

BREATHING MOVEMENTS IN *ENTOBDELLA SOLEAE* (TREMATODA, MONOGENEA) FROM THE SKIN OF THE COMMON SOLE

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(Text-figs. 1-3)

During August 1960 and April 1961, a number of living specimens of *Entobdella soleae* (van Beneden & Hesse, 1863) Johnston, 1929, were recovered from the blind (= 'ventral') surfaces of common sole (*Solea solea* L.) caught at Plymouth. A characteristic undulating movement of the flattened leaf-like body was noticed in all individuals examined. No previous record of this movement is available for *Entobdella soleae*, but in *E. squamula* from the bastard halibut (*Paralichthys californicus*) of the Pacific, Heath (1902) noted very briefly that body undulations occurred, while both extremities of the animal were attached, and that these movements probably aided the process of respiration. Heath, however, produced no evidence to support his suggestion, and the purpose of the present work is to investigate the nature and function of the undulating movements.

THE UNDULATING MOVEMENTS

Undulating movements were observed, with the aid of a stereo-microscope, in specimens of *Entobdella soleae* from 2 to 6 mm in length, kept in sea water in a glass vessel. On removal from the fish a parasite lived for 2 or 3 days in filtered, fresh sea water and continued to undulate until it became moribund (i.e. until the animal could no longer attach itself to the surface of the vessel). However, even in this condition the parasite continued to move the detached haptor (= the adhesive organ; see Fig. 1, A, B) rhythmically. In order to examine *E. soleae* while attached to a living fish, a sole was kept in a glass-bottomed tank just large enough to accommodate it, and a mirror system, similar to that used by Smyth (1946) for the study of tapeworms *in vitro*, was employed to view the blind surface. Undulating movements, similar to those in detached specimens, were seen.

The movement consisted of a wave passing always from posterior to anterior along the parasite, with the wave front transverse with respect to the body. In life the parasite always attached itself with the haptor nearer to the anterior end of the host, and therefore the wave passed antero-posteriorly

with respect to the fish. At 10°C the rate of undulation in normally oxygenated sea water (i.e. water containing about 6 ml. of oxygen per litre) was approximately thirty-five waves per minute (average of ten readings), and under stable conditions a particular behaviour pattern was usually maintained by one parasite for long periods, although different animals under the same conditions often showed very different behaviour. For instance, the undulations were sometimes continuous, but in many animals they were broken by pauses, normally of 1-2 sec, in which the waves ceased and the body contracted back slightly on the peduncle (= the stalk uniting the haptor and body; see Fig. 1, B). Pauses occurred at fairly regular intervals, but they often disappeared for some time; the number of waves between pauses varied from 3 to 40. A second type of interruption in the movement occurred less commonly and lasted 30 sec or more, during which the anterior extremity of the body actively searched the surrounding water. Often a large parasite undulated at a slower rate than a small one under the same conditions but this was not always so. Again the amplitude of the waves varied very considerably between different animals in the same conditions.

Temperature changes did not affect the form of the waves, but their frequency was approximately doubled by a rise in temperature of 10°C .

THE EFFECT OF THE UNDULATIONS ON THE AMBIENT SEA WATER

The parasite attached itself to the bottom of a glass vessel containing normally oxygenated sea water. In order to investigate possible water movements produced by the body undulations, droplets of a suspension of fine carmine particles were placed close to the body margin of the parasite with a microsyringe. The attitude of the parasite appeared to be similar to that adopted on the host in that the leaf-like body was closely applied to the glass with the haptor firmly attached. The thin edge of the body formed a curtain which was raised as the wave began at two points, one on each side of the peduncle in the postero-lateral regions (Fig. 1, A, B, *f*). At these two points, but at no other position around the curtain, carmine suspension was drawn rapidly beneath the body, into the cavity between the animal and the substrate (Fig. 1, B, *c*). This cavity was closed posteriorly by the haptor and peduncle, and sealed peripherally by the body curtain. The wave then passed forward, flushing the carmine suspension out in the 'shoulder' region of the animal (B, *B'*). Some carmine particles were seen emerging from beneath the head.

Since the water currents passed beneath the parasite, the glass vessel was sealed and inverted so that the ventral surface of the animal could be examined. Very small, light carmine particles in the water drifted upwards around the animal and were drawn in by the waves. The paths of these currents are illustrated in Fig. 1, A, B. *AB* and *A'B'* represent strong laterally

situated currents, which carried most of the carmine particles forward and out at the shoulder region. Alternatively, some particles were deflected and passed across the mouth and out beneath the head (AC and $A'C'$) but although the mouth was seen to open occasionally, no particles entered. Sometimes a leakage of particles along the lateral edge of the body occurred (AD and $A'D'$). Particles passed regularly to the centre of the posterior end of the animal (AE and $A'E$). This was the highest point in the cavity beneath the body of the parasite and a great deal of turbulence was observed here. Particles from this region were eventually pushed forward in a rather jerky manner and expelled beneath the head and shoulder regions (EF).

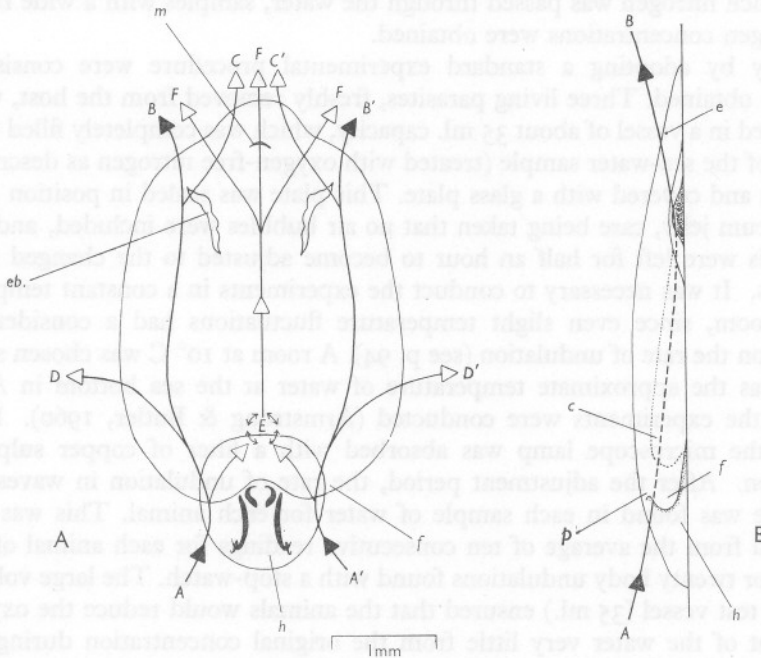


Fig. 1. Diagrams of the paths of water currents produced by undulations of the body in *Entobdella soleae*. A. Ventral view of the parasite. B. Lateral view, showing the path of the main water current only. See text for explanation of water currents. *c*, cavity between the parasite and the substrate; *e*, eye; *eb*, excretory bladder; *f*, postero-lateral projecting flap of the body; *h*, haptor; *m*, mouth; *p*, peduncle.

THE EFFECT ON THE UNDULATING MOVEMENTS OF CHANGING THE CONCENTRATIONS OF RESPIRATORY GASES IN THE MEDIUM

It seemed quite likely that the undulating movements of *Entobdella soleae* might have a breathing function and this possibility was investigated by varying the amounts of dissolved respiratory gases in the ambient sea water.

The effects were examined of, first, decreased content of dissolved oxygen, and, secondly, excess dissolved carbon dioxide, on the rate of undulation.

The effect of lack of oxygen on the undulating movements

Two methods were used to lower the oxygen content of sea water. First, the pressure above a sample of fresh sea water was reduced by means of a filter pump, and secondly, oxygen-free nitrogen was bubbled through the sample. The second method was found to be the more convenient since it was possible to reduce the oxygen content of half a litre of sea water to a very low level (less than 1 ml. per litre) in only 10 min. By varying the length of time for which nitrogen was passed through the water, samples with a wide range of oxygen concentrations were obtained.

Only by adopting a standard experimental procedure were consistent results obtained. Three living parasites, freshly removed from the host, were enclosed in a vessel of about 35 ml. capacity, which was completely filled with some of the sea-water sample (treated with oxygen-free nitrogen as described above) and covered with a glass plate. This plate was sealed in position with petroleum jelly, care being taken that no air bubbles were included, and the animals were left for half an hour to become adjusted to the changed conditions. It was necessary to conduct the experiments in a constant temperature room, since even slight temperature fluctuations had a considerable effect on the rate of undulation (see p. 94). A room at 10° C was chosen since this was the approximate temperature of water at the sea bottom in April when the experiments were conducted (Armstrong & Butler, 1960). Heat from the microscope lamp was absorbed with a filter of copper sulphate solution. After the adjustment period, the rate of undulation in waves per minute was found in each sample of water for each animal. This was calculated from the average of ten consecutive readings for each animal of the time for twenty body undulations found with a stop-watch. The large volume of the test vessel (35 ml.) ensured that the animals would reduce the oxygen content of the water very little from the original concentration during the course of the experiment, and it was assumed that the results from the experiments with water samples of different oxygen contents would be closely comparable. The oxygen content of each sample was determined by the macro-Winkler method (Ministry of Agriculture, Fisheries and Food, 1954).

Table 1 illustrates the results of all experiments in which animals were subjected to changing oxygen concentrations. It can be seen that on forty-two occasions (involving, in all, nineteen animals) out of a total of fifty-four, in each of which a parasite was transferred to water of lower oxygen content or to water of higher oxygen content, the rate of body undulation increased or decreased, respectively. A sample experiment, showing the changes in the rates of body undulations in three individuals of *E. soleae* exposed together to changing oxygen concentrations, is illustrated graphically in Fig. 2. This

graph clearly shows that as the oxygen content of the medium decreased, an increase in the rate of body undulation occurred.

During the course of these experiments it became evident that the parasite was able to adjust itself to changing oxygen conditions not simply by

TABLE 1. SUMMARY OF RESULTS OF ALL EXPERIMENTS IN WHICH ANIMALS WERE EXPOSED TO CHANGING OXYGEN CONDITIONS

Specimen no.	Sea water deoxygenated by means of a filter pump			Specimen no.	Sea water deoxygenated by means of nitrogen		
	+	.	.		+	-	+
1	+	.	.	11	+	-	+
2	+	.	.	12	+	-	+
3	+	.	.	13	+	-	+
4	+	+	.	14	+	+	+
5	+	+	.	15	+	+	+
6	+	.	.	16	-	+	+
7	+	.	.	17	+	+	+
8	+	-	-	18	+	+	+
9	+	-	+	19	-	+	+
10	+	-	+				

+ Indicates that when the animal was placed from water of high to low or low to high oxygen content the rate of undulation increased or decreased, respectively.

- Indicates that when the animal was treated as above the rate of undulation decreased or increased, respectively.

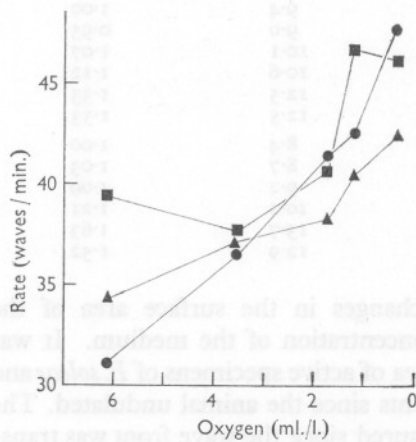


Fig. 2

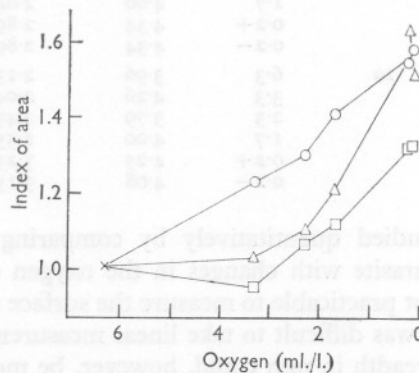


Fig. 3

Fig. 2. The relationship between the rate of body undulation and the oxygen content of the medium in three individuals of *Entobdella soleae*. ▲, Specimen no. 14 (see Table 1); ●, no. 15; ■, no. 16. The approximate lengths of these specimens in sea water containing 6.3 ml. of oxygen per litre were 4, 5 and 4 mm, respectively.

Fig. 3. The relationship between 'surface area' (see p. 98) and the oxygen content of the medium in three individuals of *Entobdella soleae*. ○, Specimen no. 17. □, No. 18. △, No. 19. Values of the 'surface area' are represented as a proportion of the 'surface area' of the animal in sea water containing 6.3 ml. of oxygen per litre (\times on graph).

changing the rate of undulation alone, but by a complex of changes. An animal in sea water of high oxygen concentration (e.g. 10 ml. of oxygen per litre) showed a low rate of undulation with frequent pauses distributed regularly, and a small wave amplitude. On the other hand, a parasite in water of low oxygen content (e.g. 1 ml. per litre) undulated very rapidly with no pauses and had a large wave amplitude. It also appeared that the exposed surface area of the body of the parasite was greater and the thickness much smaller under conditions of low oxygen content than under conditions of high concentration. 'Wrinkles', visible on the surface of the body in high oxygen concentrations, were no longer seen, and the internal organs, blurred in outline in high oxygen conditions, were sharply defined. This phenomenon was

TABLE 2

Specimen no.	O ₂ content (ml./l.)	'Length' (mm)	Breadth (mm)	'Length' × breadth = index of surface area	'Surface area' as a proportion of that in sea water with an O ₂ content of 6.3 ml./l.
17	6.3	5.07	2.93	14.9	1.00
	3.3	5.36	3.40	18.2	1.23
	2.3	5.53	3.49	19.3	1.30
	1.7	5.62	3.74	21.0	1.41
	0.2+	5.79	3.91	22.6	1.53
	0.2-	5.87	4.00	23.5	1.58
18	6.3	4.17	2.26	9.4	1.00
	3.3	3.91	2.30	9.0	0.95
	2.3	3.96	2.55	10.1	1.07
	1.7	4.00	2.64	10.6	1.12
	0.2+	4.34	2.89	12.5	1.33
	0.2-	4.34	2.89	12.5	1.33
19	6.3	3.96	2.13	8.4	1.00
	3.3	4.26	2.04	8.7	1.03
	2.3	3.79	2.43	9.2	1.09
	1.7	4.00	2.55	10.2	1.21
	0.2+	4.25	3.23	13.7	1.63
	0.2-	4.08	3.15	12.9	1.52

studied quantitatively by comparing changes in the surface area of the parasite with changes in the oxygen concentration of the medium. It was not practicable to measure the surface area of active specimens of *E. soleae* and it was difficult to take linear measurements since the animal undulated. The breadth in mm could, however, be measured since the wave front was transverse with respect to the body, but an accurate measure of the length could not be obtained because the body was at no time completely flat along the longitudinal axis. Therefore, an index of length was found by measuring the maximum distance from the posterior extremity of the haptor to the anterior extremity of the head. It was then assumed that the product of 'length' and breadth was proportional, for comparative purposes, to the surface area of the animal (see Table 2). The results are illustrated graphically in Fig. 3, where the index of surface area of each animal under each set of conditions is

expressed as a proportion of the surface area of the same animal in sea water containing 6.3 ml. of oxygen per litre (see Table 2). It can be seen from the graph that a decrease in the oxygen concentration of sea water results in an increase in the exposed surface area of the animal.

Values of the rate of undulation for each animal under each set of conditions were also obtained during the course of this experiment and were found to be very closely comparable with the results in Fig. 2.

The effect of excess carbon dioxide on the undulating movements

The carbon dioxide content of the medium was increased by passing the gas, obtained from a Kipp's apparatus and tested to ensure that it contained no acid impurities, into sea water. Fresh, living animals were placed in the sample and observed. It was found that carbon dioxide, even very little in excess of that dissolved in fresh sea water (i.e. sufficient to change the pH from 7.8 to 6.0) almost immediately had a strong narcotic effect on the parasites, the rates of undulation decreasing rapidly. The animals then detached from the substrate and contracted considerably. Recovery was rapid in fresh sea water.

Similar behaviour was observed when attempts were made to deprive animals of oxygen by sealing them in a very small glass container (about 5 ml. capacity) and allowing them to exhaust the water of oxygen by their own respiratory activity. Possibly the carbon dioxide produced by the respiratory processes and other products of metabolism accumulated in the confined space and produced narcosis.

OBSERVATIONS ON OTHER SKIN-PARASITIC ANIMALS AT PLYMOUTH

Since *E. soleae* is a parasite of the skin of a fish (*Solea solea*) other skin parasites (particularly monogeneans) of Plymouth fishes were examined for undulating movements. All parasites were observed in normally oxygenated sea water in a glass vessel, and no attempt was made to vary the oxygen concentration of the water.

Undulations were observed in three species of skin-parasitic Monogenea from Plymouth: first in a species of *Acanthocotyle* common on the ventral surface of *Raia clavata*, secondly, in a single specimen of *Pseudocotyle squatinae* from the ventral skin of *Squatina squatina*, an elasmobranch flat-fish rarely caught at Plymouth, and lastly in *Leptocotyle minor* common on the skin of a dogfish (*Scyliorhinus canicula*). In *Acanthocotyle* the strap-shaped body was not closely applied to the substrate as in *Entobdella soleae* and the movement was mainly confined to the posterior third of the body. At 18° C animals of about 4 mm in length produced 63.2 waves per minute (average of five readings). The individual of *Pseudocotyle squatinae* was about 6 mm long and

produced 17.4 undulations per minute at 18° C (average of three readings), but the specimen was moribund since it had been separated from the host for a long period. In *Leptocotyle minor* the movement consisted of a gentle oscillation of the whole body, which was itself kept quite rigid, about the attached haptor. The rate of undulation in an individual about 3 mm long was found to be 29.2 waves per minute at 17° C (average of seven readings), and the movement was punctuated at intervals of about 1 min by short periods of searching activity.

Undulations were not observed in three living individuals of an unidentified, parasitic leech, collected from the ocular (= 'dorsal') surface of *Solea solea*.

DISCUSSION

It is concluded that in *Entobdella soleae* the body undulations almost certainly function as an aid to respiration. This conclusion is strongly supported since the undulating movements produced continuous currents which followed definite paths across the ventral surface of the parasite (Fig. 1) and the rate of these undulations increased when the oxygen concentration of the environment was lowered and, conversely, decreased when the oxygen content was raised (Fig. 2).

It was thought possible that the current produced by the movements supplied the parasite with food. However, during the experiments on the effects of the undulations on the ambient water (p. 94), although carmine particles were often carried across the mouth of the parasite, none were seen to enter. Similarly, it was thought that the current could serve to carry excretory products away from the animal, but, first, the excretory pores are dorsal and the current produced by the parasite passed across the ventral surface of the body, and secondly, expulsion of material from the excretory bladders has been seen to be sporadic, whereas the current produced by the undulating movements was continuous.

The occurrence of breathing movements in *E. soleae* indicates that the sea water surrounding the parasite in its natural habitat, attached to the blind surface of a bottom-living flat-fish, is depleted of oxygen and stagnant. Conditions of abnormally low oxygen concentration appear to prevail over large areas of the ocean bottom, particularly in the sediment but also often above it if there are no disturbing bottom currents. (Present knowledge on the oxygen content of marine sediments and bottom waters has been summarized by Perkins, 1957 and Richards, 1957.) It is also significant that *Solea solea* spends most of the day buried (except for the eye-bearing surface of the head) in mud or sand, swimming freely above the sediment only at night (Kennedy, 1954). In the aquarium at Plymouth, soles, when not buried in the substrate, rarely rise more than a few centimetres from the bottom and swim with gentle body undulations. On the few occasions when they were seen away

from the bottom, the blind surface was usually kept in close contact with the vertical walls of the tank, in contrast with, for example, specimens of plaice (*Pleuronectes platessa*), which usually swam freely and vigorously, well clear of the bottom. Hence for most of their lives soles and the parasites they carry are exposed to low oxygen concentrations, but forward locomotion and the gill-ventilating current of the blind side of the sole might serve to prevent stagnation around the parasite. However, it has already been stated that *Solea solea* rests for most of the day and the application of carmine particles near the opercular valves of the fish showed that the exhalant current of the blind side spread over only a small area of this surface and would be even more restricted when the fish was buried in mud. It is therefore highly likely that a parasite living on the blind surface of a sole would be living in stagnant water of low oxygen content and would need to resort to a breathing device to change the water around itself and obtain sufficient oxygen. This is an interesting example of an ectoparasite being directly influenced by the environment of the host.

It is probable that variations in the rate of undulation, the frequency of pauses, the amplitude of the waves and the exposed surface area of the animal are adjustments to the fluctuating oxygen concentration of the environment. The availability of oxygen is likely to vary during the day since the parasite is exposed to stagnant, deoxygenated conditions when the host is buried in mud and to conditions of higher oxygen content when the fish is moving about above the sediment. Thus, if the oxygen concentration is lowered, increases occur in the undulation rate, the amplitude of the waves and the exposed surface area of the body, and a decrease occurs in the frequency of pauses. These changes enable the animal to move a greater volume of water in a given time and hence to bring more oxygen into contact with the body. If the surface area proper was increased by a stretching of the integument and the volume of the body remained constant, not only would more water be moved but also dissolved oxygen would be more readily available to the deeper seated tissues. It is not clear why *Entobdella soleae* should require more than one response to oxygen fluctuations or how these phenomena are co-ordinated.

The breathing movements of *E. soleae*, which involve no specialized breathing organs, can be compared with 'tail' movements in two freshwater, mud-dwelling, tubificid worms (*Tubifex rivulorum* and *Limnodrilus hoffmeisteri*) which were studied in detail by Alsterberg (1922). In both *Entobdella soleae* and the tubificids the breathing movements are controlled by oxygen and not by carbon dioxide. Alsterberg has shown that in *Tubifex rivulorum* and *Limnodrilus hoffmeisteri* the rate of undulation increased as the oxygen concentration was lowered, as in *Entobdella soleae*, but carbon dioxide excess had no stimulating effect on the movement and often stopped the undulations completely (again as in *E. soleae*). The reaction to changing oxygen

concentration in tubificid worms is not a simple change in undulation rate, but involves also an increase in the length of the undulating tail exposed above the mud. Thus as in *E. soleae*, in which not only undulation rate but also exposed surface area change with fluctuations of oxygen content, adjustments to new oxygen conditions are made in several ways. Alsterberg also found that the frequency of the undulations in *Limnodrilus hoffmeisteri* doubled for a rise in temperature of 10° C as in *Entobdella soleae* (see p. 94). These close similarities between the behaviour of *E. soleae* and the breathing movements of mud-dwelling annelids such as the Tubificidae, leave no doubt that *E. soleae* does indeed use its undulating movements to aid respiration.

It has been mentioned above (p. 93) that undulating movements have been observed in *E. squamula* from *Paralichthys californicus*, a bottom-living teleost flat-fish from the Pacific. Recently, Dr J. Llewellyn (personal communication) observed characteristic undulations in *Entobdella diadema* from the ventral surfaces of *Dasyatis pastinaca* and *Myliobatis aquila*, elasmobranch flat-fishes from the Mediterranean. It will be interesting to discover whether species of the genera *Benedenia*, *Pseudobenedenia* and *Ancyrocotyle* (which, together with the genus *Entobdella*, form the subfamily Benedeniinae) produce body undulations. Most species of the first three genera do not occur on flat-fish but *Benedenia macrocolpa* lives on the skin of *Rhinoptera javanica*, and *Benedenia pacifica* on *Myliobatis californicus*. Many species of the subfamily have been recorded from the gills of fishes (e.g. *Benedenia convoluta* on *Epinephelus akaara*), and since monogeneans living in this habitat would be washed continuously by a current of water, they would be expected not to possess undulating movements to aid respiration.

Undulations have been observed in two species of non-entobdellid skin parasites of bottom-living flat-fishes: *Acanthocotyle* sp. from *Raia clavata* and *Pseudocotyle squatinae* from *Squatina squatina* (see p. 99). It was surprising to find that rhythmical movements occurred in *Leptocotyle minor* from the skin of the dogfish, *Scyliorhinus canicula*, since the dogfish is not in the habit of burying itself in mud at the sea bottom. Ford (1921) and Rae (1961) however, both regard *Scyliorhinus canicula* as a bottom feeder and the work of Richards & Redfield (1954) and Brouardel & Vernet (1958) suggests that oxygen content of sea water immediately above wide areas of the bottom sediments is very low. Hence the occurrence of rhythmical movements in *Leptocotyle minor* might also be associated with low oxygen concentration of the environment.

Hence it appears that the possession of undulating movements is a character which does not reflect in all instances close phylogenetic relationships. An interesting example of convergent evolution has emerged in which three apparently unrelated groups of parasites, represented by *Entobdella soleae*, *Acanthocotyle* sp. and *Pseudocotyle squatinae*, all of which live in conditions

of low oxygen concentration on mud-dwelling flat-fishes, have evolved undulating movements to obtain sufficient oxygen.

I would like to acknowledge the help given to me by the staff of the Plymouth Laboratory, particularly that of Dr L. H. N. Cooper and Mr E. I. Butler. I am also greatly indebted to Dr J. Llewellyn for suggesting the problem and for helpful advice during the course of the work, and to Dr R. W. Murray for critical comments. I am also grateful to Mr A. E. Brafield of Queen Mary College, London, for information on the oxygen content of marine sands. The work was conducted during the tenure of a Fishery Research Training Grant from the Development Commission.

SUMMARY

An undulating movement of the body was observed in *Entobdella soleae*, a monogenean found on the blind surface of a mud-dwelling flat-fish, *Solea solea*, at Plymouth. The movement is described and shown to have a breathing function, the rate of undulation increasing with decreasing oxygen content of the ambient sea water and vice versa.

The relationship between the movement and micro-habitat is discussed and the phenomenon is compared with breathing movements in other mud-dwelling animals.

A similar movement was noted in three other skin-parasitic monogeneans: *Acanthocotyle* sp. from *Raia clavata*, *Pseudocotyle squatinae* from *Squatina squatina* and *Leptocotyle minor* from *Scyliorhinus canicula*.

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Additional note

Since writing the above I have had opportunity to examine living specimens of *Entobdella hippoglossi* collected from the surface of a halibut (*Hippoglossus hippoglossus*) taken in the trawl by the M.T. 'Josena' of Fleetwood, fishing on the 'hake grounds' between the Shetland Islands and the Norwegian coast in September 1961. The 20 parasites collected were all about 1.5 cm long; 16 of them were found on the blind surface and the other 4 on the ocular surface of the fish. This distribution however may not have been the usual one since the host was dead and had been in the trawl probably for more than an hour. The parasites were removed from the host and examined with the naked eye in sea water in a glass vessel. All specimens were observed to undulate regularly, and the rate of undulation for one individual was found to be 34 waves per minute at about 15°C.