more efficient in the former than in the latter species. Now, it has been shown by Fox and Simmonds (1933) that the oxygen intake of fresh-water amphipods is considerably greater than that of their marine relatives; in particular *G. pulex* consumes twice as much oxygen per gram per hour as *G. locusta*. It is interesting to find, therefore, that the former animal possesses a more efficient control of its respiratory movements than does the latter.

In both of the isopods *Asellus* and *Ligia* oxygen excess inhibits respiratory movements, but there is a difference between the two cases. In *Asellus* the rate of pleopod movements increases as the oxygen content of the water diminishes. This acceleration of the rate of beat goes on increasing until the oxygen tension is much below that of water saturated with air. In *Ligia*, however, although it is true that the pleopods beat faster in aerated water than in water through which oxygen has

<sup>1</sup> The reason why no such latent period is seen for the other changes of oxygen or pH in Figs. 1 and 2 is that the records reproduced were not made soon enough after the changes.

been bubbled, yet when the oxygen tension is reduced below that in equilibrium with air there is no further acceleration of the rhythm. In other words there is a biological control of the rate of beat in *Asellus* but not in *Ligia*. *Ligia*, however, is really a semi-terrestrial animal and it may well be that it has lost some of its adaptations to aquatic life.

#### VIII. SUMMARY.

1. The respiratory movements of the barnacle *Balanus* and of the phyllopod *Cheirocephalus* are not accelerated either by decreased oxygen or by increased carbon dioxide tension in the water.

2. The rate of movement of the scaphognathite of the crayfish *Astacus* is accelerated by a fall in oxygen but not by a rise in carbon dioxide tension of the environment.

3. The rate of movement of the pleopods of the fresh-water isopod *Asellus* is accelerated by a fall in oxygen but not by a rise of carbon dioxide tension.

4. In the sea-shore isopod *Ligia*, submerged in sea water, the rate of pleopod movement is not accelerated by a decrease in oxygen tension below that in equilibrium with air, but a rise in oxygen tension above the latter value slows respiratory movements. Carbon dioxide has no accelerating effect.

5. Both a decrease in oxygen and an increase in carbon dioxide tension accelerate the respiratory pleopod movements of the amphipods *Gammarus pulex* and *G. locusta*.

6. Whereas in the fresh-water *G. pulex* the quickened respiratory rhythm is permanent at each decreased oxygen and increased carbon dioxide tension, in the marine *G. locusta* these changes in rate of beat are transitory. This is correlated with the greater oxygen consumption of the marine species.

#### REFERENCES.

- ALSTERBERG, G. (1922). *Lunds Univ. Årssk.* N.F. **18**, No. 1.  
 — (1924). *Lunds Univ. Årssk.* N.F. **20**, No. 7.  
 BABÁK, E. (1921). In Winterstein's *Handb. der vergl. Physiol.* **1**.  
 BABÁK, E. and FOUSTKA, O. (1907). *Pflügers Arch.* **119**, 530.  
 BUDDENBROCK, W. VON and ROHR, G. VON (1922). *Z. allg. Physiol.* **20**, 111.  
 DAHR, E. (1924). *Lunds Univ. Årssk.* N.F. **20**, No. 10.  
 DARWIN, C. (1854). *A Monograph of the Cirripedia*.  
 DAUSEND, K. (1931). *Z. vergl. Physiol.* **14**, 557.  
 DEMOLL, R. (1928). *Z. Biol.* **87**, 8.  
 FOX, H. M. and SIMMONDS, B. G. (1933). *Journ. Exp. Biol.* **10**, 67.  
 GESELL, R. (1925). *Physiol. Rev.* **5**, 551.  
 HAZELHOFF, E. H. (1927). *Z. vergl. Physiol.* **5**, 179.  
 MATULA, J. (1912). *Pflügers Arch.* **144**, 109.  
 ROAF, H. E. (1912). *Journ. Physiol.* **43**, 449.  
 STAHN, I. (1928). *Zool. Jahrb.* **46**, 1.  
 TAIT, J. (1917). *Proc. Roy. Soc. Edin.* **37**, 50.  
 WINTERSTEIN, H. (1923). *Naturwissenschaften*, **11**, 625, 644.  
 — (1925). *Z. vergl. Physiol.* **2**, 315.  
 YSSELING, M. A. (1930). *Z. vergl. Physiol.* **13**, 1.