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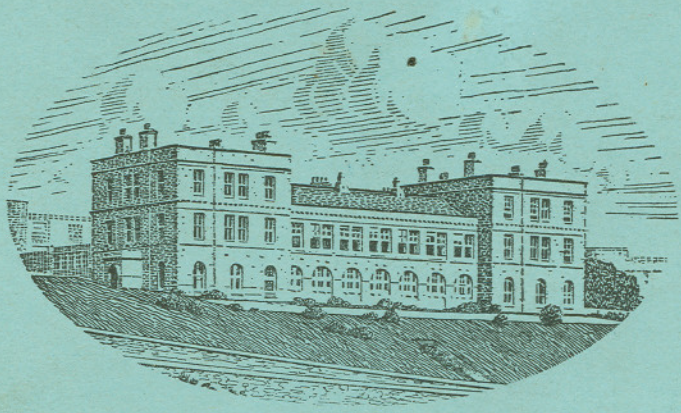

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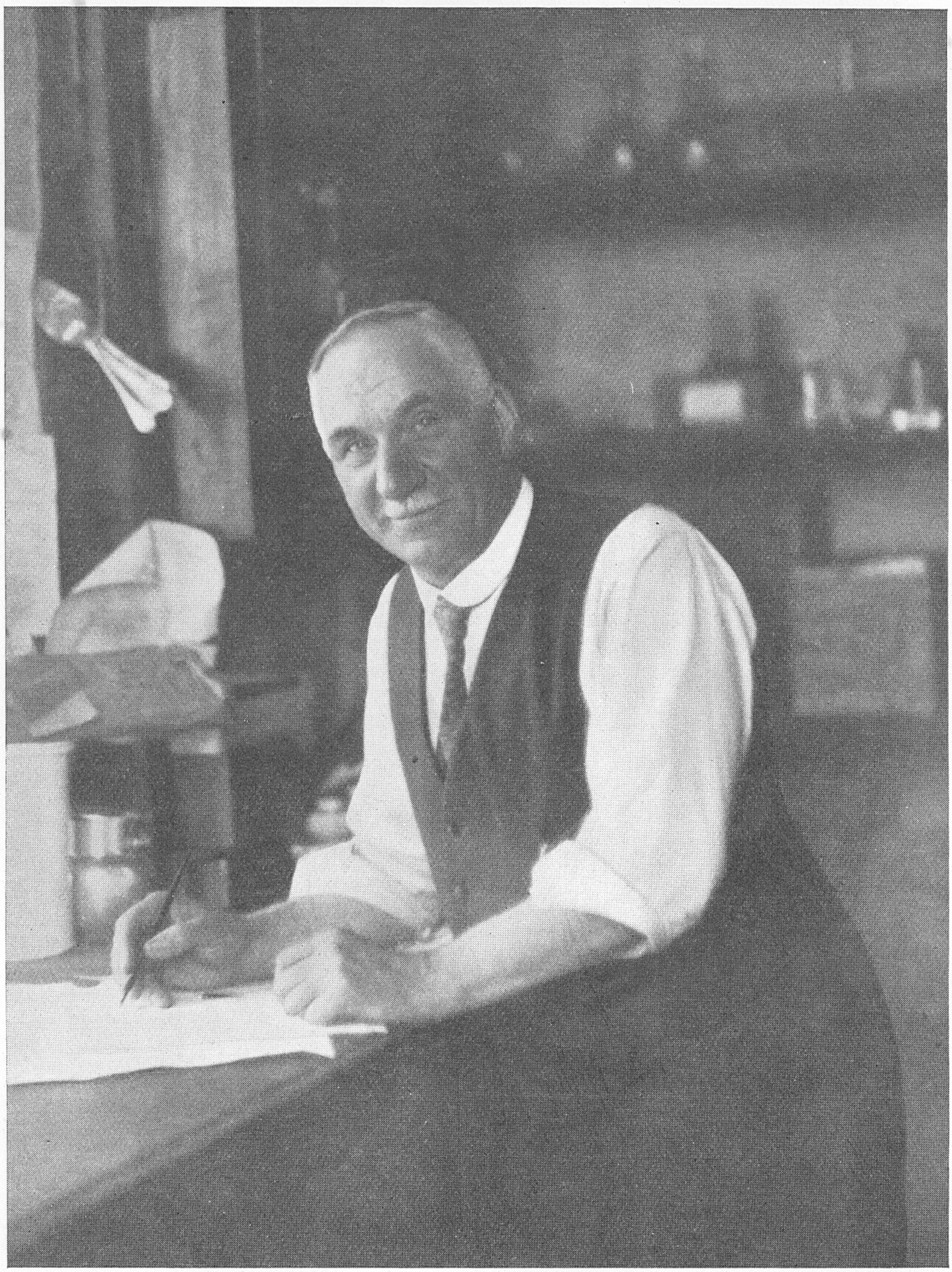

ARTHUR JAMES SMITH

ARTHUR JAMES SMITH (1871–1941)

A Personal Tribute by E. J. Allen

Those who have known the Plymouth Marine Biological Laboratory at any period during the last forty-five years will have learned with regret that our Chief Laboratory Attendant, Arthur James Smith, died on 29 January, after a trying illness which had lasted for more than a year. He took up the post which he held so successfully for so long in April 1895, about six months after my own appointment as Director. Trained at the Cambridge Zoological Laboratory, largely under the special care of Adam Sedgwick who always took a close and friendly interest in him, he owed much to the help he received from Brockett, the head attendant at Cambridge, to whom so many men in Zoological and other University Departments throughout the country were in the same way indebted.

When Smith arrived at Plymouth he already knew the Laboratory, having spent some weeks there the year before collecting and preserving material for use in Cambridge, and it was then, when I was working on the nervous system of embryonic lobsters, that I first met him.

From the beginning his work at the Laboratory was most successful and as the years passed he undertook the oversight of more and more of the routine activities of the whole establishment. He gradually acquired an extensive knowledge of the marine fauna and flora of the district and he was always eager to increase it and bring it up to date. For this he had many opportunities, since specialists in the systematics of particular groups, both visitors to the Laboratory and members of the scientific staff, were only too glad to repay the trouble he took to keep them supplied with material for their work by discussing with him the recent advances in their subjects. He had a very keen eye for discriminating species and was an excellent collector, especially on the shore and when examining material brought in from the daily work of our fishermen.

A group in which he took special interest was the Coelenterata, and the Ray Society Monograph on Anemones by Stephenson owes a good deal to his keenness and help. He made it, too, a regular practice to keep in touch day by day with the local fish market and with the fishermen and other workers there.

Smith brought with him from Cambridge a high degree of technical skill in dissection combined with considerable knowledge of the methods of preservation of animal tissues for section cutting or for museum purposes. This was greatly developed as his experience grew at Plymouth in the department for the sale of specimens, which was in his charge. It was well shown also by the ability he displayed in preserving and mounting specimens for exhibitions. An exhibit prepared largely by him attracted much attention at

the St Louis Exhibition in 1903. This was subsequently purchased and remained in the United States. Other exhibits arranged under his supervision were shown at the Yachting and Fisheries Exhibition at South Kensington in 1897 and the Oceanographical Exhibition at Marseilles in 1906. In the latter case he travelled with the exhibits to France and himself set them up there. He also took a great part in preparing and setting up the exhibits of marine animals which have been a popular feature for so many years at the Annual Soirees of the Royal Society at Burlington House. His work in all these directions was remarkable for his great care over details: nothing short of the best would ever satisfy him.

Amongst other assets was a good share of business ability, and for some years, in addition to his other duties, he had charge of the office, and himself kept the detailed accounts called for by the varied activities of the Marine Biological Association. His work in this direction always met with the full approval of the professional auditors.

Smith was essentially a man of action—a man who could do things. There was in him a fundamental honesty of mind and character which permeated his whole life, and it was this, added to a general desire to help the scientific work of the Laboratory, that made him of such great value in the post he held. He gave of his best to a long and varied succession of biologists from all parts of the world by whom he will always be remembered with gratitude, and this feeling will be shared in a very special way by those who have been fellow workers with him at the Laboratory.

Nothing I can say can express my own personal debt for his life's work and the help and kindness I have received from him. Always reliable, always willing to undertake new responsibilities, there were few things connected with the details of administration that could not safely be left in his hands.

ON CHANGES TAKING PLACE IN SEA WATER DURING STORAGE

By H. W. Harvey, Sc.D.

Hydrographer at the Plymouth Laboratory

(Text-fig. 1)

Whipple reported in 1901 that when tap water was filled into glass bottles, the number of bacteria fell during the first 3–6 hr. by 10–25 %, and later increased by many hundred per cent, with a reduction in number of species. This rise in bacterial numbers was several times greater in small than in large bottles, and was reduced or even nullified if the water was kept agitated. A similar rise in bacterial numbers takes place when sea water is stored in glass vessels. Waksman & Carey (1935) found that multiplication took place in Seitz and in colloid-filtered water which had been inoculated with raw water, and from the oxygen used calculated that the rapid growth of bacteria breaks down about one-third of the organic matter in solution. Zobell & Anderson (1936) and Lloyd (1937), found a much greater increase in numbers of bacteria when the water was stored in small than in larger bottles, or in bottles where the water-glass surface area had been increased by filling the bottle with glass beads or rods. By observing the numbers at close intervals of time, Miss Lloyd found that the increase followed the course of a population curve; the peaks were determined by the volume of the container, but were not affected by the surface area of the water exposed to the air. Zobell & Anderson found that the peaks, or maximum number of bacteria found in the water, showed a rough direct proportion to the volume/surface area of the bottles, the maxima in small vessels being about twice the maxima in vessels ten times as large. They also observed that when the surface area was increased by a shallow layer of glass beads or silica grains, the resulting increase in numbers of bacteria was less than that calculated from the volume/area ratio. They concluded that not only this ratio but also the proximity of the main body of water to the glass surface played a part. The greatest increases were found in water between sand grains where populations of some twelve million bacteria per c.c. developed in water which maintained no more than a few hundred bacteria per c.c. in the sea.

Whipple had found that the marked difference between maximum bacterial population, which arose when tap water was stored in small and in large bottles, was much reduced if a small quantity of peptone (5 mg./l.) was added to the water. Zobell & Anderson noted that if nitrite and 10 mg./l. of peptone were added to sea water, there was a greater and more rapid loss of nitrite

in smaller than in larger vessels, but no such difference when 100 mg./l. of peptone was added. It appears that the volume effect only occurs when bacteria develop in water containing food substances at very great dilution. This conclusion was also reached by Heukelekian & Heller (1940) who found no growth of the bacterium *Escherichia coli* in solutions containing 0·5 and 2·5 mg. of glucose and peptone, but growth did occur if glass beads were added. With 25 mg./l. growth did take place, and with concentrations greater than this the effect of adding glass beads faded out.

As Whipple had found for tap water, Zobell and Anderson found a reduction in number of species when sea water was stored. Some twenty-five to thirty-five species were generally found immediately after the water had been collected, falling to nine or ten species by the time bacterial numbers had reached a maximum and to no more than four or five species after the maximum population had declined. After this decline the population remained relatively high for a long period, the numbers fluctuating from a few thousand to over a hundred thousand per c.c.—a sample of sea water which had been stored at 2–6° C. for 4 years was found to contain 209,000 bacteria per c.c.

In Zobell and Anderson's investigation a series of experiments was made dealing with the effect of oxygen on the proliferation of bacteria in stored sea waters. No material effect was observed unless the water was less than 50 % saturated with air. Waksman & Carey, on the other hand, observed greater growth in fully aerated than in partially aerated water.

The oxygen content of sea water stored at 16° C. in glass-stoppered bottles of different capacities after 20 days, and the maximal bacterial population reached (after 3–5 days) in similar bottles. The water initially contained 3·46 c.c. O_2 per litre and 231 bacteria per c.c.

Volume of sea water (c.c.)	10	100	1000	10,000
O_2 per litre	2·59	2·90	3·68	4·17
Bacteria per c.c.	1,475,000	1,080,000	673,000	382,000

Zobell, 1936. *Proc. Soc. Exp. Biol.*, Vol. xxxv, p. 271.

Both Zobell & Anderson (1936) and Waksman & Renn (1936) observed that in full and stoppered bottles the consumption of oxygen continued undiminished for some time after the bacteria in the water had reached maximum numbers and while the population was falling. The former investigators have shown that great numbers of bacteria develop on the glass surfaces; one experiment indicated that within 24 hr. more than twice as many were attached to the surface of the glass as were in the water. This accounts for the continued consumption of oxygen after the number of bacteria in suspension have declined.

The proliferation of bacteria when water is enclosed in glass vessels and the effect of their size is attributed by Zobell & Anderson to the water-glass surfaces:

(i) Providing a resting place for periphytic bacteria, many marine species having periphytic tendencies and at least some being obligate periphytes. In this connexion it is pertinent that saprophytic bacteria are attached to sus-

pended particles in the sea (Lloyd, 1930) and that bacteria are most numerous where plankton is most abundant, as observed by Waksman, Reuzer *et al.* (1933) who consider that 'bacteria exist only to a very limited extent in the free water of the sea, but are largely attached to the plankton organisms'.

(ii) Concentrating organic substances from very dilute solution on the surfaces owing to adsorption or other physical attraction.

(iii) Causing the diffusion of bacterial enzymes away from the cell, where it is attached to a solid surface, to be retarded; it has been generally observed that attachment to particles exerts a favourable influence on their enzymatic activity.

The second suggestion—that organic matter in solution is adsorbed on solid surfaces—is of particular interest. The authors state that the accumulation of a film of organic matter on glass slides soon after being submerged in sea water can be demonstrated by differential stains as well as by microchemical technique. Stark *et al.* (1938) also state that an accumulation of organic matter can be detected on glass slides which have been immersed for several hours in lake water. Their method of detection was based on the oxidation of a sulphuric acid-dichromate mixture. In neither of these communications is the exact technique described. The writer has been unable to obtain definite results on these lines, but a number of observations have been made which point to adsorption taking place.

It was noticed that when offshore water was kept in glass tubes a gelatinous ring of bacteria slowly developed at the meniscus, becoming apparent after five months (Harvey, 1925). This suggested that if their growth was due to local concentration of organic matter by adsorption, the latter took place to a greater extent at the meniscus, where potent physical forces come into operation, than on other parts of the glass surface. Experiments were therefore made to test this possibility.

If a clean glass or silica tube of about 1 mm. bore is dipped into sea water, the water rises in the tube and the meniscus takes up a position at a definite height above the surface, this height corresponding to a surface tension of approximately 72 dynes/cm. When the tube is either lowered or raised, the meniscus returns to this same height; it moves freely both up and down the tube, provided the glass is clean and moist. However, if a trace of various organic substances is dissolved in the water, such as 5 mg./l. of peptone or casein, it is otherwise. The water rises in the tube to approximately the same height as before, but if allowed to remain undisturbed for a few minutes and the tube is then lowered, the meniscus is lowered and is flattened owing to the lesser pull by the column of water below; finally the meniscus breaks free and rises again to the same height as before. If the water in the tube is covered with ether or benzene and allowed to stand, on lowering the tube the water-ether meniscus is lowered and becomes flatter, while the ether-air meniscus keeps its shape. It appears that a ring of organic matter is adsorbed on the glass where the meniscus has stood and this prevents water moving

readily up the tube past the ring, but does not hinder the movement of water down the tube past the ring. Using more concentrated solutions, a series of rings were formed where the meniscus had stood in the tube for a few minutes, each ring acting as a valve allowing water to pass freely downwards but hindering its passage in the reverse direction. The contact angle of water advancing past the ring is considerable, while the angle when the water retreats remains at or near zero.

At somewhat higher concentrations, such as 50 mg. of sodium caseinate per litre, the solution rose in a clean tube to almost the same height as sea water without this addition, but when allowed to stand the column of liquid gradually fell. It appears that the water is repelled by the ring of adsorbed protein; a similar gradual fall took place when the column of liquid in the tube was covered with benzene.

The addition of 0·2 mg./l. of sodium caseinate, or less, could be detected by filling a short column into a clean capillary tube and allowing this to rest horizontally for a few minutes, after which a tilt of several degrees was required to start the column of liquid moving, whereas similar tubes with similar columns of filtered sea water required a tilt of *circa* one-twentieth of a degree. Using this method of detecting adsorbable substances in a liquid, the following experiment was made. A shrimp was ground with water, filtered, and the filtrate added to filtered sea water in the proportion of extract from 0·2 g. wet weight of shrimp per litre of water. A quantity of glass-wool, previously cleaned with concentrated sulphuric acid, washed and dried, was added to a part of this liquid. After standing for half an hour, a column about 1 cm. long was drawn into a clean glass capillary and moved to and fro in order to wet the glass surface. This capillary, and also one filled in the same way with a similar column of the liquid which had not been in contact with the glass-wool, were adjusted on a horizontal plate, so that the columns of liquid lay in the central parts of the tubes which were placed alongside each other. The plate was then slowly tilted and the angle of tilt at which each column started to move was noted. The plate was again levelled and a fresh pair of readings obtained:

Liquid ex glass-wool required	2° 36′	0° 39′	1° 18′	1° 3½′ tilt
Liquid not treated required	5° 12′	3° 24′	3° 24′	>3½°

On repetition with a fresh pair of capillaries:

Liquid ex glass-wool required	0° 26′	1° 18′	0° 39′	1° 44′ tilt
Liquid not treated required	2° 10′	5° 12′	5° 12′	7°

Two hours later, that is, after the liquid had stood 2½ hr. in contact with the glass-wool, fresh tubes were prepared and gave results as follows:

Liquid ex glass-wool required	0° 13′	0° 13′	0° 26′	0° 26′	0° 13′ tilt
Liquid not treated required	0° 52′	2° 10′	2° 23′	0° 39′	0° 52′

This shows a marked falling off in the capability of the liquid which had stood in a glass vessel, and of that which had been in contact with glass-wool, to form a ring. A similar effect has been found in other experiments.

These experiments are interpreted as indicating that organic matter is adsorbed on a glass-water surface, the most rapid adsorption taking place where there is a glass-water-air or a glass-water-benzene or ether interface forming a meniscus. There is no indication of how much of the added organic substance is so adsorbed; it is not known to what particular substances the ring effect is due, but experiment suggests that it is not due to traces of oily impurities.

An attempt was made to evaluate the quantity of organic matter which was adsorbed from very dilute solution, such as 10 mg. of peptone per l., on a large surface exposed by glass-wool, about 50 sq. cm./c.c. of liquid. The most delicate method found for estimating the change, due to contact with glass-wool, was the quantity of dilute alkaline permanganate oxidized in 24 hr. at 30° C. by the liquid, the excess of permanganate being estimated by titrating the iodine set free on adding potassium iodide and a buffered mixture of sulphuric and boric acid. By acidifying with such a mixture, the trace of chlorine set free on acidifying permanganate in sea water was certainly reduced, if not eliminated; consistent triplicate titrations were obtained. The results of these experiments showed that only a small proportion of the oxidizable organic matter was adsorbed, possibly no more than the experimental error. The values obtained in the different experiments ranged from 0 to 7 % less in the liquid which had been in contact with glass-wool than in the control. Only a minute fraction of the added organic matter would be required to give a monomolecular layer; on the other hand, layers many molecules thick are known to build up on solid surfaces (Blodgett, 1935). Whether the presence of a monomolecular layer of nutrient on a surface would provide a sufficient local concentration of food to allow bacteria to grow, assuming it was rapidly renewed after being used, is not obvious.

It is an outstanding question why offshore sea water, which contains sufficient nutriment for the production of several million bacteria per c.c., and will in fact rapidly produce this population when in contact with clean sand grains, normally supports a population of no more than 10–200 bacteria per c.c. In addition to lack of solid surfaces, protozoa and other animals keep the bacterial fauna eaten down; this has been stressed by both Zobell and by Waksman & Hotchkiss (1937). The former investigator (1936) has also concluded that natural sea water contains a bacteriophage, or heat-labile substances inimicable to the growth of bacteria; added bacteria grew more rapidly in autoclaved than in Berkefeld filtered sea water.

Changes brought about by bacteria developing in stored sea water are the setting free of ammonia, phosphate and carbon dioxide, the interconversion of ammonia, nitrite and nitrate, and the utilization of oxygen.

The ammonia may either increase or decrease (Keys *et al.* 1935). When plankton is added to the water and it is stored in the dark, there is an increase in ammonia which later decreases as nitrite is formed; later nitrite decreases with an increase in nitrate (Von Brand *et al.* 1937). In a great number of

cases no change has been found in the nitrate and nitrite content of water from the open sea during storage, even if enriched with ammonia, unless it was obtained close to the bottom or bottom deposit had been added to it (Harvey, 1926; Cooper, 1937). It appears that nitrite-forming and nitrite-oxidizing bacteria are not usually present in offshore water, free from plankton, unless collected close to the bottom.

The phosphate in solution increases slowly during storage, the increase being sometimes preceded by a decrease.

These changes are brought about by bacteria; however, there is evidence that both the setting free of phosphate and changes in the nitrogen cycle may take place to some extent without their aid. Kreps (1934) found that changes in ammonium and nitrate took place in inshore water which had

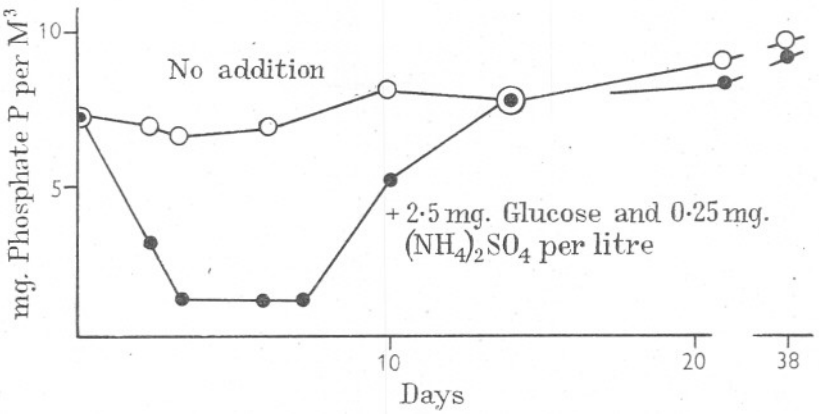

Fig. 1. Changes in phosphate concentration during storage in a sample of sea water, filtered through Whatman No. 3 paper. The lower curve shows the effect of increased bacterial growth due to the addition of phosphorus-free nutrient.

passed a Seitz filter or been poisoned with mercuric chloride, and suggested that sea water, particularly water near the bottom where organic matter was decomposing, contains enzymes which cause these changes. Keys *et al.* have also noted changes in ammonium content of water which had been sterilized with mercuric chloride, while Newcombe & Brust (1940) have noted that saturating water with chloroform reduces but does not stop phosphate being set free during storage.

Several investigations concerning the bacterial decomposition of organic matter added to sea water throw light on changes taking place during storage. Waksman & Carey (1935) have come to the following conclusions. Bacterial growth which occurs in clear water from the open sea consumes a similar quantity of oxygen as that brought about by the addition of $2\frac{1}{2}$ mg. of glucose per litre, and there are sufficient nitrogen compounds available for double the growth of bacteria which normally occurs when such water is stored. They

found sufficient available phosphorus or nearly sufficient in the waters they used for the requirements of the bacteria; adding phosphate had no effect or only slight effect upon their growth. On the other hand, Keys *et al.* have noted instances where the addition of phosphate has increased the oxygen consumption of stored waters. Renn (1937) found that when glucose was added to sea water the resultant growth of bacteria assimilated significant quantities of phosphate, and that this was soon regenerated following their death. This accounts for the decrease in phosphate which sometimes precedes an increase when sea water is stored. The writer has made similar experiments, adding glucose and an ammonium salt in order to provide ample organic matter and nitrogen for bacterial growth, and in some experiments enriching the water with phosphate. The results showed rapid and complete, or almost complete, regeneration of the phosphorus which the bacteria had utilized—that is, dephosphorulation of their body substances during autolytic breakdown. Previous experiments had shown that bacteria in a sample of sea water rapidly set free phosphate from nucleic acid and from casein in solution, but not from glycerophosphate, suggesting that the phosphatase enzymes of the bacteria in these samples of water could only deal with certain types of organic compounds.

The breakdown of organic phosphorus compounds and setting free of orthophosphate is of particular interest. Russell (1935, 1936) has shown a close correlation between the maximum concentration of phosphate which has been found in the water of the English Channel off Plymouth during winter and the subsequent abundance of young fish. Indeed, the fluctuations in maximum phosphate and in abundance of animal life have now followed each other rather closely for a number of years in this area. Redfield *et al.* (1937) have succeeded in showing by chemical analysis that there is a considerable accumulation of organic phosphorus in solution in the water of the Gulf of Maine during the summer, nearly all breaking down to phosphate during the ensuing winter. However, in an experiment by the writer when summer sea water was filtered and stored, no such quantity of phosphate was set free during succeeding months as would be expected to take place if the water had remained in the sea with a bacterial population at least several hundred times less. Some observations by Cooper (1935) point in the same direction; two out of four samples of sea water which had only been freed from larger plankton organisms showed little change in phosphate content when stored for several months, yet, where animal plankton had been added to this water, not only was all the phosphorus added in this form regenerated as phosphate but a considerable quantity in excess. He concluded that this excess was set free from dissolved organic compounds present in the sea water. It seems reasonable to surmise that some species of bacteria grow on the relatively rich food provided by the added animal plankton, which do not proliferate when plankton-free sea water is stored, and that these are able to dephosphorulate a greater variety of organic compounds.

The observations which have been discussed suggest a problem which

awaits, and would repay, solution. Russell's correlation has shown the value of a knowledge of the winter phosphate maximum of the water occupying an area, when studying the fluctuations in its total fauna from year to year; the same probably holds when studying differences between two bodies of water, due account being taken of such physical differences as the extent of vertical mixing. This winter maximum reflects the biological history and future of the water mass. The concentration of a conservative constituent such as chloride (salinity) reflects its physical past. To obtain the winter maximum requires the use of a ship over the period when the maximum is likely to occur, and exact colorimetric comparisons within a day or two of the samples being collected. The analysis of total organic and inorganic phosphorus, as made successfully by Redfield *et al.*, would require a team of workers to deal with the large number of samples necessary for a survey. Hence it would be particularly useful to reproduce in vitro the changes from organic phosphorus into ortho-phosphate which take place in the sea, presumably due, or mostly due, to the agency of bacteria, and which become apparent during autumn and winter when phosphate is not used as quickly as it is formed.

Summary

The rapid growth of bacteria when sea water is stored in glass vessels, and the possibility that this is brought about by the concentration of food by adsorption on the solid surface, are discussed. Some evidence is presented bearing upon such adsorption.

The regeneration of phosphate from dissolved organic phosphorus compounds in the sea and in stored water is considered.

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THE SOLUBILITY OF CALCIUM CARBONATE IN TROPICAL SEA WATER

By C. L. Smith, Ph.D.

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Introduction

In a previous paper (Smith, 1940*b*) the chemical changes observed in ocean sea water flowing across the shallow banks on the west coast of Andros Island (Bahamas) were reported. High salinities were produced by evaporation and calcium carbonate was precipitated. From the data obtained under natural conditions a maximum value of the solubility product constant of calcium carbonate in sea water was suggested. There was no reason to believe, however, that this value of the constant represented the true equilibrium conditions, and experiments have since been made in the laboratory with a view to bringing water from these banks into equilibrium with solid calcium carbonate.

Before describing this work it is necessary to point out two errors in the calculation of the ionic product, $Ca^{++} \times CO_3''$, in the previous paper (Smith, 1940*b*). In the first place, the calcium contents there given are uncorrected for strontium, which is usually included in the analysis as calcium. Assuming a calcium/strontium ratio of 30/1 (Lyman & Fleming, 1940), the true calcium content of the water may be found. Secondly, the quantities, carbonate alkalinity, total carbon dioxide, and carbonate ion, were found by reference to Buch's tables (1933). These tables are based on a constant relation between chlorinity and titration alkalinity (Titr. Alk. 0·123 Cl $^{o}/_{oo}$), a relation which holds for most open-ocean sea waters. On the Bahama Bank, however, a marked increase in chlorinity may be associated with a considerable decrease in titration alkalinity. For this reason Buch's tables are not applicable to such water, as different values of pK_B', pK_1' and pK_2' must be used in the calculations. When these errors are corrected for, the value of the ionic product, $Ca^{++} \times CO_3''$, becomes $1{\cdot}62 \times 10^{-6}$ at 30° C. and 36 $^{o}/_{oo}$ instead of $1{\cdot}27 \times 10^{-6}$ as previously reported. Similarly, at 20° C. and 36 $^{o}/_{oo}$, $Ca^{++} \times CO_3''$ becomes $1{\cdot}87 \times 10^{-6}$ instead of $1{\cdot}77 \times 10^{-6}$.

Experimental method

The principle adopted for attaining equilibrium was that of shaking sea water in sealed bottles with a quantity of the naturally precipitated bottom deposit, which was washed with distilled water and dried at 120° C.; about 1 g. was added to each bottle. Standard sampling bottles of 500 ml. capacity were used, leaving about 100 ml. air space. The bottles with solid, but without water, were sterilized in an autoclave. Save for four bottles in the first experiment, the sea water was filtered into the bottles through Berkefeld

filters. It had previously been ascertained that there was no change in the titration alkalinity of a sample on filtering (cf. Gee, 1932), although the *p*H increased slightly, owing to the escape of carbon dioxide from solution under reduced pressure.

The bottles were then mounted in a constant temperature shaking apparatus, which was maintained at above or below air temperature, constant to $\pm 0{\cdot}2^{\circ}$ C. Bottles were removed from time to time and estimations of the *p*H exponent, titration alkalinity, calcium content, and chlorinity, were made. On removing a bottle from the shaker, it was left in the thermostat for 24 hr. to settle; 10 ml. was then run into a test-tube, and the *p*H estimated colorimetrically in the usual way with cresol red. The remainder of the sample was filtered, and the titration alkalinity, calcium content and chlorinity then estimated. In the calculations which were necessary to obtain these values use was made of various constants, such as K_1', K_2' and K_B'. The constants K_1' and K_2' represent the stoichiometric dissociation constants of carbonic acid in sea water, and they are defined by Wattenberg (1933). K_B' represents the stoichiometric dissociation constant of boric acid in sea water as defined by Buch (1933). The relation between *p*H and the activity of the hydrogen ions (a_H) is also defined by the same two authors.

The following analytical methods were employed:

(1) *Hydrogen-ion concentration.* Palitzsch boric acid-borate buffers were used for making up the standard buffer tubes, which covered the range at *p*H 0·02 interval. The *p*H in situ (pH_W) was obtained by applying the corrections given by Buch (1937).

(2) *Titration alkalinity* was estimated in duplicate by Wattenberg's (1930) method.

(3) *Calcium content.* Kirk and Schmidt's double precipitation method as adapted for sea water by Kirk & Moberg (1933) was used. More reliable results could thus be obtained than by Gripenberg's (1937) single precipitation method. Estimations were made in duplicate, and the maximum deviation from the mean was 2·0 mg./l., or 0·05 m. mol./l.

(4) *Chlorinity* was determined by Knudsen's standard method.

(5) *Carbonate alkalinity* was calculated directly from the titration alkalinity and *p*H by means of the equations

$$\text{Carb. Alk.} = \text{Titr. Alk.} \frac{K_B' C_{\Sigma H_3BO_3}}{a_{H^+} + K_B'}, \qquad \text{(i)}$$

$$C_{\Sigma H_3BO_3} = 0{\cdot}0225 \text{ Cl } {}^{o}/_{oo} \text{ (m. mol.)} \quad \text{(Buch, 1933).} \qquad \text{(ii)}$$

Values of pK_B' were taken from the table given by Buch (1933).

(6) *Total carbon dioxide*, ΣCO_2, was calculated from the equation derived by Moberg *et al.* (1934) from the equilibria equations for the carbonic acid system:

$$\Sigma CO_2 = \frac{\text{Carb. Alk.}}{1 + (2K_2'/a_{H^+})} \left(1 + \frac{a_{H^+}}{K_1'} + \frac{K_2'}{a_{H^+}}\right).$$

Values of K_2' were taken from Buch (1933), and of K_1' from Wattenberg (1933).

(7) *Carbonate ion*, $C_{CO_3''}$, was similarly calculated directly from the equation

$$C_{CO_3''} = \frac{K_2'}{a_{H^+}} \frac{\text{Carb. Alk.}}{\text{I} + (2K_2'/a_{H^+})}.$$

Experimental Results

The first experiment was made with water collected 22 miles due north from Williams Cay on the central part of the Bank (cf. Smith (1940 *a*) for general description of the Great Bahama Bank). Ten bottles of water were collected together with a sample of the bottom deposit. Four of these were prepared as described above, while a second four were placed in the shaker without previous filtration through Berkefeld filters. These were subsequently removed in pairs, one unfiltered and one filtered sample, at intervals of 11, 24, 33 and 38 days. The temperature was $29{\cdot}7 \pm 0{\cdot}2°$ C. One sample of sea water was analysed as an initial control, while a second was allowed to stand for 19 days before analysis. The analyses are shown in Table I.

The most striking feature of these results is the continuous increase in titration alkalinity, calcium content and total carbon dioxide in all the bottles. Closer inspection of these figures, however, reveals that while the gain in calcium and titration alkalinity is practically identical (within the limits of error of the calcium estimation), the total carbon dioxide content has increased more rapidly. One must conclude, therefore, that carbon dioxide has been produced in the bottles by biological activity. Owing to the fact that there was only a slight gain in total carbon dioxide content when sea water alone was allowed to stand for 19 days, it is more probable that the source of the marked increase in the shaken bottles was due to the added solid. The effect of this increasing carbon dioxide content was to depress the *p*H and consequently the carbonate-ion content to such an extent that the water became unsaturated with respect to calcium carbonate. This caused calcium carbonate to be dissolved from the solid phase as shown by the increasing carbonate alkalinity and calcium content. As each analysis is based on a separate bottle of water, the results cannot be regarded as a progressive series, and considerable variation in the final state is to be expected. The column in Table I showing values of the ionic product, $Ca^{++} \times CO_3''$, corrected to 36 ‰ S, shows that, despite considerable variation in *p*H and carbonate alkalinity, this quantity is practically a constant. The fact that the calcium carbonate in solution has increased in all bottles precludes the possibility of this value of the ionic product representing an over-saturated condition, while its constancy within such narrow limits is indicative of conditions close to the true equilibrium between the solid and liquid phases. This experiment shows, also, that there is no systematic difference in the behaviour of the filtered and unfiltered water, and in subsequent experiments filtered water only was used.

TABLE I. EXPERIMENT I. THE SOLUBILITY PRODUCT CONSTANT, K'_{CaCO_3} IN SEA WATER. WATER FROM THE GREAT BAHAMA BANK, 22 MILES N.N.W. FROM WILLIAMS CAY, SHAKEN WITH THE NATURAL BOTTOM DEPOSIT.
TEMPERATURE = 29·7 ± 0·2° C.

No.	Date of analysis	Description	S ‰	pH$_W$	Tit. Alk. m. eq./l.	$C_{Ca^{++}}$ m. mol./l.	Carb. Alk. m. eq./l.	ΣCO_2 m. mol./l.	$C_{CO_3''}$ m. mol./l.	$Ca^{++} \times CO_3'' \times 10^{-6}$ at 36 ‰
10	18. vii. 40	Experiment started not shaken, no solid added	36·18	8·15	1·781	10·35	1·671	1·445	0·221	2·27
12	6. viii. 40	Not shaken, no solid added, stored 19 days	36·26	8·08	1·779	10·36	1·682	1·489	0·198	2·03
1	30. vii. 40	Unfiltered. Shaken with solid 11 days	36·22	7·79	1·786	10·48	1·729	1·648	0·120	1·24
2	13. viii. 40	Unfiltered. Shaken with solid 24 days	36·37	7·70	1·921	10·41	1·875	1·808	0·108	1·09
3	21. viii. 40	Unfiltered. Shaken with solid 33 days	36·43	7·69	2·022	10·42	1·977	1·905	0·111	1·13
4	26. viii. 40	Unfiltered. Shaken with solid 38 days	36·42	7·69	1·943	10·45	1·898	1·829	0·107	1·09
5	30. vii. 40	Filtered. Shaken with solid 11 days	35·88	7·73	1·905	10·40	1·856	1·775	0·113	1·18
6	13. viii. 40	Filtered. Shaken with solid 24 days	36·32	7·65	2·269	10·56	2·230	2·164	0·115	1·19
7	21. viii. 40	Filtered. Shaken with solid 33 days	36·29	7·72	2·048	10·43	2·000	1·919	0·120	1·23
8	26. viii. 40	Filtered. Shaken with solid 38 days	36·08	7·72	1·880	10·35	1·832	1·758	0·110	1·14

TABLE II. EXPERIMENT II. THE SOLUBILITY PRODUCT CONSTANT, K'_{CaCO_3} IN SEA WATER. COMPOSITE WATER SAMPLE FROM THE GREAT BAHAMA BANK, SHAKEN WITH THE NATURAL BOTTOM DEPOSIT. TEMPERATURE = 29·7 ± 0·2° C.

No.	Date of analysis	Description	S °/₀₀	pH$_W$	Tit. Alk. m. eq./l.	$C_{Ca^{++}}$ m. mol./l.	Carb. Alk. m. eq./l.	ΣCO_2 m. mol./l.	$C_{CO_3''}$ m. mol./l.	$Ca^{++} \times CO_3'' \times 10^{-6}$ at 36 °/₀₀
C	6. viii. 40	Not shaken and no solid added	38·22	8·05	2·105	11·01	2·003	1·781	0·228	2·25
A	19. viii. 40	Filtered. Shaken with 1 g. solid 14 days	37·79	7·71	1·946	10·84	1·895	1·816	0·113	1·12
B	19. viii. 40	Filtered. Shaken with 1 g. solid 14 days	37·83	7·73	1·957	10·80	1·903	1·821	0·119	1·17

TABLE III. EXPERIMENT III. OPEN-OCEAN SEA WATER SHAKEN WITH BOTTOM DEPOSIT FROM THE GREAT BAHAMA BANK. TEMPERATURE = 29·7 ± 0·2° C.

No.	Date of analysis	Description	S °/₀₀	pH$_W$	Tit. Alk. m. eq./l.	$C_{Ca^{++}}$ m. mol./l.	Carb. Alk. m. eq./l.	ΣCO_2 m. mol./l.	$C_{CO_3''}$ m. mol./l.	$Ca^{++} \times CO_3'' \times 10^{-6}$ at 36 °/₀₀
F	4. ix. 40	Open-ocean water. Not shaken and no solid added	36·33	8·24	2·495	10·67	2·367	1·989	0·362	3·80
A	9. ix. 40	Filtered. Shaken with 1 g. solid 5 days	36·20	7·88	2·120	10·41	2·053	1·919	0·168	1·74
B	9. ix. 40	Filtered. Shaken with 1 g. solid 5 days	36·22	7·90	2·136	10·44	2·067	1·905	0·175	1·82
C	19. ix. 40	Filtered. Shaken with 1 g. solid 12 days	36·13	7·80	2·065	10·40	2·008	1·901	0·141	1·46
D	21. ix. 40	Filtered. Shaken with 1 g. solid 16 days	36·17	7·79	2·022	10·39	1·966	1·872	0·136	1·40
E	21. ix. 40	Unfiltered. Shaken without solid 16 days	36·17	8·22	2·491	10·61	2·369	2·002	0·351	3·70

The second experiment was carried out on a small composite sample made up from a number of water samples taken at several different places on the Bahama Bank (Table II). The initial titration alkalinity was higher and the *p*H lower than that of the water in Experiment I. After 14 days' shaking with solid there was a fall in titration alkalinity and *p*H, although as in the previous series the total carbon dioxide content increased. In these samples, therefore, the predominant process has been precipitation of calcium carbonate despite the liberation of carbon dioxide by some other agency. At the end of the experiment the value of the product, $Ca^{++} \times CO_3''$, lies well within the limits of variation found in Experiment I. The average value of the ionic product based on the ten observations made in these two experiments is $1{\cdot}16 \pm 0{\cdot}05 \times 10^{-6}$. This figure is put forward as a new value for the solubility product constant (K'_{CaCO_3}) of calcium carbonate in sea water at 30° C. and 36 °/₀₀.

A third experiment in which open-ocean sea water was shaken with added solid was made to check the previous results. The data (Table III) show that at the start of the experiment the water had the normal characteristics of ocean water in these latitudes. On shaking with solid there was a marked fall in titration alkalinity, *p*H, and total carbon dioxide content, which was continuous to the end of the experiment. Comparison of the amounts of calcium carbonate precipitated as shown by the reduction in carbonate alkalinity and calcium content, and the associated decrease in total carbon dioxide again show that there has been a slight carbon dioxide production by biological activity. After 16 days' shaking the ionic product, $Ca^{++} \times CO_3''$, had fallen to $1{\cdot}40 \times 10^{-6}$, indicating that final equilibrium had probably not been attained, though the water at that point is only 20 % supersaturated. From this experiment it is apparent that equilibrium is more readily attained when sea water is used which has precipitated the greater part of its excess calcium carbonate under natural conditions. The two samples F and E in Table III show the effect of shaking sea water for 16 days with no solid phase present. On correcting for the slight difference in chlorinity, it can be seen that the carbonate alkalinity and total carbon dioxide have increased slightly. At least a part of the rise in carbonate alkalinity may be attributed to the solvent action of the water on the glass bottle, but the increase is so small that it would have no significant effect on the product $Ca^{++} \times CO_3''$.

Discussion

The behaviour of the open-ocean water on shaking with the calcareous bottom deposit in Experiment III follows very closely the pattern observed under natural conditions when ocean water flowed across the shallow banks (Smith, 1940*b*). Owing to the continuous diffusion of carbon dioxide from the water on the bank, however, the *p*H does not fall to the same extent as in the sealed bottles, and consequently more calcium carbonate must be precipitated before equilibrium is attained. It is therefore to be expected that

a steady state would only be reached under natural conditions after prolonged contact with the solid phase.

Values for the solubility product constant of calcium carbonate in sea water have previously been put forward by Revelle & Fleming (1934) and Wattenberg & Timmermann (1936). Revelle & Fleming concluded that tropical sea water could only be slightly supersaturated with calcium carbonate. This view has already been shown by the writer to be untenable by direct observations on the Bahama Bank, and the present work confirms this view. Wattenberg & Timmermann, however, found a value of $0{\cdot}52 \times 10^{-6}$ for K'_{CaCO_3} at 30° C. and 36 ‰. Such a value would suggest that tropical ocean water is as much as 700 % saturated with calcium carbonate. Even the lowest values observed on the Bahama Bank after extensive precipitation has taken place would represent approximately 300 % saturation. Considering the conditions under which the present experiments were conducted, it is difficult to see how the value of K'_{CaCO_3} can lie below $1{\cdot}16 \times 10^{-6}$. Owing to the fortuitous production of carbon dioxide in the bottles, the water in Experiment I became unsaturated with respect to calcium carbonate, and it was in fact being dissolved from the solid phase. In this case if the average value from these observations does not represent the equilibrium, it should be too low and not too high. In Experiment III, however, the ocean water was still slowly depositing calcium carbonate when the ionic product, $Ca^{++} \times CO_3''$, had fallen to the neighbourhood of $1{\cdot}40 \times 10^{-6}$. On this evidence it is suggested that the true value of the solubility product lies very close to $1{\cdot}16 \times 10^{-6}$ under the given conditions of salinity and temperature. Combining such a value with the observations made on the Great Bahama Bank in 1939 shows that the water was in some cases only 140 % saturated with calcium carbonate, which is a more probable figure than 300 %. It is possible that differences in the material used as the solid phase may account for some of the discrepancies in the recorded values of K'_{CaCO_3}. The material used in the present work contains large numbers of minute aragonite needles. Mechanical analyses of similar bottom samples made by Vaughan (1918) show that these deposits contain up to 57·6 % of clay and silt-sized particles (0·05–0 mm. diameter).

It is interesting to note that Gee *et al.* (1932) carried out experiments in which sea water was shaken with solid calcium carbonate in sealed bottles. Calcium content, *p*H, and total carbon dioxide content were estimated after periods of shaking up to 17 days. A very marked fall in *p*H was observed, associated with a reduction of total carbon dioxide and a slight fall in calcium content. From the total carbon dioxide and *p*H values observed by these authors it is possible to compute the carbonate alkalinity and hence the carbonate-ion content by the following equation:

$$\text{Carb. Alk.} = \frac{\Sigma CO_2 . \mathrm{I} + (2K_2'/a_{H^+})}{\mathrm{I} + (a_{H^+}/K_1') + (K_2'/a_{H^+})}.$$

In one experiment the results for five bottles which had been shaken for periods of from 5 to 17 days give an average value for K'_{CaCO_3} of $1{\cdot}10 \times 10^{-6}$ at 30° C. and 36 °/₀₀ when recalculated in this way. Thus this work may be regarded as providing close confirmation of the value found by the writer.

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THE FECUNDITY OF *OSTREA EDULIS*

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(Text-figs. 1–4)

Introduction and Review of Previous Work

Galtsoff (1930) has shown that females of the American oyster (*Ostrea virginica*) may discharge from 15 million to 114 million eggs in a single spawning period, while even after such heavy spawning the gonad still contains a large quantity of eggs. A female of *Ostrea gigas* was shown by the same writer to be capable of producing up to 92 million eggs. These oysters both belong to the group of large oviparous species, which Ranson (1938) and Nelson (1938) have proposed should be separated from the so-called flat oysters under the generic name of *Gryphaea*, with the Portuguese oyster *Gryphaea angulata* as the type. Nelson (1938) and Elsey (1935) have shown that in addition to their fundamental difference in process of reproduction the oviparous and viviparous types differ considerably in the relations between the mantle and the visceral mass. The differences between the two types appear to the writer to warrant the adoption of the two generic names as suggested. The viviparous oysters of the genus *Ostrea* are usually much smaller than the oviparous species, and those which have been investigated have been found to produce far fewer eggs, generally discharging the gonad completely in one spawning act. The earliest known estimate of the number of embryos produced by a European oyster is that of Eyton (1858), who arrived at the figure 1,800,000 for very large oysters. Buckland in 1865 (quoted by Philpots, 1890) stated that he had examined several native oysters of average size and weight, and had never found 'the highest number of spat to be more than 829,655 and the lowest 276,555'; his method of estimation is not recorded. Moebius (1883), by weighing separately the total broods of five individuals and also a small fraction of each, and by counting the embryos contained in the small fraction, arrived at the conclusion that adult females of *O. edulis* produced on the average a brood of 1,012,955 embryos. It is probable that Moebius was dealing with what would be considered to-day to be very large oysters. This estimate has been extensively quoted, for no further detailed observations of the production of adult oysters have since been published. Dantan (1913) has, however, published details of a series of counts of the broods of 1-, 2- and 3-year-old oysters from Arcachon and Brittany, in France. Dantan employed a satisfactory method of counting aliquot parts and gives figures for the broods of six 1-year-old, three 2-year-old and eight 3-year-old

oysters; his mean values are 95,500, 247,000 and 725,000 respectively. Gaarder & Bjerkan (1934) also give figures for 1-, 2- and 3-year-old oysters and also for older oysters, viz. 100,000, 250,000, 800,000 and about 1,000,000 respectively, but no details are given of the method of arriving at these figures, or of the number of broods counted. It is not clear whether these represent new estimates or are merely a quotation of the work of Dantan and Moebius. Orton (1937) states that a 1-year-old native oyster from Essex was found to be carrying a brood of about 240,000, while a small 3- or 4-year-old oyster, also from Essex, carried 525,000.

The only other species of flat oyster of which the size of brood is known is *O. lurida*, for which Hopkins (1937) has made some careful observations. Hopkins concludes that the average market-size individual of this species, i.e. one about 5 cm. across the shell, produces a brood of from 250,000 to 300,000 larvae. This figure is the result of examining, and estimating by counting aliquot parts of the whole brood, the broods of twenty-five individuals. Hopkins found that the range of variation was considerable, the smallest brood he found being only 69,490 while the largest was 355,500.

In the course of a study of the sexual cycle and breeding in 1- and 2-year-old oysters, the writer found several with broods of embryos, and it was thought worth while to preserve these broods and to make estimates of the numbers present, inasmuch as the age of the parent oysters was known with certainty, for they had been reared from spat setting on limed tiles in the breeding tanks at Conway, N. Wales (see Cole, 1938). As Dantan (1913) points out, it is especially desirable in work of this kind to have oysters other than native oysters from the natural beds, the age of which is always uncertain. The work of estimating the fecundity of oysters in their first few seasons was later extended, as it became necessary to determine the average size of the brood produced by adult oysters kept under tank conditions at Conway, and to compare this figure with the productivity of different types of oysters from the natural beds.

Material and Methods

The material from which our own observations are drawn consists of several distinct categories of oysters.

In the first place there are groups of 1-, 2- and 3-year-old oysters from our relaying ground in the Menai Straits, all of which had settled on tiles in the Conway tanks before being transferred to the Menai Straits.

Secondly, there is a group of young oysters from the Helford River, Cornwall, the age of which could be determined with more or less certainty, comprising both French relaid oysters of 3 and 4 years, and a number of small native brood oysters whose age was believed to be 2 years. It is possible, however, that one or two of the latter may have been 3 years old.

Thirdly, there is a group of large oysters taken from those used as breeding

stock in the Conway tanks, and obtained by us from the Yealm River, S. Devon. The majority of these oysters originated in France, being laid down for a year or more in the Yealm River before dispatch to Conway.

Fourthly, there are two groups of large oysters from the Helford River, Cornwall, one consisting of those native to the river, the other comprising Brittany oysters, which are laid down annually in large quantities in the Helford River.

The following method was adopted in determining the number of embryos comprising a brood. The embryos were carefully washed free from the gills, palps and mantle of a gravid oyster, and were killed with formalin. They were then collected by pipette and placed in a round-bottomed flask or a beaker, and the volume made up to a fixed amount, either 250 or 400 c.c. The contents of the flask or beaker were thoroughly agitated and five, or on some occasions ten, exactly determined samples were removed with an automatic pipette. The pipettes used delivered 0·52 and 0·45 c.c. respectively. The embryos contained in each of these aliquot parts were counted under a binocular microscope on a squared counting plate and a mean value determined; multiplied by the appropriate factor, this gave a figure for the total brood. At the same time, the colour, to the naked eye, of the brood as a whole and the stage of development reached by the embryos were noted. Where oysters were dredged from the beds they were opened as quickly as possible, as abortion of embryos may occur through rough handling.

Observations

One-year-old oysters. Gerbe (1876) was the first to observe that oysters may produce eggs even in the first year of their life, i.e. in the summer following that in which they attached themselves. He found thirty-five with embryos out of a total of 435 1-year-old oysters from Arcachon. This observation was followed by the work of de Lacaze-Duthiers (1893), who, after several years' experience of oysters held in the aquaria of the biological station at Roscoff, concluded that only a very few oysters bred during their second and third years, and then only to a slight extent, and that reproduction was not fully assured until their fourth year. Gerbe's work has, however, since been supported by Dantan (1913), who found that of 133 1-year-old oysters from Arcachon, opened on 8 August, six contained broods of embryos. There is good reason for believing that Dantan was able to determine exactly the age of the material with which he was dealing, always a difficult matter unless the oysters have been reared on tiles or have been under observation since settlement. Orton (1922) has recently recorded also that of oysters settling in 1920 in English waters, certain of the larger individuals liberated larvae in the summer of 1921, but the same author later states (Orton, 1937) that in his opinion this state of affairs is not usual in England 'where young oysters may not usually produce the first batch of eggs until their third or even fourth

summer'. Work which I have at present in hand shows that the proportion of young oysters functioning as females in the summer following attachment may in some years be larger than was formerly suspected.

Dantan was fortunate in obtaining six 1-year-old oysters with broods of embryos, the mean number in a brood being 95,500 with a range of from 69,000 to 144,000. The average length of the shell of these oysters was 3·4 cm. I have unfortunately only opened one such oyster which was actually carrying a brood of embryos, although ripe females have been of fairly frequent occurrence. This one oyster measured 3·8 cm., and carried a brood of 91,628 when opened on 25 October 1938. The embryos were in the late gastrula stage.

TABLE I. BROODS OF EMBRYOS OBTAINED FROM 2-YEAR-OLD OYSTERS FROM THE MENAI STRAITS, NORTH WALES

Date	No. of embryos	Stage of development of embryos	Colour
29. vi. 38	432,200	Trochophores	White
29. vi. 38	320,900	Trochophores	White
29. vi. 38	272,400	Shelled veligers: 0·15–0·165 mm.	White
29. vi. 38	259,100	Gastrulae	White
29. vii. 38	270,900	Trochophores	White
29. vii. 38	189,300	Shelled veligers: 0·15–0·165 mm.	Light grey
Average	290,800		

TABLE II. BROODS OF EMBRYOS OBTAINED FROM SUPPOSED 2-YEAR-OLD OYSTERS FROM THE HELFORD RIVER, CORNWALL

Date	Length of shell cm.	No. of embryos	Stage of development of embryos	Colour
2. viii. 39	5·0	257,800	Shelled veligers: 6 % 0·14 mm., 76 % 0·15 mm., 18 % 0·16 mm.	Light grey
16. viii. 39	4·5	108,800	Shelled veligers: shell not completely enclosing body, *ca.* 0·14 mm. in length	White
16. viii. 39	5·3	161,300	Trochophores	White
21. viii. 39	4·7	41,900	Shelled veligers: 18 % 0·14 mm., 38 % 0·15 mm., 36 % 0·16 mm., 8 % 0·17 mm.	Light grey
1. ix. 39	4·1	84,400	Shelled veligers: 20 % 0·16 mm., 66 % 0·17 mm., 14 % 0·18 mm.	Medium grey
Average	4·72	130,800		

Two-year-old oysters. During 1938 small samples of 2-year-old oysters from our relaying ground in the Menai Straits were opened at monthly intervals from June to September, and from twenty-six oysters opened on 29 June four were found carrying broods, while a further lot of twelve oysters opened on 29 July yielded two more with broods of embryos; the details of these broods are set out in Table I. Those lots of thirty-one and thirteen oysters opened in August and September respectively gave none with broods. All these oysters formed part of the crop obtained on tiles during 1936 in the breeding tanks at Conway. They were transferred to the Menai Straits about

a month or so after settlement and were detached from the tiles when about 12 months old.

In Table II are shown details of the broods of embryos obtained from a number of small native oysters from the Helford River, Cornwall. From a knowledge of the normal growth rate in this river, it was possible to say with fair certainty that practically all of these oysters were 2 years old, but it is possible that one or two were unusually small 3-year-old oysters.

TABLE III. BROODS OF EMBRYOS OBTAINED FROM 3-YEAR-OLD OYSTERS FROM THE MENAI STRAITS, NORTH WALES

Date	Length of shell cm.	No. of embryos	Stage of development of embryos	Colour
19. vii. 39	6·0	374,600	Shelled veligers: shell not completely enclosing body, *ca.* 0·14 mm. in length	White
19. vii. 39	6·2	601,600	Shelled veligers: shell not completely enclosing body, *ca.* 0·13 mm. in length	White
19. vii. 39	6·3	519,800	Shelled veligers: shell not completely enclosing body, *ca.* 0·13 mm. in length	White
22. vii. 39	5·0	440,000	Trochophores	White
22. vii. 39	5·5	416,300	Trochophores	White
22. vii. 39	6·0	349,400	Trochophores	White
22. vii. 39	6·0	562,800	Shelled veligers: 84 % 0·17 mm., 16 % 0·18 mm.	Dark grey
22. vii. 39	6·5	496,100	Shelled veligers: 48 % 0·17 mm., 52 % 0·18 mm.	Dark grey
22. vii. 39	6·5	693,700	Gastrulae	White
22. vii. 39	7·0	555,400	Gastrulae	White
Average	6·1	501,000		

TABLE IV. BROODS OF EMBRYOS OBTAINED FROM 3-YEAR-OLD RELAID BRITTANY OYSTERS FROM THE HELFORD RIVER, CORNWALL

Date	Length of shell cm.	No. of embryos	Stage of development of embryos	Colour
1. viii. 39	5·5	482,800	Shelled veligers: 0·15–0·16 mm.	Light grey
1. viii. 39	6·2	275,900	Shelled veligers: 8 % 0·17 mm., 28 % 0·18 mm., 52 % 0·19 mm., 12 % 0·20 mm.	Dark grey
16. viii. 39	5·7	216,200	Shelled veligers: shell not completely enclosing body, *ca.* 0·13 mm. in length	White
16. viii. 39	6·4	492,200	Shelled veligers: shell not completely enclosing body, *ca.* 0·13 mm. in length	White
Average	5·95	366,800		

Three-year-old oysters. During 1939 a number of 3-year-old oysters from our relaying ground in the Menai Straits were opened and any broods found were preserved. The details of these broods are shown in Table III. These oysters formed part of the crop obtained in 1936 in the tanks at Conway. Unfortunately it was not possible to collect gravid oysters at different times during the season, as the writer was away from Conway during the greater part of the summer.

A small number of individuals carrying embryos were found among samples of 3-year-old relaid Brittany oysters, opened on the Helford River, Cornwall, during the summer of 1939. The details of these broods are shown in Table IV. These oysters had been obtained in April 1939 from Locmariaquer in the Gulf of Morbihan and were stated to be '2-year olds', i.e. 1936 crop, when bought. There is no reason to doubt that they were correctly described, since they were originally caught on tiles, so that the time of settlement would be known. When opened they were of course in their fourth summer, i.e. they were a month or so over 3 years old.

TABLE V. BROODS OF EMBRYOS OBTAINED FROM RELAID BRITTANY OYSTERS, 4 YEARS OR MORE OF AGE, FROM THE HELFORD RIVER, CORNWALL

Date	Length of shell cm.	No. of embryos	Stage of development of embryos	Colour
18. vii. 38	7·5	1,163,700	Shelled veligers: 25 % 0·17 mm., 65 % 0·18 mm., 10 % 0·19 mm.	Light grey
19. vii. 38	8·0	567,400	Shelled veligers: 16 % 0·16 mm., 32 % 0·17 mm., 48 % 0·18 mm., 4 % 0·19 mm.	Medium grey
5. viii. 38	6·5	1,393,300	Trochophores	White
5. viii. 38	7·0	181,800	Gastrulae	White
5. viii. 38	9·5	1,730,200	Trochophores	White
8. viii. 38	6·5	1,189,400	Shelled veligers: 30 % 0·16 mm., 70 % 0·165 mm.	Light grey
*5. viii. 39	6·4	1,239,100	Shelled veligers: 26 % 0·16 mm., 74 % 0·17 mm.	Medium grey
Average	7·34	1,066,400		

* 4 years old.

Oysters 4 years old, or older. An oyster when 4 years old has frequently reached marketable size and, for practical purposes, may be regarded as adult. The only oyster actually known to be 4 years old from which I have obtained a brood of embryos was a single relaid Brittany oyster from the Helford River. This individual measured 6·4 cm. across the shell and carried a brood estimated at 1,239,100. This oyster is included in Table V, which also gives details of the broods of a number of relaid Brittany oysters, 4 years or more of age, from the Helford River, Cornwall. It is probable that most of the oysters whose broods are detailed in this table were actually 4 or 5 years old when opened, as Brittany oysters are usually sold from the Helford River at this age.

In addition to those from relaid Brittany oysters, broods of embryos were also obtained from a number of oysters native to the Helford River, and the details of these are shown in Table VI. It is possible that one or two of the smallest of these native oysters may have been only 3 years old, but the majority would be 4 or 5 years of age.

From time to time broods of embryos have been collected from adult oysters used as breeding stock in the Conway tanks. Such oysters are brought

into the tanks early in May of each year and are taken out again in September and relaid on our own grounds in the Menai Straits, where they remain until taken up again early in the following May. All were originally obtained from

TABLE VI. BROODS OF EMBRYOS OBTAINED FROM ADULT OYSTERS NATIVE TO THE HELFORD RIVER, CORNWALL

Date	Length of shell cm.	No. of embryos	Stage of development of embryos	Colour
19. vii. 38	6·0	571,600	Shelled veligers: 20 % 0·165 mm., 10 % 0·17 mm., 60 % 0·18 mm., 10 % 0·19 mm.	Medium grey
27. vii. 38	5·75	944,200	Segmenting eggs and morulae	White
27. vii. 38	6·0	353,100	Trochophores	White
27. vii. 38	7·0	1,075,900	Trochophores	White
27. vii. 38	8·5	1,401,500	Shelled veligers: shell not completely enclosing body, *ca.* 0·15 mm. in length	White
5. viii. 38	8·0	322,200	Gastrulae	White
Average	6·88	778,100		

TABLE VII. BROODS OF EMBRYOS OBTAINED FROM OYSTERS, 4 YEARS OR MORE OF AGE, FROM THE CONWAY BREEDING TANKS

Date	Length of shell cm.	No. of embryos	Stage of development of embryos	Colour
17. viii. 38	6·35	1,172,800	Shelled veligers: shell not completely enclosing body, *ca.* 0·14 mm. in length	White
17. viii. 38	6·5	559,100	Shelled veligers: shell not completely enclosing body, *ca.* 0·14 mm. in length	White
19. viii. 38	8·0	1,115,800	Shelled veligers: 10 % 0·15 mm., 20 % 0·16 mm., 70 % 0·165 mm.	Black
20. viii. 38	7·0	1,245,800	Shelled veligers: 15 % 0·165 mm., 65 % 0·17 mm., 20 % 0·18 mm.	Black
31. viii. 38	6·7	544,000	Morulae	White
31. viii. 38	7·3	663,600	Early gastrulae	White
8. ix. 38	7·0	588,700	Shelled veligers: 5 % 0·165 mm., 90 % 0·17 mm., 5 % 0·18 mm.	Dark grey
8. ix. 38	7·0	749,000	Shelled veligers: 5 % 0·16 mm., 95 % 0·165 mm.	Dark grey
3. vii. 39	No record	1,091,400	Shelled veligers: 40 % 0·16 mm., 60 % 0·17 mm.	Medium grey
Average	*6·98	858,900		

* Average of eight only.

the Yealm River, S. Devon, from which a new consignment is added each year. The majority of these oysters are French oysters from the Gulf of Morbihan, and were laid down in the Yealm River for at least 12 months before being sent to Conway. Details of the broods of embryos obtained are shown in Table VII. The majority of these oysters were probably 5 or 6 years old when opened.

The data derived from all the above year-groups of oysters are summarized in Table VIII.

TABLE VIII. SUMMARY OF THE DATA FROM ALL BROODS OF EMBRYOS

Age	No.	Mean length of shell cm.	Range of variation in number of embryos	Average number of embryos per brood	Origin of parent oysters
1 *year*	1	3·8	—	**91,600**	Conway oyster from Menai Straits
2 years	6	No record	189,300–432,200	290,800	Conway oysters from Mena Straits
*2 years	5	4·72	41,900–257,800	130,800	Helford River natives
All 2-year-old oysters	**11**			**218,100**	
3 years	10	6·1	349,400–693,700	501,000	Conway oysters from Menai Straits
3 years	4	5·95	216,200–492,200	366,800	Brittany oysters, relaid Helford River
All 3-year-old oysters	**14**	6·06	—	**462,600**	
4 years	1	6·4	—	1,239,100	Brittany oyster, relaid Helford River
4 years and over	6	7·5	567,400–1,730,200	1,066,400	Brittany oysters, relaid Helford River
4 years and over	6	6·88	181,800–1,401,500	778,100	Helford River natives
4 years and over	9	†6·98	544,000–1,245,800	858,900	Oysters from Conway tanks, mainly relaid Brittanys from Yealm River, S. Devon
All oysters 4 years old and over	**22**	‡7·07		**902,900**	

* Probable age, may have been 3 years old in few cases.
† Average of eight only (see Table VII).
‡ Average of twenty-one only (see Table VII).

DISCUSSION

In Fig. 1 length of shell is correlated with brood strength. It will be seen that the number of embryos produced varies rather widely in oysters of similar age and length of shell. A comparable variation has also been recorded in *Ostrea lurida* by Hopkins (1937). Such a variation may in part be due to the fact that length of shell alone is not a completely satisfactory criterion of reproductive capacity, as in oysters with shells of similar length the volume of the meat and the degree of fatness, upon which the reproductive capacity presumably depends, may vary rather considerably. In recent work on the sexual cycle of young oysters I have adopted the weight of the soft parts as giving a better measure of the reproductive capacity.

This variation in brood size among oysters of similar age is well brought out in Fig. 2, in which the observations have been grouped in year-classes, those observations appertaining to adult oysters being grouped arbitrarily

opposite the 5-year mark. This method of treating the data shows that the 2- and 3-year-old oysters apparently belong to more or less homogeneous groups, but the observations of brood size among adult oysters appear to group themselves about two points, and this suggests that we are dealing with two distinct classes of adult oysters varying considerably in brood strength. The reason for this peculiar grouping is not clear, but it may perhaps be due to a considerable reduction in the number of embryos produced by oysters

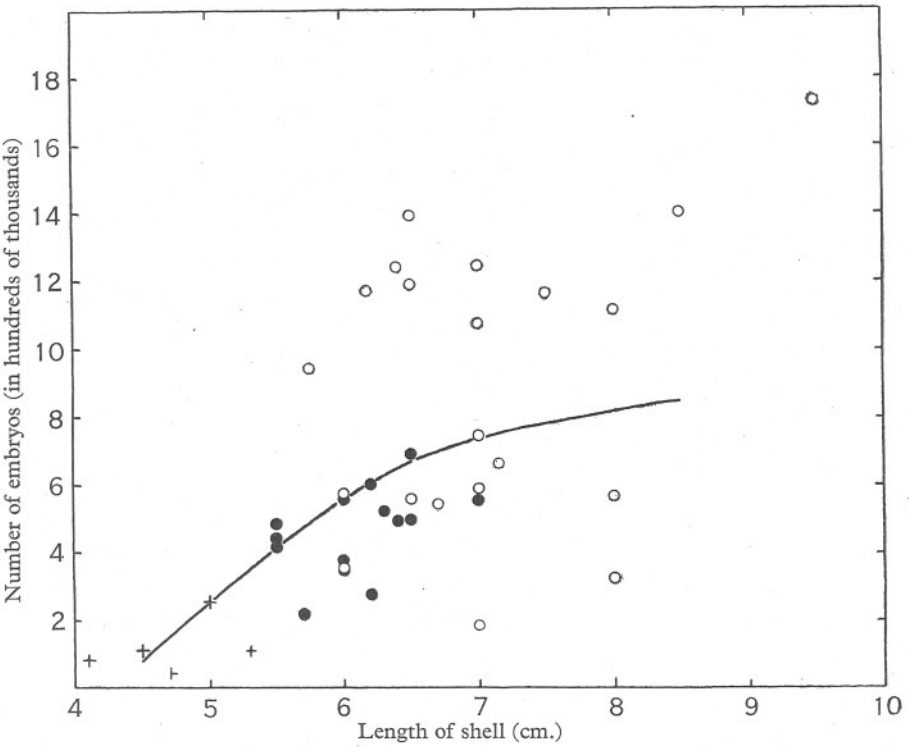

Fig. 1. The relation between length of shell and the number of embryos produced. The curve showing the general trend of the relationship has been drawn through points representing the mean number of embryos per brood in groups of oysters of all ages in the size categories 4·0–4·9, 5·0–5·9, 6·0–6·9 cm., etc. The single observation in the group 9·0–9·9 cm. has been ignored. + = 2-year-old. ● = 3-year-old. ○ = 4-year-old and over.

maturing as females in the second half of the season after an early male phase, when compared with individuals which enter on the female phase as soon as the season begins. This possibility is further discussed later in this paper (p. 255).

The curve showing the general relationship between shell length and the number of embryos produced (Fig. 1) has been drawn through points representing the mean brood size of groups of oysters of shell length 4·0–4·9, 5·0–5·9, 6·0–6·9 cm., etc.; the single oyster in the size-group 9·0–9·9 cm. has

been omitted. The trend of this curve appears to indicate that it is unlikely that an average brood of more than 1,000,000 will be produced throughout the whole season, even by a population of very large oysters.

The colour to the naked eye of a brood of embryos as it lies in the mantle cavity of the parent oyster is not a very reliable guide to its state of development, for, as can be seen from the tables, certain broods recorded as being light or medium grey in colour contained larger larvae than those which

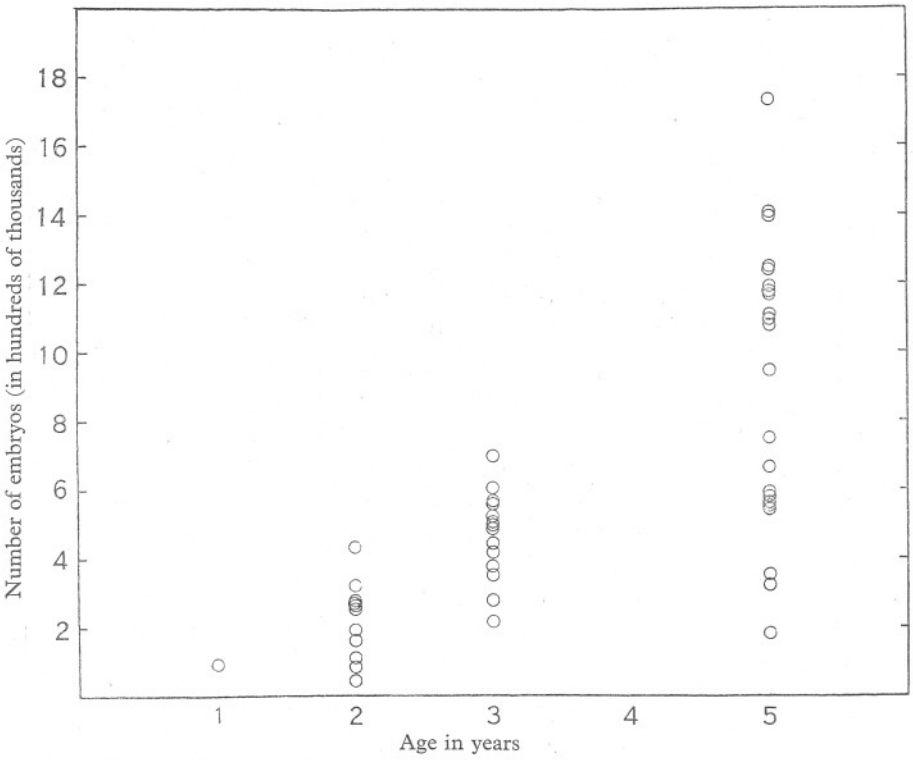

Fig. 2. The relation between the number of embryos produced and the age of the parent oyster. All oysters over 3 years of age have been grouped arbitrarily at 5 years.

appeared almost black to the naked eye. As anyone who has an extensive experience of oyster larvae will confirm, the degree of pigmentation of individual larvae after liberation is very variable.

The figures given in this paper tend to show that as regards the production of larvae an oyster practically doubles its capacity each year during the first 4 years of its life and that it is only when 4 years old that an oyster may be looked upon as adult, and playing its full rôle in the reproduction of the species. This is in close agreement with the opinion of most practical oystermen who are agreed that a 3-year-old oyster is readily distinguishable as a juvenile, while one 4 years old is not.

The data obtained from 1- and 2-year-old oysters do not differ markedly from those of Dantan (1913) and Gaarder & Bjerkan (1934), if the latter represent separate estimates, but the figure for 3-year old oysters given by these authors seems rather high and approximates to that of many adult oysters.

The data presented above provide a fairly satisfactory answer to the query as to whether breeding oysters held under tank conditions produce larvae as freely as similar oysters living on the natural beds. The oysters from the Conway tanks are themselves mainly relaid oysters from Brittany and are best compared with similar relaid Brittany oysters from the Helford River. The results of such a comparison are slightly in favour of the oysters from the Helford River, with an average brood of 1,066,400, as against 858,900 for those from the Conway tanks, but the average size of the former lot was some 5 mm. greater. Compared with Helford River natives of about the same size, the oysters from the Conway tanks are slightly superior in the production of larvae. We may therefore conclude that the retention of breeding oysters in large tanks such as those at Conway for a period of 4 months does not impair their reproductive capacity, for it has already been shown (Cole, 1939) that the percentage maturing as females under tank conditions may reach or slightly exceed 50 %, the figure arrived at by Orton (1936) working on the natural beds. In making the above estimate (viz. 50 % females) I had assumed that each oyster produced a brood of 1,000,000 embryos; had I employed the average figure of 858,900 arrived at in this paper, the percentage maturing as females would be slightly increased (see Cole, 1939, Table XXI, p. 39).

There is no indication from the figures presented in the tables of any substantial loss or mortality of embryos during the period of development within the mantle cavity, for very large broods of both early embryos and shelled veligers occur in practically all groups. The amount of variation in shell size between the larvae of a brood is itself variable, although in those broods carried by adult oysters the variation rarely exceeds 0·015 mm. In some of the younger oysters the larvae of a brood varied however as much as 0·03 mm., but a marked difference in development between parts of a brood, such as would indicate successive partial spawnings at a few days' interval, was never observed, although Orton (1936), who has examined a great many oysters carrying larvae, records that such a phenomenon is occasionally observed.

Only in one instance were veligers measuring 0·20 mm. across the shell found in the mantle cavity (see Table IV), the maximum size being usually 0·19 mm., although Orton (1927) has recorded larvae up to 0·22 mm. The usual size of larvae when liberated in the Conway tanks is 0·18 or 0·19 mm., but both slightly smaller and slightly larger larvae are not uncommon. Under natural conditions in the Helford River during 1939 I frequently observed larvae as small as 0·15 mm. in the plankton, such larvae appearing very pale.

As can be seen from the tables, those broods which showed most variation in size amongst the veligers occurred principally late in August and more

frequently among immature oysters (see Tables III and IV). It has been observed both at Conway and Port Erin (Bruce, Knight & Parke, 1939) that all broods of larvae are not equally viable and that, as a general rule, those broods which show least variation in size amongst the individual larvae show also the best survival under experimental conditions.

A comparison between 2- and 3-year-old oysters bred in the tanks at Conway and oysters of similar age from the Helford River shows the Conway

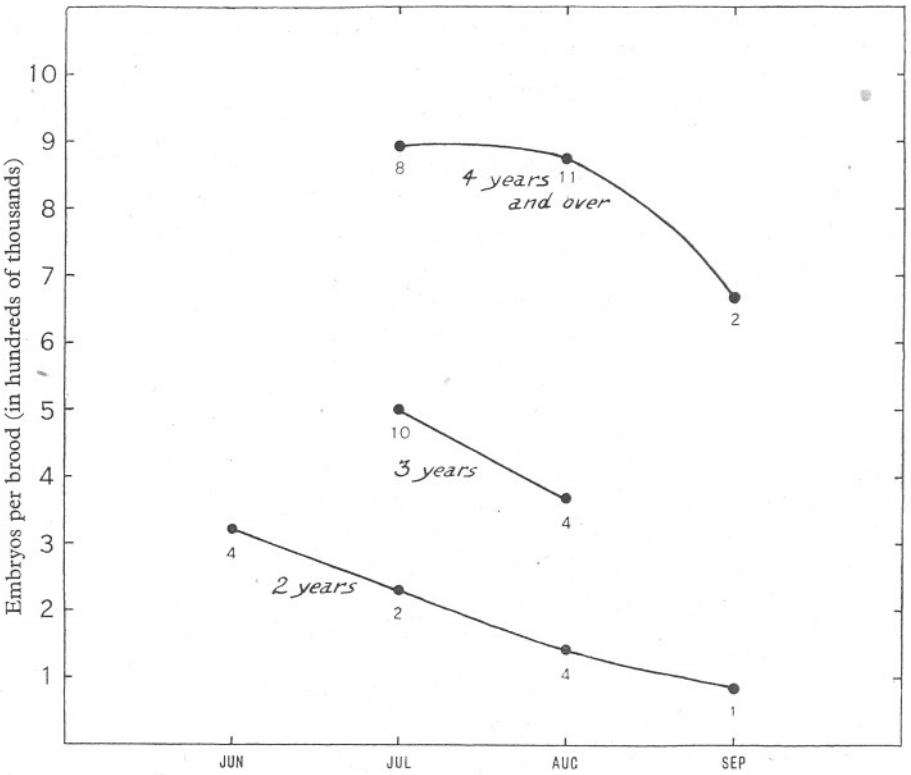

Fig. 3. The variation in the mean number of embryos produced per oyster as the season advances. The observations have been grouped according to the month in which they occur. The figures under the points on the curves indicate the number of observations in each group.

oysters to be distinctly in advance, and is an indication of the viability and general soundness of oysters bred under enclosed conditions. In making this comparison it should not be forgotten, however, that the oysters from the Helford River were collected rather late in the season, during August, and there is a possibility that such late breeding oysters may produce smaller broods than those breeding during June and July. In Fig. 3 the observations have been grouped according to the month in which they occur and curves

have been drawn through points representing the mean size of brood for each month in the three year-classes of oysters. The single adult oyster with an unusually large brood has been omitted, as its inclusion produced an abnormal displacement of the mean. All three curves fall more or less sharply in the second half of the season and, although the number of observations is small, it suggests that in oysters of all ages there is a general falling off in the number of embryos in a brood as the season advances.

The peculiar grouping of the observations of brood strength in adult oysters, as shown in Fig. 2, strongly suggests that we are dealing with two distinct categories of female spawners differing considerably in brood strength. By taking the mean of each apparent group of observations, the figures obtained for the average number of embryos produced by oysters in the two classes

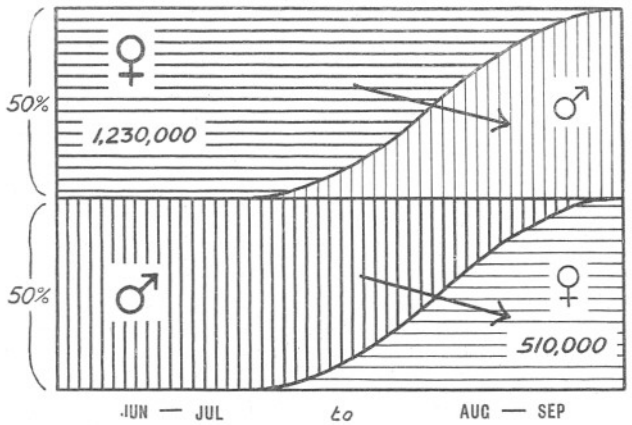

Fig. 4. Diagrammatic representation of the possible effect of sex change during the breeding season on the relative intensities of the male and female phases. The data show the probable mean number of embryos produced by each of the two categories of females.

are 1,230,000 and 510,000 respectively. Taken in conjunction with Fig. 3, which shows a marked decline in brood strength as the season advances, these figures suggest that those oysters spawning as females during the second half of the season produce, on the average, rather less than half as many embryos as those spawning earlier. If this is so, and it is recognized that the observations are few, the most likely explanation appears to be that oysters maturing as females after an early male phase have expended a part of their reserves in maturing a crop of sperm, and consequently are only able to ripen a comparatively small batch of eggs, whereas those oysters maturing as females from the outset of the season, without a preliminary male phase, are able to draw upon the whole of the reserves accumulated during the spring.

As the season advances, the percentage of the population spawning either as males or females, without a preceding phase of the other sex, must clearly decline, thus accounting for the shape of the curves in Fig. 3; but it is not

possible to fix a definite date after which, for instance, all oysters spawning as females will have experienced a previous male phase early in the season. In unfavourable circumstances, for example in abnormally cold seasons, it is possible that the ripening of those oysters maturing as females from the outset of the season may be delayed until late July or even August. Experience of breeding oysters held in tanks supports this view, for in 1938, for example, although the Conway tanks contained over a thousand oysters no substantial spawning of females occurred until the third week in July (see Cole, 1939).

We arrive therefore at the conclusion that the majority of adult oysters probably experience a sex-change during favourable seasons, and that the early phase, either male or female, is a strong one, quantitatively speaking, and is followed by a very much weaker phase of the other sex. Fig. 4 represents an attempt to give diagrammatic representation to these possibilities. For the purpose of this diagram it has been assumed that each adult oyster experiences a sex-change every season and functions as both male and female each season. The evidence at present available suggests that this occurs only in favourable circumstances, but further investigation is likely to show that the percentage of female spawners is higher than at present supposed. It should perhaps again be stressed that the observations are few and the conclusions are therefore extremely tentative. They are advanced with the particular desire to call attention to the singular grouping of the observations of brood strength among adult oysters, and the interesting lines of inquiry which these observations suggest.

It is interesting to compare the fecundity of adult *Ostrea lurida*, which rarely exceed 5 cm. in diameter, with that of juvenile *O. edulis* of the same shell size. A 3-year-old Conway oyster of 5 cm. (see Table III) gave a brood of 440,000 embryos, while the 2-year-old Conway oysters averaged 290,800 (Table I). Although the sizes of the oysters composing the latter group were not noted at the time, the usual size of oysters of this age is about 5 cm. The broods produced therefore compare favourably with those of adult *O. lurida*, for which the average figure given by Hopkins (1937) is 250,000–300,000. *O. lurida* has, however, a distinct biological advantage for, due principally to the lengthy breeding season, each individual may be expected to mature as a female during each season, while in favourable years the proportion maturing as females *for the second time* may reach 75 % (see Hopkins, 1937).

Now that the practice of selling four grades of oysters, instead of one or two as in former years, seems well established in this country, it is as well to take stock of the effect of this change on the oyster fisheries. On the majority of British oysterages, except those few which still deal exclusively in the true natives, the stock of marketable oysters is maintained to a very large extent by relaying annually in March or April large quantities of imported French oysters. These oysters may in some instances remain on the beds for two or even three or four breeding seasons before being dispatched to market, but a great many are sold during the winter following that in which they are laid

down. Those carried forward to another winter season are usually young oysters 2 years and 8 months old when purchased. The contribution made by such relaid oysters from France towards the natural spatfall on our beds is a matter for dispute among practical oystermen. It is possible that the passage from France, and the disturbance which accompanies taking up and replanting during the spring, may so upset the maturation of the gonads of these oysters that they do not breed effectively during the following summer; this point has not yet been fully investigated. It is, however, probable that during subsequent summers spent on English beds they behave in a very similar manner to native oysters. Evidence collected by the writer appears to indicate that this is so. Protagonists of the view that such Brittany relaid oysters do not give rise to any quantity of spat point to the fact that the natural spatfall obtained in rivers where Brittany oysters are laid down shows little, if any, improvement since the practice of importing these oysters began. This is undoubtedly true, but such poor spatfalls may be the result of the operation of a great many adverse factors the nature of which is not at present understood.

Since four grades of oysters are now sold almost everywhere, the beds are practically swept clean every winter of large oysters, those remaining in early spring being mainly very small stunted oysters or young oysters which were '2-year-olds' (actually 2 years and 8 months) when laid down. In March or April the grounds are replanted with a fresh consignment of '2- and 3-year-old' oysters from France, but, as indicated above, the rôle played by these oysters is uncertain. Therefore the stock of acclimatized oysters consists in the main of very small rejected adult oysters and beds of oysters 3 years old, together with such native half-grown oysters as the ground carries. It is therefore of some interest to know to what extent these young oysters may be expected to contribute to the stock of larvae in the rivers and hence to the spatfall. As already noted, the observations given in this paper tend to show that a gravid oyster doubles the number of embryos it produces each year during the first 4 years of its life, and that only when it is 4 years old does it play its full part in the reproduction of the species. We may therefore say that as regards the number of embryos which it produces a 4-year-old oyster is equivalent to at least two 3-year-old or four 2-year-old oysters. The balance is probably in fact more heavily weighted in favour of the use of adult oysters for breeding stock, for it is not yet established that among such 2- and 3-year-old oysters the percentage maturing as females is as high as among adults; the evidence at present available suggests quite the reverse. Although work which I have now in hand tends to show that the percentage of oysters maturing as females in their first year (i.e. their second summer) may, in favourable seasons, be larger than hitherto supposed, yet it is not likely that it will be found to exceed 25 %, and it is probable that on investigation the percentage of 2-year-old oysters maturing as females will be found to be less than 50 %; on the other hand, among well-nourished adult oysters it is likely that more

than 50 % of the population will spawn as females in favourable seasons and perhaps, in exceptional circumstances, the whole population. If one assumes that the proportions of female spawners among populations of 2-, 3- and 4-year-old oysters are 40, 60 and 80 % respectively, in any given season, then, taking into account the known differences in brood strength, it is clear that as regards its contribution to the stock of larvae one 4-year-old oyster is equivalent to nearly three 3-year-old, or to eight 2-year-old, oysters. Further investigation is therefore likely to lend additional weight to the view that adult oysters are much to be preferred as breeding stock.

Thus it will be seen that the answer to the question whether it is wise to denude a bed of practically all large oysters each winter will depend very much upon whether the newly relaid Brittany oysters breed effectively during their first summer on English beds. If these oysters can be shown to breed early enough and freely enough to give a crop of spat capable of surviving the winter, then there is no reason for supposing that the removal of the major part of the stock of acclimatized adult oysters is likely to lead to any diminution in the natural spatfall. As mentioned earlier, the breeding of these *freshly laid* oysters has not yet been fully investigated, but the general opinion seems to be that they breed very late in the year on our beds during their first summer and that the spat settling is rather too small to survive in any numbers over the winter.

On beds where French oysters are not laid down, and which at the present day generally carry a rather meagre stock of native oysters, it is probable that the selling of several grades of oysters, resulting as it does in practically clearing the beds of all adult oysters, is definitely detrimental to the continuance of the fishery and the proper replenishment of the stock by the natural spatfall. On such beds it seems advisable to establish a reserve of large oysters for breeding purposes, and to replenish this reserve each year, to the extent of about 20 % of the stock, so as to allow for the annual mortality.

Summary

It is shown that the average number of embryos produced by a gravid oyster is approximately doubled each year until the oyster is 4 years old, when it may be regarded as adult. Estimates have been made of the quantities of embryos produced by one 1-year-old, eleven 2-year-old, fourteen 3-year-old and twenty-two adult oysters; the mean values for these year-classes are 91,600, 218,100, 462,600 and 902,900 respectively.

Since it is probable that the proportion of a population of oysters which matures in the female phase increases progressively up to the age of 4 years, it follows that adult oysters are for this reason superior to 2- or 3-year-old oysters as breeding stock, quite apart from the increased number of embryos produced by each oyster.

As the season advances there appears to be a marked decrease in the average

number of embryos produced by oysters of all ages maturing as females. In adult oysters the observations of brood strength appear to be grouped around two points and this suggests the existence of two distinct categories of adult female-spawning oysters differing considerably in the average number of embryos produced. No such separation into two groups can be observed in the observations of brood strength in 2- and 3-year-old oysters. The average brood strengths of the two classes of adult females appear to be about 1,230,000 and 500,000. It is suggested that this great difference is due to the existence of two types of female spawners, the one maturing as females from the outset of the season, and the other functioning as males early in the season and, following a sex-change, spawning again as females towards the second half of the season. In the first class of females all the reserves accumulated during the spring are available and the batch of eggs ripened is consequently very large, whereas in the second class a part of the reserve materials has already been utilized in maturing an early crop of sperms, with a consequent marked reduction in the number of eggs ripened. The tentative nature of these conclusions is stressed.

In the light of the data obtained concerning the relative fecundity of adult and half-grown oysters, the effect is considered of the practice, now general on British beds, of selling several grades of oysters, thereby depleting the beds of large oysters during the breeding season. It is deduced that on beds where French oysters are relaid each spring little diminution in the natural spatfall is likely to result, provided that it can be shown that these relaid French oysters breed satisfactorily during their first summer on British beds. This matter has not yet been fully investigated. On beds where new stocks of oysters are not laid down each year, it is suggested that a breeding reserve of large oysters should be established if the normal winter marketing operations result in serious depletion of the stock of such large oysters, as undoubtedly often happens on some beds.

Acknowledgement

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METRIC VARIATIONS IN POPULATIONS OF *CARCINUS MOENAS*

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(Text-figs. 1–8)

Introduction

In the years 1893, 1895 and 1898 careful measurements were made by Weldon and Thomson (Weldon, 1894, 1898) of the dimensions 'frontal width' and 'carapace length' (Fig. 2) in large numbers of young male *Carcinus moenas* from the beach at Plymouth. From a consideration of these data Weldon found that there was a continuous decrease in the ratio, frontal width/carapace length, with increase in body size, and further that at any particular body size the ratio was progressively smaller in succeeding years. He concluded that natural selection was at work on the population, differentially removing individuals with relatively wide frontal aperture. He suggested that since the building of the breakwater across Plymouth Sound in 1813 the amount of silt in the waters of the Sound had been continuously increasing, so that a relatively wide frontal aperture to the branchial chamber of *Carcinus* became increasingly deleterious to the animal. Weldon thought that his laboratory experiments bore out his conclusions. Both conclusions and experiments have been severely criticized by Cunningham (1928) and others, and there seems little doubt that the criticisms are sound.

The problem of the undoubted decrease in the ratio frontal width/carapace length remained. However, Huxley's demonstration (1932) of the widely occurring phenomenon of differential growth between different dimensions of the same body suggested a possible explanation of the change in the ratio in question, with increase in body size. The graph obtained by plotting frontal width against carapace length on double logarithmic paper (Needham, 1935) showed the straight line characteristic of most cases of differential growth. The graphs for the three years were three parallel straight lines, indicating that differential growth was essentially the same in all. There remained the problem of the vertical separation of the curves, that is, the successive decrease in the initial ratio frontal width/carapace length in succeeding years. The present work, an extension of Weldon's work on natural populations, was intended chiefly as an investigation of this problem. In addition, the suggested explanation of the change in the ratio with increase in body size was tested by measurements of the growth of actual individuals in the laboratory (Fig. 3).

Material and Methods

Three different populations were studied in three successive years, in the neighbourhood of Belfast. It was hoped that by choosing a locality in Larne Lough (Mill Bay) with much silt, one at Greencastle, in Belfast Lough, with

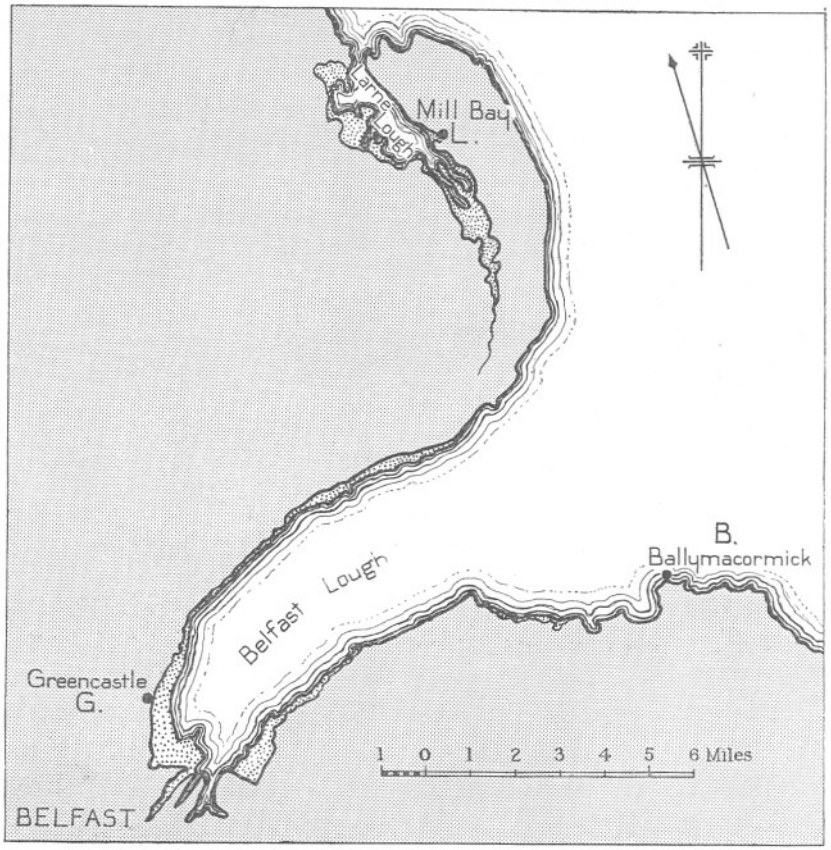

Fig. 1. Outline map of the north-eastern coast of Ireland, in the Belfast district, to show the three localities selected for the study of *Carcinus* populations, and the differences in their situation.

a moderate amount of silt, and Ballymacormick, facing the open waters of the Irish Sea and therefore comparatively free of silt, to detect any possible effect of this factor on the dimensions studied. The three localities will subsequently be referred to as L, G, and B respectively (see Fig. 1), followed by '36, '37 or '38 for the three years (1936, 1937, 1938). Samples of male *Carcinus* were collected from the three localities as nearly as possible at the same time each autumn. Orton's observations on the growth of individuals

in the field (Orton, 1936) indicated that they reach maturity in a single year, so that the bulk of each sample belonged to one season and was therefore homogeneous in this respect. The samples varied between 180 and 450 in number and covered a range of body size from 4 to 50 mm. Weldon's samples were much larger and covered a much smaller range of body size (10–15 mm.). The same dimensions as those of Weldon were measured (Fig. 2), but 'dentary margin' has not been extensively used in the subsequent analysis. Measurements were made with fine callipers on all individuals above 7 mm. carapace length. The smaller specimens were measured under a binocular microscope with a micrometer eyepiece. The error of measurement is estimated at not more than 1·5 %.

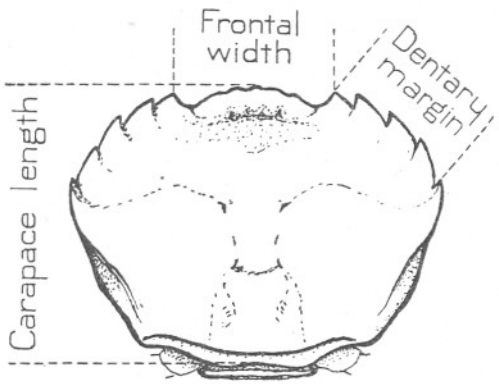

Fig. 2. Outline of carapace of *Carcinus moenas* to show dimensions measured.

The two dimensions, frontal width and dentary margin, were plotted against carapace length on double logarithmic paper. Group points were plotted, the grouping of individuals being at equal intervals on a logarithmic scale, except in the case of G '36 where the grouping was on a linear scale. There appeared to be no essential difference in the resulting graphs, but the logarithmic grouping gives even spacing of the points all along the logarithmic graph.

In the case of frontal width/carapace length the significance of possible differences between populations, in their growth curves, was tested mathematically. The Plymouth graphs (Needham, 1935) had taken the form of parallel straight lines corresponding to the equation of simple allometry (Huxley & Teissier, 1936), $y = bx^{\alpha}$, where α (the slope of the line) is constant in the three years, but b (the initial ratio y/x) decreases in succeeding years. In a case like this it is legitimate to test only for the significance of differences in b and assume that α is quite constant. An appropriate test, originally due to Teissier (1935), has been devised by Reeve and is here applied to the Belfast data.

The data for the nine populations were pooled and plotted on a single graph, double-logarithmically (Fig. 6). From this a mean value of α for all

nine, was obtained. Actually it was thought preferable to divide up the data into three sections according to body size, the sections to contain approximately equal numbers of individuals. The sections will subsequently be indicated by the suffixes 1, 2 and 3 to the appropriate population (L '36_1 and so on). Thus the data are divided into twenty-seven sections altogether. In correspondence with the division of the data three consecutive straight lines have been fitted to Fig. 6, their slopes giving the values of α appropriate to the three sections; the probability that α did, in fact, change with increase in body size was the main reason for dividing up the data. Although representing equal numbers of individuals the three sections do not cover equal ranges of body size (Fig. 6).

Using the appropriate mean values of α in each section, the value of b for every individual (about 3500 in all) was calculated from the allometry equation $y = bx^{\alpha}$, the calculation being performed in two steps (x^{α} and then y/x^{α}) using a log-log slide rule. From the series of values of b the mean, $\bar{b}$, was calculated for each of the twenty-seven sections and also the corresponding variance of b (Tables I–III). Any two populations were then considered to be significantly different in any section, if the difference, $\bar{b}_2 - \bar{b}_1$, between their mean values of b was greater than twice the standard error of the difference (i.e. more than twice the square root of the sum of their variances of b). The test was applied in two ways, which may be referred to as the general and the detailed tests. In the former, annual differences were tested by pooling the data of the three localities, and local differences in the same way by pooling the data for three years (Table I). This has the advantage of eliminating minor irregularities. In the detailed tests, the data for single populations were compared (Tables II, III). A significant difference is indicated by S and a non-significant difference by o, together with the sign of the difference. Differences greater than bare significance are marked by more S's, three times the standard error by SS, four times by SSS and so on.

Mr G. M. Spooner has kindly checked the results by an alternative test based on analysis of the variance.

Results and Conclusion from the Graphical Methods

Measurements of the growth of individuals show clearly that a decrease in the ratio frontal width/carapace length with increase in body size is manifest in the growth of the individual (p. 261). The data are shown in graphical form in Fig. 3, the short lines joining points representing the dimensions of successive exuviae. The slope of these lines varies around that of the whole population (dotted line), and the individual lines themselves are more or less normally distributed around the population curve. The extent of individual variation is perhaps worth noting. All measurements were carefully made by both workers, and there is little doubt that most of the variation is real. The population curve is a mean curve only (cf. Davenport, 1934; Needham, 1937).

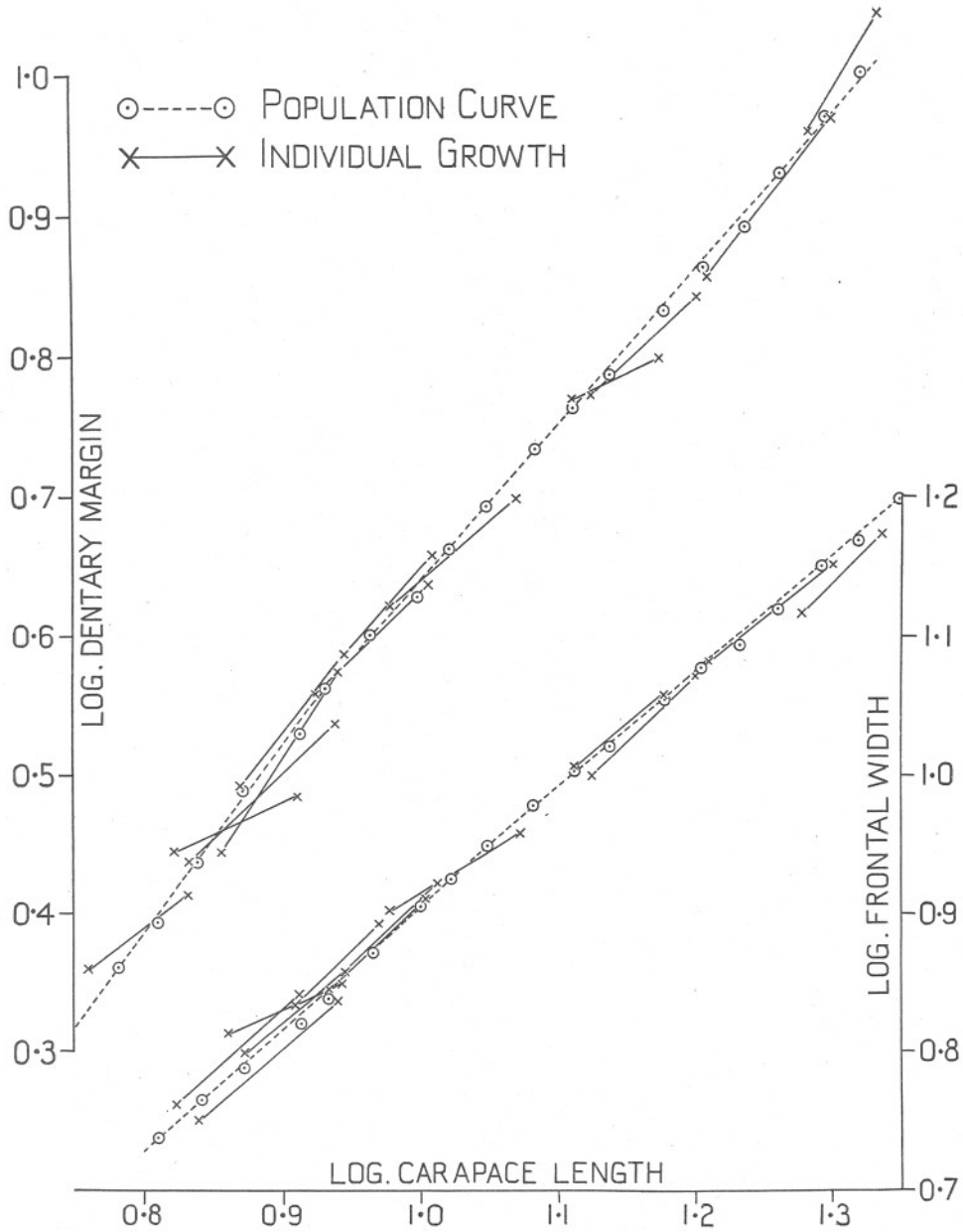

Fig. 3. Growth in frontal width and dentary margin relative to carapace length in individual male *Carcinus* kept in the laboratory, plotted for comparison, on the curve (dotted line) for the whole population in the same year and locality (Greencastle, 1937). Double logarithmic plotting. The lines join dimensions of successive exuviae.

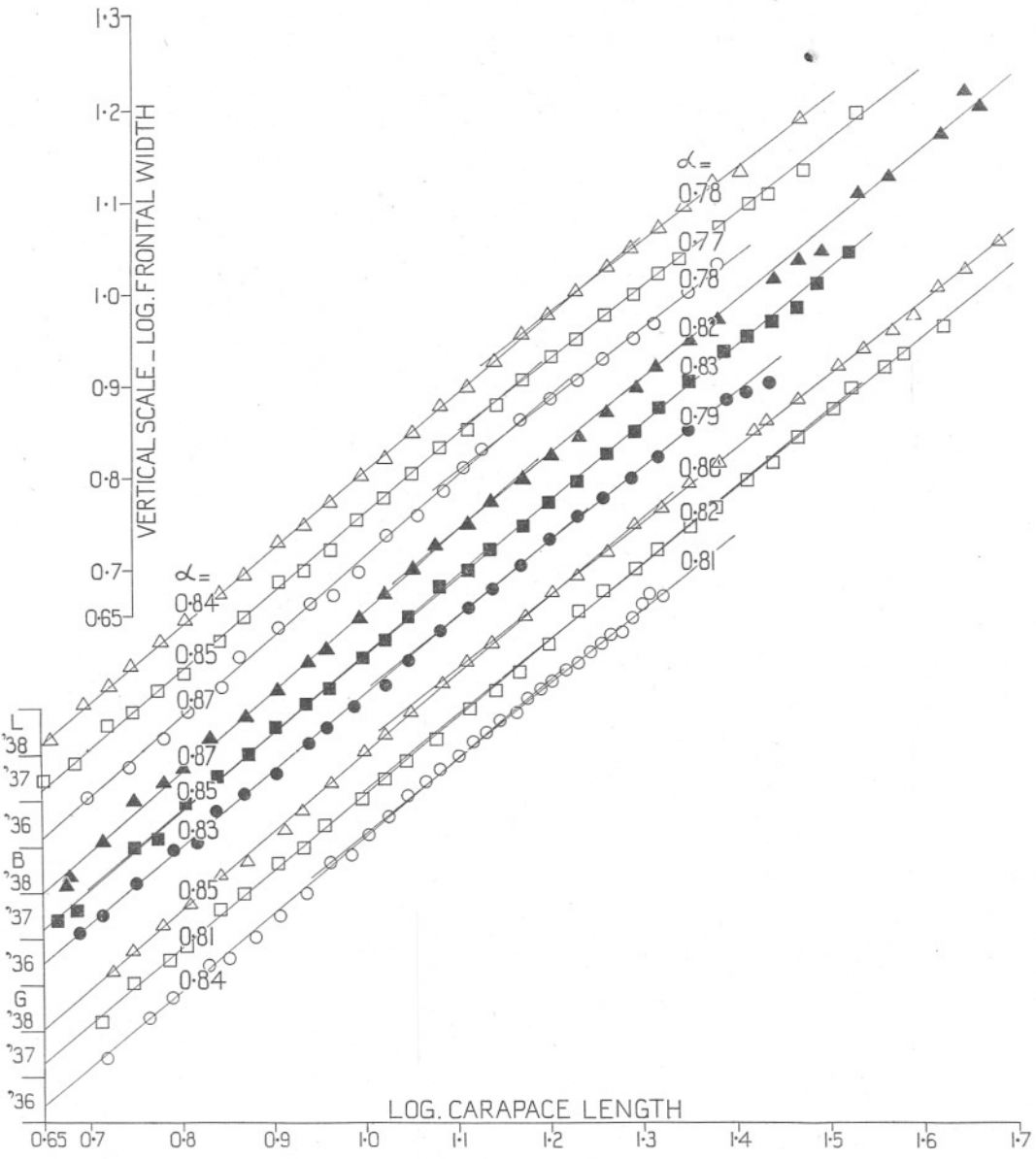

Fig. 4. Growth in frontal width relative to carapace length in nine populations of *Carcinus moenas* (males), from three localities, Greencastle (G), Larne (L), and Ballymacormick (B), in three successive years, 1936, 1937, 1938, to show the differences between the populations. Double logarithmic plotting. The curves are separated out, for clarity, by one large square of the graph paper, in the direction of the ordinate.

The curves of relative growth, frontal width/carapace length, for the Belfast populations (Fig. 4) are essentially similar to the Plymouth curves (Needham, 1935). It is clear, however, that the value of α is not absolutely constant over their much greater range of body size (p. 263), but decreases at larger body sizes. Two consecutive straight lines give reasonable fitting to the

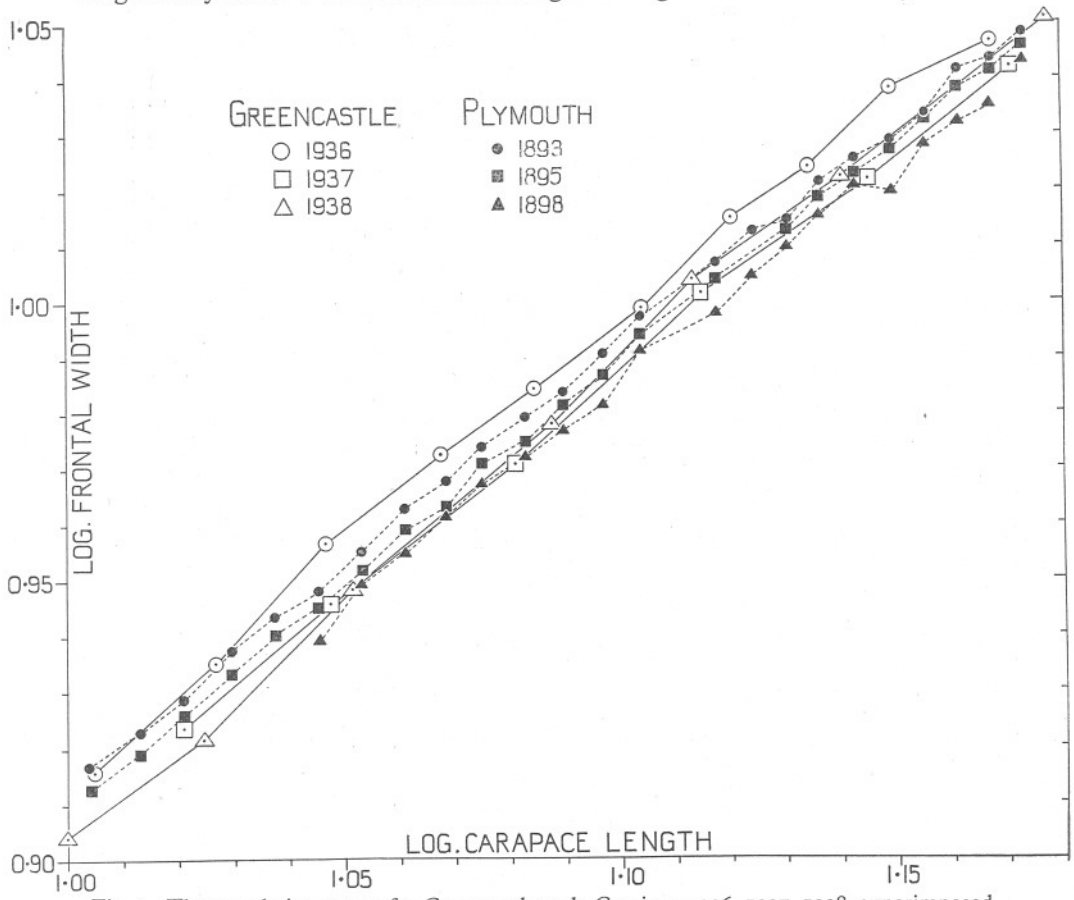

Fig. 5. The population curves for Greencastle male *Carcinus*, 1936, 1937, 1938, superimposed on those for Plymouth males, 1893, 1895, 1898, to show how the two sets overlap, and the parallel annual trend in the two localities. Double logarithmic plotting.

points, but it is probable that there is a continuous fall in α and that the three lines of Fig. 6 give a nearer approximation. For the sake of clarity the curves in Fig. 4 have been separated by one large square of the graph paper, in a vertical direction. At G and B there is a clear fall in b in '37, '38. The annual decrease is also shown by the wider gap between the curves L '38 and B '36 and between B '38 and G '36 (2-year interval) than between the curves in each locality, spaced at 1-year intervals. The value of α varies

somewhat in the different curves (0·81–0·87 for the first, and 0·77–0·83 for the second section), but an estimate of the standard error for one population (B '36) indicated that variation up to 0·08 was not significant. The division of the curves into two sections prevents a direct comparison with the Plymouth curves, but this has been done graphically in Fig. 5, where the latter are superimposed on the corresponding section of the G curves. The latter are possibly slightly higher than the Plymouth curves (higher value of b) and α is also possibly higher, but the differences are much less than the annual differences in the two localities. Since there is an interval of 40 years between the two groups of data it seems probable that the annual trends are not maintained over such long periods. At the same time they were, however, steadily maintained over a 5-year period at Plymouth, and the 2- and 3-year intervals show a correspondingly greater fall in b than the 1-year intervals of the Belfast data. The greater scatter among the points on the graphs of the latter is explained by the much smaller samples (p. 263).

Fig. 6 shows clearly that there is a real difference between the different populations in mean frontal width at corresponding body sizes. The grouping by body size was the same for all populations (except G '36, which is very distinct from the rest, therefore), so that the variation in carapace length (horizontal scatter of each group point) is due solely to chance variation in small samples. This variation is very small in the central region of the graph where there were large samples in the group. The variation in frontal width (vertical scatter of the points), on the other hand, shows considerable variation even in this region.

There is an interesting difference between different groups in the extent of this variation in frontal width, even in the central region. It is small in the groups with log carapace length = 1·0, 1·3 respectively, while in intermediate groups it shows a regular increase up to the group with log carapace length = 1·17, followed by a regular decrease.

The relative growth of dentary margin/carapace length is also essentially as at Plymouth, the value of α being about 1·2. Dentary margin shows positive allometry, therefore, whilst frontal width shows negative allometry. Carapace width shows virtual isometry relative to carapace length (plotting from the data of Day (1935)), so that the growth of the two sections of the carapace width (Fig. 2) is, as it were, compensatory. The possibility that b for dentary margin/carapace length shows regular trends as in the case of frontal width has not been investigated; it might possibly be expected to show an annual increase, in view of the 'compensatory' tendency noted above.

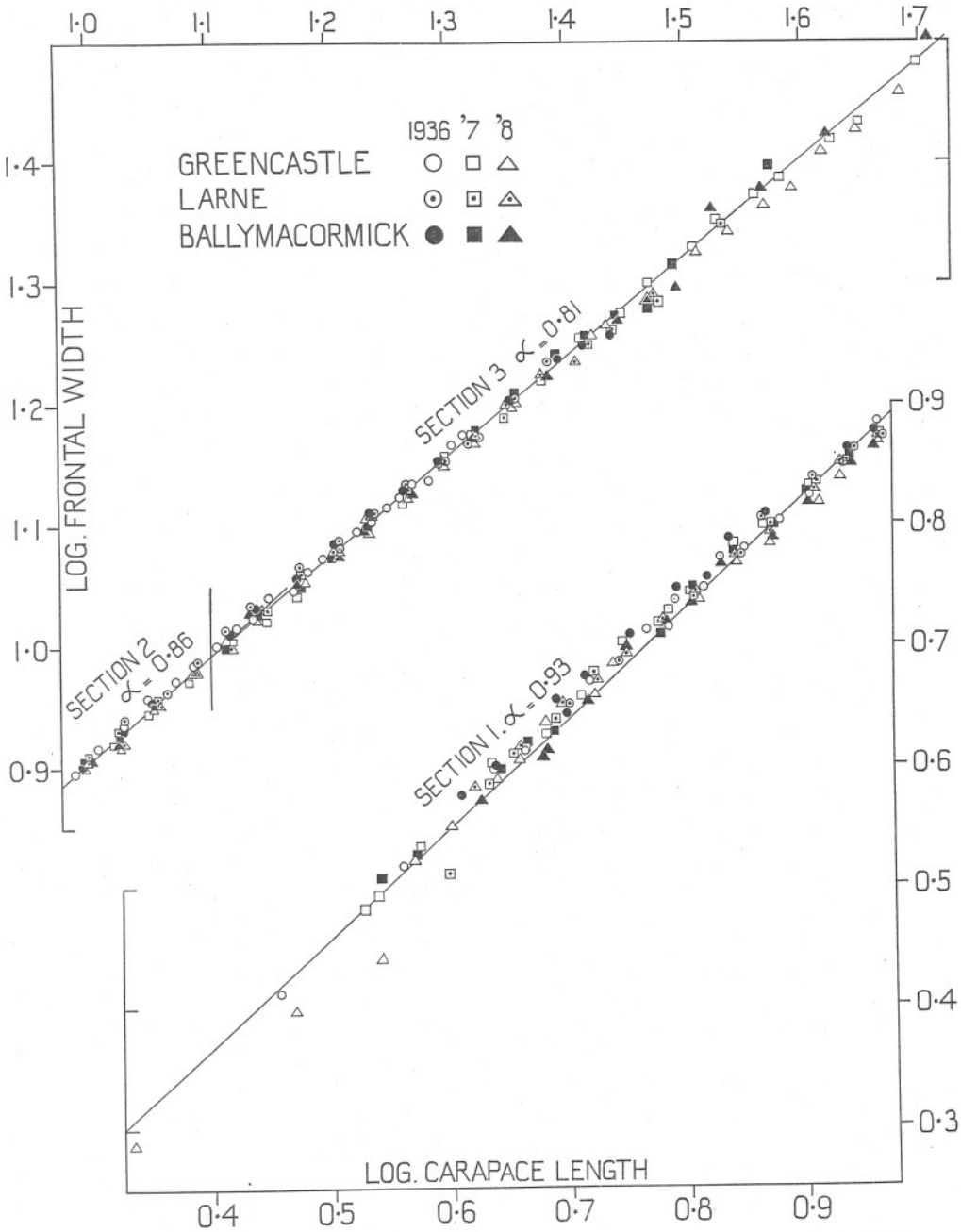

Fig. 6. Relative growth, frontal width/carapace length. Data (group points) for all nine Irish populations plotted on a single graph. Double logarithmic plotting. The curve is divided into three sections (1 in the lower graph and 2 and 3 above) containing approximately equal numbers of individuals. The slope of the line best fitting each section gave the values of α (0·93, 0·86, 0·81) used for the mathematical tests. It also shows: (1) variation in frontal width (vertical scatter of points) in each group is much greater than that in carapace length (horizontal scatter) due to chance variation in small samples only; (2) considerable differences between different groups in the extent of this variation in frontal width.

Notes on the Mathematical Tests

The assumption that α is the same for all nine populations, in any section, is fundamental to Teissier's test (p. 263); but, as indicated above (p. 267, Fig. 4), it is probable that α shows slight variations between the different populations, and it seems advisable to inquire how this might affect the validity of the tests. For an individual of average size (12 mm. carapace length) the substitution of $\alpha = 0{\cdot}85$ for $\alpha = 0{\cdot}80$ would produce a change in b of about 0·05, far greater than any difference recorded between the $\bar{b}$ values for any two populations. The range of values of α shown on Fig. 4 indicates that the error in applying a mean value of α to a particular curve might well be of this order. Moreover, every point on the curve will be subject to this error, since the method of calculating b amounts to drawing a line through each point parallel to the mean curve. Again, in comparing two population curves, one having a real α value above the mean and the other below the mean, the difference $\bar{b}_2 - \bar{b}_1$ will be doubly affected by this error. If the true $\bar{b}_2$ were not less than the true $\bar{b}_1$ the apparent difference would be too large, while if it were less the errors would tend to cancel each other out; recorded differences may therefore be too large or too small. What they actually represent is a difference in b and α combined, the effect of α being often considerable (cf. Reeve, 1940, Text-fig. 4). Where two curves diverge their difference will be exaggerated, and where they approach or cross the difference may be reduced to insignificance. It seems probable that much of the apparent irregularity in significance from one section to another in the detailed test (Table II) may be due to this cause; the considerable reduction in this irregularity in the general test is also explained—the actual irregularities are partly smoothed out and therefore not so grossly exaggerated by subsequent treatment.

There are, however, a number of reasons for not discarding the tests as valueless. The contrast revealed between the magnitude of annual differences and that of local differences is independent of the factor just considered. The consistent trends in annual, local and sectional differences in variability of $\bar{b}$ and in $\bar{b}$ itself must also be real. The differences clearly show the relation between any two curves, whether they diverge, approach or cross, by the sign and magnitude of the difference in successive sections, so that an accurate picture is obtained which could not be accepted with confidence from the graphs alone. Moreover, since both b and α do apparently vary between populations, a figure based on both may legitimately be considered the best estimate of the extent of the difference between populations. In any case b depends on the value of α in previous sections of the growth curve, and the two parameters are interdependent (cf. Lumer, 1939). The values of $\bar{b}$ shown in the tables give the mean position of that section of the curve in a vertical direction (i.e. direction of the ordinate)—the 'positional' value of Reeve (1940, p. 69). The differences between these $\bar{b}$ values give the extent to which the curves are separated in the vertical direction, throughout the section, and not

merely at the beginning as would be the case with true values of $\bar{b}$. Thus the tests give valuable information about the differences between populations. Their only shortcoming is that they do not distinguish between differences due to b and those due to α. The Plymouth graphs strongly suggest that $\bar{b}$ is much the more variable, and there is support from the Belfast data. Teissier (1936) found that local races of *Homarus*, *Haliotis*, *Littorina*, etc., differ in b but not in α, and the greater variability of b has been demonstrated by many workers.

In further justification of the tests it should be borne in mind that the use of a value of α (i.e. the adopted value) other than the true value increases the

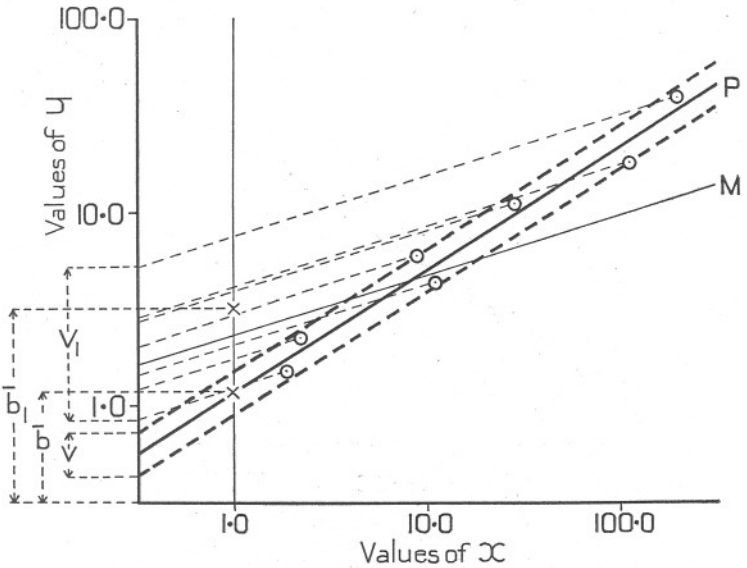

Fig. 7. Diagram to show the effect on $\bar{b}$ and on the variation of b produced in a population curve by the substitution of the mean value of α for the true value. M = mean curve for all populations. P = curve for population in question. b and v = true value of b and of its range of variation. $\bar{b}_1$ and v_1 = corresponding values obtained by using the mean value of α.

variance of b whether it increases or decreases the value of b itself (Fig. 7). The variance involves the square of the standard deviation, and since a difference, $\bar{b}_2 - \bar{b}_1$, must exceed $2 \times$ the square root of the sum of the two variances to be significant, there is every reason to believe that the test of significance is much more demanding than it would be with the true values of $\bar{b}$.

A possible source of error exists where the distribution of individuals is not uniform along the whole curve (or section). In the case of two curves not parallel to the mean curve, if one has many individuals near the beginning of the section and few in the second half, whereas the reverse holds for the other curve, then even if the two are virtually coincident (their α and b values both being the same) they will show very different apparent values of $\bar{b}$ (Fig. 8).

In the present work every effort was made to ensure even distribution of individuals, and in any case those body sizes which were poor in individuals (the smallest and the largest) corresponded fairly closely in all populations.

If α is not constant over any considerable range of body size, but changes continuously throughout each section, as seems very probable, the value of $\bar{b}$ obtained from the tests will be affected by this too. Here again the tests are of value; they still give an estimate of the *average* positional difference between two curves, in each section.

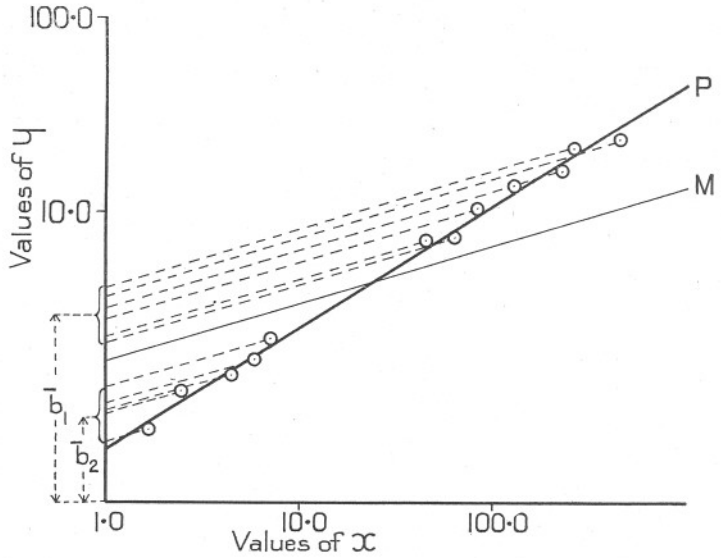

Fig. 8. Diagram to show how, when using the mean value of α instead of the true value, two coincident population curves give widely different values of b if there is not an even distribution of individuals along the curve. M = mean curve for all populations. P = coincident curves of the two populations in question. $\bar{b}_1$ and $\bar{b}_2$ = the mean values of b for the two populations.

Results of the Mathematical Tests

The value of b is given by y when $x = 1$ in the allometry equation. Since the unit of x is a purely arbitrary quantity it may be taken as the value of x at the beginning of the particular section of the graph under consideration. In any case it will increase from section $1 \rightarrow 2 \rightarrow 3$, following the fall in α. The change is far greater than the differences between the populations within any section, but this does not affect the significance of the latter (Tables I–III), which depend only on the values of b within that section.

From the general test (p. 264) it is seen (Table I) that the value of $\bar{b}$ shows a regular annual decline, '36 > '37 > '38, and that there is a comparable regular sequence among the three localities, L, B, G, the widest frontal aperture being

TABLE I. THE GENERAL TEST

A. Yearly differences in $\bar{b}$, all localities combined

Year	Section	No. of individuals	$Sb/n=\bar{b}$	Variance of $b\times n$	Variance of $\bar{b}\times 10^6$	Comparison between years	Section	Difference $\bar{b}_2-\bar{b}_1$	Standard error of difference	Sign and significance of difference
1936	1	228	0·9798	0·3588	6·93330	1936–7	1	−0·002142	0·003027	−0
	2	458	1·1342	0·4558	2·17791		2	−0·011701	0·001913	+SSSSS
	3	320	1·2712	0·2360	2·31211		3	−0·008552	0·001921	+SSS
1937	1	421	0·9819	0·3939	2·22758	1937–8	1	−0·012239	0·002032	+SSSSS
	2	496	1·1225	0·3647	1·48542		2	−0·004127	0·001723	+S
	3	381	1·2627	0·1990	1·37464		3	−0·007568	0·001726	+SSS
1938	1	404	0·9697	0·3101	1·90477	1936–8	1	−0·010097	0·002973	+SS
	2	374	1·1183	0·2069	1·48313		2	−0·015828	0·001913	+SSSSSSS
	3	368	1·2551	0·2172	1·60852		3	−0·016120	0·001980	+SSSSSSS

B. Local differences in $\bar{b}$, all years combined

Locality	Section	No. of individuals	$Sb/n=\bar{b}$	Variance of $b\times n$	Variance of $\bar{b}\times 10^6$	Comparison between localities	Section	Difference $\bar{b}_2-\bar{b}_1$	Standard error of difference	Sign and significance of difference
Greencastle	1	425	0·9760	0·5562	3·08657	G–L	1	−0·002285	0·002345	−0
	2	535	1·1233	0·4993	1·74787		2	−0·003970	0·001957	−0
	3	423	1·2573	0·2921	1·63658		3	−0·009509	0·001726	−SSSS
Larne	1	327	0·9782	0·2574	2·41440	G–B	1	−0·000299	0·002492	−0
	2	347	1·1273	0·2504	2·08576		2	−0·003022	0·001849	−0
	3	387	1·2668	0·2004	1·34126		3	−0·007752	0·002095	−SS
Bally-macormick	1	301	0·9763	0·2817	3·11949	L–B	1	+0·001986	0·002316	+0
	2	446	1·1263	0·3318	1·67204		2	+0·000948	0·001939	+0
	3	259	1·2651	0·1840	2·75299		3	+0·001757	0·002022	+0

found in the most silty locality. However, whereas the annual differences are quite significant (except '36_1–'37_1), local differences are not (except G–L_3 and G–B_3). Mr Spooner's analysis of variance leads to the same conclusions: there are significant annual differences in all sections but local differences are only significant in section 3. The annual differences on the other hand decrease slightly, section $1 \rightarrow 2 \rightarrow 3$.

The detailed tests (p. 264) reveal a number of irregularities which repay further investigation. The analysis of variance shows that the irregularities are greater than the variance within samples and are therefore significant. They are of the order of magnitude of the local differences but much smaller than the annual differences. The irregularities are greatest in section 1 and about half as great in 2 and 3. It seems possible that they are of the same nature as the local differences, that is, due to quite local causes.

The differences between '36 and '38 are consistently more significant than over either of the 1-year periods (Table II), showing that the annual differences are definitely progressive. This is most marked at B; at G, L there are often marked differences over a 1-year interval. In no 1-year interval is the difference significant in all three sections, but only at G '36–'37 is it insignificant in two of the three sections and usually it is far in excess of the limit of significance. In both G and B the order of the significance for 1, 2 in '36–'37 corresponds to that for 2, 3 in '37–'38 ($-$o and $+$SSSSS for G, and $+$o and $+$SSS for B), and this might be taken to indicate that the annual growth of *Carcinus* was approximately one section of the range of body size. However, the observations of Orton (1936) definitely disprove this. In any case the feature is not shown by L and is probably quite fortuitous.

L is anomalous in a number of respects. Its annual differences are smaller and less consistent than at G and B. In L_1 there is a significant increase in b from '36 to '38, instead of the usual decrease, and similarly in L_3 '37–'38.

Annual differences are greatest at Greencastle, the intermediate locality, and least at Larne.

The detailed tests bear out the general conclusion that local differences are less marked than annual differences: only 14/27 differences are significant as against 21/27 for the latter (Table II, B). Four of the nine comparisons show two sections insignificant (one only in the annual tests) and in seven of the nine the sign of the difference changes between sections 1 and 3, indicating that the two curves in question have crossed each other. In three sections ('36_2, '37_3, '38_2) none of the differences are significant, and only in '37_2 and '38_3 are all the three differences significant.

The value of the standard deviation of $\bar{b}$ has been calculated by Mr Spooner and shows interesting features. It decreases regularly '36 > '37 > '38 as does $\bar{b}$ itself, but the decrease is relatively greater; the decrease is shown in all sections (1 > 2 > 3) and in all localities (L least). Similarly there is a regular sequence in the localities G > B > L, involving all sections (1 most) and all years ('38 least). On the other hand, it is noteworthy that the locality sequence

TABLE II. THE DETAILED TEST. TEST FOR SIGNIFICANCE OF DIFFERENCES BETWEEN MEAN VALUES OF b FOR DIFFERENT POPULATIONS

Year, place and section			Difference $\bar{b}_2 - \bar{b}_1$	Standard error of difference	Sign and significance of difference
A. Yearly differences in each locality					
Greencastle		$'36_1 - '37_1$	−0·008889	0·0050170	−o
		$'37_1 - '38_1$	+0·024508	0·0033407	+SSSSSS
		$'36_1 - '38_1$	+0·015619	0·0047223	+SS
		$'36_2 - '37_2$	+0·021141	0·0030878	+SSSSSS
		$'37_2 - '38_2$	−0·000728	0·0028660	−o
		$'36_2 - '38_2$	+0·020413	0·0028940	+SSSSS
		$'36_3 - '37_3$	+0·001239	0·0030720	+o
		$'37_3 - '38_3$	+0·017874	0·0028627	+SSSSS
		$'36_3 - '38_3$	+0·019113	0·0029460	+SSSSS
Larne		$'36_1 - '37_1$	−0·012786	0·0057155	−S
		$'37_1 - '38_1$	−0·003160	0·0032420	−o
		$'36_1 - '38_1$	−0·015946	0·0055009	−S
		$'36_2 - '37_2$	+0·005934	0·0030020	+o
		$'37_2 - '38_2$	+0·009408	0·0030578	+SS
		$'36_2 - '38_2$	+0·015342	0·0031353	+SSS
		$'36_3 - '37_3$	+0·012393	0·0031780	+SSS
		$'37_3 - '38_3$	−0·004677	0·0023130	−S
		$'36_3 - '38_3$	+0·007716	0·0033029	+S
Ballymacormick		$'36_1 - '37_1$	+0·006632	0·0041770	+o
		$'37_1 - '38_1$	+0·017908	0·0039674	+SSS
		$'36_1 - '38_1$	+0·024540	0·0045804	+SSSS
		$'36_2 - '37_2$	+0·008349	0·0029770	+S
		$'37_2 - '38_2$	+0·004014	0·0028480	+o
		$'36_2 - '38_2$	+0·012363	0·0031220	+SSS
		$'36_3 - '37_3$	+0·012938	0·0039740	+SS
		$'37_3 - '38_3$	+0·009298	0·0037840	+S
		$'36_3 - '38_3$	+0·022236	0·0036730	+SSSSS
B. Local differences in each year					
1936	G–L	1	+0·013672	0·0067446	+S
		2	−0·000385	0·0044283	−o
		3	−0·009889	0·0036235	−S
	G–B	1	−0·007756	0·0054313	−o
		2	+0·003628	0·0031804	+o
		3	−0·011891	0·0035270	−SS
	L–B	1	−0·021428	0·0062193	−SS
		2	+0·004013	0·0044710	+o
		3	−0·002002	0·0039560	−o
1937	G–L	1	+0·009775	0·0036729	+S
		2	−0·015592	0·0029880	−SSSS
		3	+0·001265	0·0025690	+o
	G–B	1	+0·007765	0·0036222	+S
		2	−0·009164	0·0028770	−SS
		3	−0·000192	0·0036030	−o
	L–B	1	−0·002010	0·0036470	−o
		2	+0·006428	0·0028050	+S
		3	−0·001457	0·0032016	−o
1938	G–L	1	−0·017893	0·0028600	−SSSS
		2	−0·005456	0·0029326	−o
		3	−0·021286	0·0026702	−SSSSSS
	G–B	1	+0·001165	0·0036121	+o
		2	−0·004422	0·0028300	−o
		3	−0·008768	0·0031180	−S
	L–B	1	+0·019058	0·0036959	+SSS
		2	+0·001034	0·0030968	+o
		3	+0·012518	0·0030692	+SSS

is the reverse of that due to $\bar{b}$ itself (p. 272). In the same way the regular decrease in the standard deviation through the three sections (1 > 2 > 3) is accompanied by a considerable increase in $\bar{b}$ itself.

The standard deviation is a measure of absolute variability; by dividing it by $\bar{b}$ the coefficient of variability is obtained. The annual differences in S.D. are so great ('38 only 75 % of the value for '36) that the much smaller decline in $\bar{b}$ itself (1½ % only) has little effect and the coefficient of variability also

TABLE III. THE DETAILED TEST. THE MEAN OF b AND ITS VARIANCE IN THE DIFFERENT POPULATIONS

Year, locality and section			No. of individuals	$\bar{b}$	$(Sb)^2/n$	$S(b^2)$	Variance of $b \times n$	Variance of $\bar{b} \times 10^6$
Greencastle	'36	1	119	0·9788	114·0038	114·2587	0·2549	18·1527
		2	218	1·1356	281·1373	281·3675	0·2302	4·8662
		3	118	1·2644	188·6444	188·7129	0·0685	4·9616
	'37	1	146	0·9877	142·4222	142·5707	0·1485	3·7015
		2	176	1·1145	218·6014	218·7452	0·1438	4·6688
		3	159	1·2632	253·6925	253·8050	0·1125	4·4781
	'38	1	160	0·9632	148·4291	148·5347	0·1056	4·1509
		2	141	1·1152	175·3583	175·4276	0·0693	3·5106
		3	146	1·2453	226·4045	226·4832	0·0787	3·7175
Larne	'36	1	29	0·9652	27·0117	27·0339	0·0222	27·3399
		2	51	1·1360	65·8153	65·8529	0·0376	14·7451
		3	104	1·2743	168·8738	168·9613	0·0875	8·1684
	'37	1	145	0·9779	138·6606	138·7959	0·1353	6·4799
		2	160	1·1301	204·3277	204·4361	0·1084	4·2610
		3	149	1·2619	237·2610	237·3037	0·0427	1·9363
	'38	1	153	0·9811	147·2579	147·3516	0·0937	4·0291
		2	136	1·1207	170·7989	170·8923	0·0934	5·0871
		3	134	1·2666	214·7989	215·0213	0·0608	3·3386
Bally-macormick	'36	1	80	0·9865	77·8605	77·9322	0·0717	11·3449
		2	189	1·1320	242·1835	242·3700	0·1865	5·2488
		3	98	1·2763	159·6314	159·7025	0·0711	7·4795
	'37	1	130	0·9799	124·8281	124·9305	0·1024	6·1061
		2	160	1·1236	202·0098	202·1016	0·0918	3·6085
		3	73	1·2633	116·5105	116·5542	0·0437	8·3143
	'38	1	91	0·9620	84·2150	84·2939	0·0709	9·6337
		2	97	1·1196	121·5951	121·6370	0·0419	4·4996
		3	88	1·2540	138·3914	138·4374	0·0460	6·0084

shows a marked annual decline. The difference between localities in the S.D. is much less marked than the annual differences, but the coefficient of variability shows more marked differences from the fact that the value of $\bar{b}$ itself shows the reverse order (L > B > G). This effect is even more marked in the sequence between sections where the inverse increase in $\bar{b}$, section 1 → 2 → 3, is much greater.

This last feature, a decrease in the variability of b with increase in body size, does not necessarily imply a corresponding decrease in the variability of y. It can be shown by calculation that a decrease in the variability of b is not inconsistent with a constant *relative* variability of y (i.e. with an increasing *absolute* variability of y, the S.D. increasing as $\sqrt{y}$). It would be interesting to know whether, in the present case, y does show constant relative

variability—for a decreasing variability of b is consistent with a considerable range in the variability of y, both above and below the standard of constant relative variability.

It is clear that the above phenomenon results from the fact that the value of b depends not on that of x or y alone but on the ratio between them. A decreasing variability of b with increase in body size implies an increasing correlation between y and x (i.e. a more constant ratio y/x). This result seems to demand an explanation but cannot be considered further at this point.

Discussion

The results of the present work show a close similarity to those of Weldon (1894); the ratio frontal width/carapace length decreases with increase in body size in *Carcinus moenas*, and it may further show a progressive decrease, at corresponding body size, from year to year. The observations on individual growth (Fig. 3) are sufficient to show that the former is a feature of differential growth in the individual, as the population curves, derived from Weldon's Plymouth data, had suggested (Needham, 1935). In this connexion, however, it is only fair to point out that since the population curve is only the mean of very variable individual curves (p. 264) it is possible that its essential form might not be changed if some natural selection agency were systematically eliminating individuals with relatively wide frontal aperture, or with a relatively high value of α. At present, however, there is no evidence concerning the possible effect of such a factor on the curve of relative growth.

Previous criticisms (Cunningham, 1928, etc.) have probably been sufficient to prove that the amount of silt in the water could not be such a factor in the present case, but the deliberate choice of localities in the Belfast studies, according to the amount of silt, has enabled a clear demonstration of this. Frontal width is consistently greatest at Larne, where, according to Weldon's hypothesis, it should be smallest; it is smallest at Greencastle, where it should be intermediate, so that silt cannot be a determining factor.

The yearly decline in the ratio is undoubtedly the most interesting feature of the data. The previous suggestion (Needham, 1935) that it depended simply on an initial difference in the ratio at the outset of growth seems reasonable. Teissier (1934) has suggested similar differences in proportion in the Zoea larva to account for the same phenomenon in the closely allied genus *Portunus*. Since local differences are so insignificant in comparison with annual differences (p. 274) it seems probable that the cause of the annual differences in larval proportions must be sought in factors which affect all localities equally, for example, climatic, affecting the atmosphere, or hydrographic. The annual changes are clearly not continuous over long periods; at Plymouth b decreased by 0·007 in 5 years, so that in about 650 years it would approximate to zero! That the Belfast data overlap the Plymouth data of 40 years earlier also suggests that periods when b decreases are offset by others showing an annual

increase. Whether the change follows a regular cyclic course, as in the case of fluctuations in animal numbers in many species (Elton, 1924), or is quite irregular cannot be decided without more data. There is strong evidence for the latter alternative however (Kemp, 1938). Hydrographic conditions in the Atlantic show considerable annual changes which appear to be quite irregular. It seems a priori probable that for marine animals hydrographic conditions may be the most important influence, far more so than direct climatic conditions.

It is of interest that there is a distinct sex difference in b in the same year (evidence from the data of Weldon), since local differences in any year are so small. However, this is presumably a genetic difference, quite independent of environmental factors.

The insignificance of local differences is indeed remarkable. The three localities differ in respects other than that of silt. Larne is very sheltered, with a very muddy substratum and with an admixture of fresh water, while B is a rocky coast exposed to the open sea; in all respects G is intermediate. Thus a number of local differences might be expected to combine to produce distinct local differences in relative proportions. The absence, in fact, of any such marked effects suggests that the animals may be relatively insensitive to most differences in local conditions. Considerable differences between local populations of terrestrial species are often recorded (Dice, 1940), but there it is probable that genetic differences may be at work; there is much less isolation among marine forms.

Such small local differences as there are (Table II B) do not follow the expected sequence; G is not intermediate and the order is L → B → G (p. 272). There is only one obvious factor which distinguishes G from the other two and might account for its position in the sequence: there is a local effluent from Belfast, and the general effect of a large city on the Lough may be considerable. The sequence G → B → L does, further, agree with their geographical latitude.

The anomalous features of the populations at L (p. 274) may well be due to its sheltered position, causing a general damping down of all environmental fluctuations. The constant outflow of fresh water might also have some effect, particularly on marine hydrographical changes. The tendency towards annual increases in b in some sections of the L data may well be due to these factors.

Local differences clearly exist (p. 274), but they are smaller and less consistent than annual differences. The fact that they tend to be more marked in section 3 than at smaller body sizes (p. 274) suggests a continuously operating type of influence, that is, a local environmental factor. At the same time it is just possible that initial differences are alone responsible: they would automatically increase with body size.

The probability that α changes continuously with body size means that the law of simple allometry (p. 267) does not strictly apply. This does not detract from the essential value of the law, however, and the assumption of a constant

value of α over a section of the growth curve is justified by the facilities it provides for testing differences between populations, varieties and species. Reeve (1940) and others have already shown the value of the law, even as an approximation, in solving taxonomic problems. The idea of a regular change in proportions with increase in body size, and the very simple equation which effectively expresses the relation, have opened up possibilities which have not yet been fully exploited.

Another apparent criticism (p. 264), namely, that the curve of relative growth obtained by plotting the data of a large sample (population curve) is only a statistical mean and not an expression of individual growth (Davenport, 1934) does not detract from the value of the curve. The growth of an individual is as much a statistical mean (of the activity of its cells) as that of a population or of a species. The population curve is a population characteristic and may be used with the same confidence as any recognized character, morphological or otherwise. Again, the superiority of a mathematical estimate over purely descriptive morphological characters, as a permanent record, is undoubted.

No reason for the change in relative frontal width with increase in body size has been advanced. It may be a secondary effect of the contrasted (and apparently complementary) increase in relative size of the dentary margin (p. 268). The dentary margin corresponds roughly to the branchial region of the carapace and if, as seems probable, the surface area of the gills increases in simple proportion to the volume of the body as a whole, the dimension in question might be expected to increase relative to other dimensions of the body (roughly to the power $\sqrt{\frac{3}{2}} = 1{\cdot}23$ approx., which is roughly the value of α actually recorded for dentary margin/carapace length). If the relative decrease in frontal width is a compensatory result of this it must further be supposed that other considerations demand approximate isometry between total carapace width and carapace length.

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It is hoped to deposit the original data, too bulky to be included here, with the library of the British Museum, Natural History.

Summary

1. Measurements of *Carcinus*, comparable to those taken by Weldon at Plymouth 40 years ago, have been made on material from three Irish localities, the observations in each area extending over three years. The three localities afford different environments, one having no silt, one a moderate amount and the other much silt.

2. The results support the view that the change in the ratio frontal width/carapace length with increase in body size is due to differential growth in the individual and is not caused, as Weldon supposed, by the continuous removal through natural selection of those crabs with a relatively wide frontal aperture. Measurement on the growth of individuals confirms this view.

3. A striking fact which emerged from Weldon's work was that the ratio mentioned above showed a successive diminution in each of the three years covered by the observations. A precisely similar diminution has been found at each of the Irish localities, and it is shown that the annual differences are mathematically significant.

4. The results disprove Weldon's hypothesis that the change in the ratio is correlated with the slow accumulation of silt in Plymouth Sound. The Irish locality with most silt has the widest frontal aperture and the intermediate locality the narrowest. It is also shown that the yearly trend towards a lower value for the ratio cannot be continuous, for the rate of change is too rapid to be maintained indefinitely, and the values obtained in the Irish localities overlap those at Plymouth 40 years earlier. Possible explanations of the changes are discussed.

5. The differences between populations from the three localities in any year are much less marked than the annual differences at one locality, and are not generally significant. Though small, however, they do show a consistent sequence among the three localities (but not corresponding to the order for siltiness).

6. The equation of simple allometry, $y = bx^{\alpha}$, applies to the data, at any rate as a useful approximation. The mean of b, and the variance of b, show consistent annual, and local differences in magnitude, and a regular change with increase in body size. The sequence for b itself may either correspond with that for its variance or run precisely counter to it.

7. The relation of individual growth to the mean curve of growth for a whole population is indicated and the value of the latter discussed.

8. A possible explanation of the differential growth of the dimension frontal width is advanced.

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SACCOGLOSSUS HORSTI SP.N., AN ENTEROPNEUST OCCURRING IN THE SOLENT

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(Text-figs. 1–8)

CONTENTS

INTRODUCTION

The species described in this paper was found at Lymington by one of the authors (C.B.G.) in the summer of 1940. It differs from the three species of this genus that have been described from the British coasts and from *Saccoglossus kowalevskyi* Agassiz, the American species, which Caullery & Mesnil (1916) claimed to have found on the French coast of the English Channel at St Martin, near Cap de la Hague. Its characters, though distinctive, are such as might lead to confusion with *S. kowalevskyi*. It seems possible, therefore, that the record from the coast of France may refer to this and not to the American species. Since St Martin is directly opposite Lymington and only some 75 miles distant, this explanation appears more probable than that the French record really refers to the American species, especially when it is remembered that, so far as is known (van der Horst, 1927–39), all other species of *Saccoglossus* have very restricted distributions. Doubtless the local character of the species of this genus, and indeed of the other members of the Harrimanidae, is associated with their direct development and it contrasts sharply with the wide distribution of several members of the Ptychoderidae, in which a free-swimming stage in development, the *Tornaria* larva, provides for dispersal.

The specific name is chosen in recognition of Prof C.J. van der Horst's many contributions to the subject and in particular his exhaustive account of the Enteropneusta in Bronn's *Klassen und Ordnungen des Tier-Reichs*.

Environment

S. horsti occurs on the Hampshire coast, near the mouth of the Lymington River which runs into the western end of the Solent. The species extends about half a mile both east and west of the mouth of the river, but does not go up the estuary, where the mud soon becomes foul and black below the surface. It occurs in the deep grey mud (see Appendix for analysis), which is of the most glutinous type, from just above low-water mark spring tides to a short way above low-water mark neap tides. The animals live about 4–8 in. below the surface in rather diffuse burrows and no 'casts' could be distinguished at the surface. They occur also higher up the shore at about half-tide mark in the chunks of dead rhizomes of *Spartina Townshendii* Groves which are scattered on the surface of the soft mud, having broken away from the edge of the *Spartina* flats which fringe the Solent. Their burrows in this situation are in the under surfaces of those chunks of rhizomes which are not deeply embedded in the mud. Such pieces lie in pools of water and so the animals below them are never completely uncovered at low tide. The proboscis was found protruding from the mouth of the burrow when the chunk was lifted, while the tail went vertically upwards into the mass of dead roots. They do not occur in those masses so deeply embedded in the mud as to have their under surfaces foul and black, nor in the mud itself at this level.

The environment of *S. horsti* thus is strikingly different from that of *S. cambrensis*, Brambell & Cole (1939*a*), which occurs in Wales in relatively clean sand and fine shell gravel and is absent from adjoining stretches of mud flats. Moreover at Hurst Castle, 4 miles to the west of the Lymington River, there are flats uncovered at low-water spring tides, grading from quite clean to distinctly muddy sand, in which *S. horsti* could not be found. It was observed in the laboratory that when living specimens of *S. horsti* were put in a dish over mud they soon burrowed down, while over sand they lay on the surface and made no attempt to burrow but formed loose tubes of sand grains and mucus in which, however, they seemed to live quite happily. It appears, therefore, that *S. horsti* is essentially a mud-living species whereas *S. cambrensis* is sand-living.

S. horsti occurs in association with the amphipod, *Corophium volutator* (Pallas). It is very common in the mud and more numerous than all the annelids put together; in a good patch there may be half a dozen specimens in a trowelfull of mud.

Material and Technique

S. horsti is fragile and liable to fragment when handled, yet it is more robust and fragments less readily than *S. cambrensis*. Moreover, it is much more amenable to narcotization and fixation. Although each of the authors is well acquainted with either one or other of these two species neither has had an

opportunity of examining both in the living condition. Therefore these conclusions are based on comparison of preserved specimens and of notes on the preservation. The specimens show in a striking manner that much more perfect examples can be obtained of *S. horsti* than of *S. cambrensis* and that they can be narcotized and preserved in a much more expanded state. Commercial specimens of *S. kowalevskyi* indicate that probably this species is still more readily preserved intact and in a flaccid condition. It may be significant

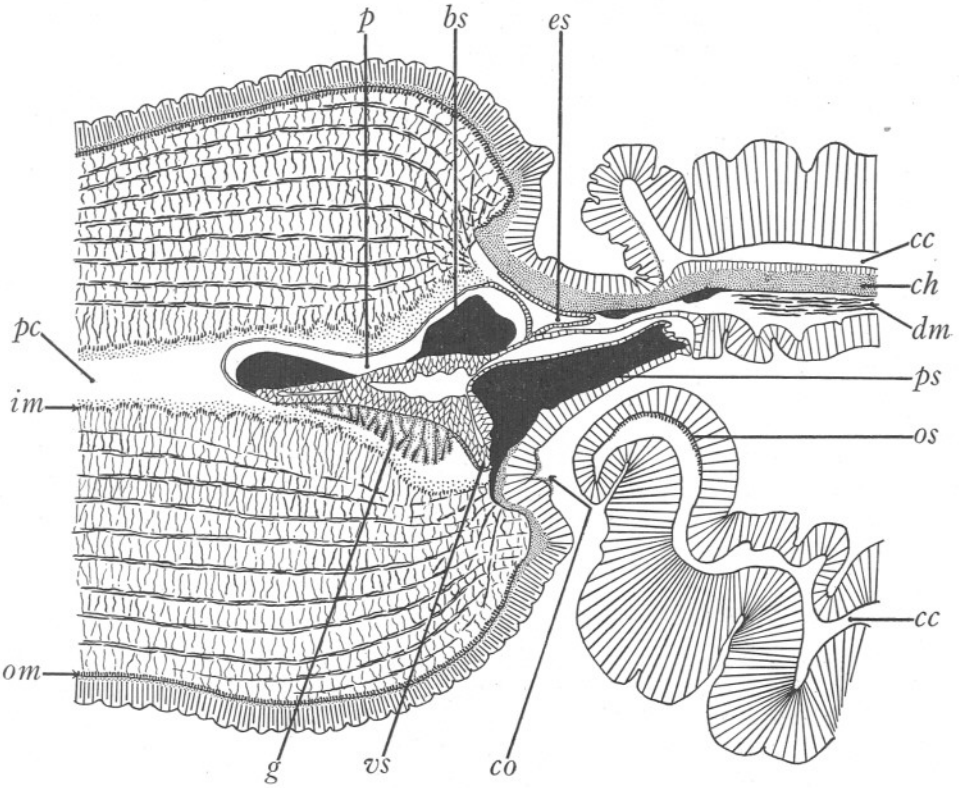

Fig. 1. Longitudinal sagittal section of the base of the proboscis. ×37. *bs.* blood sinus; *cc.* coelomic cavity of collar; *ch.* nerve cord of collar; *co.* preoral ciliary organ; *dm.* longitudinal muscle fibres of trunk in perihaemal cavity; *es.* end-sac; *g.* glomerulus; *im.* inner circular muscle fibres of proboscis; *om.* outer circular muscle fibres of proboscis; *os.* oral sphincter; *p.* pericardium; *pc.* coelomic cavity of proboscis; *ps.* body of proboscis skeleton; *vs.* ventral diverticulum of stomochord.

that these differences are associated with differences in the epidermis and in the development of the musculature, which in turn may be related to the nature of the substratum in which the animal lives.

Narcotization was effected readily both by the menthol and by the gradual alcoholization methods, provided the animals were treated soon after collection. Specimens kept in the laboratory for some hours, though apparently

healthy, responded to narcotization by copious secretion of mucus and by autolysis. Specimens, both narcotized and without narcotization, were fixed either in formalin or in Bouin's fluid. Thus both expanded and contracted examples were available for comparison. Both fixatives gave excellent results. Serial sections were cut at a thickness of 10μ and were stained with Ehrlich's haematoxylin and either eosin or Tischutkin's orange G-erythrosin. The

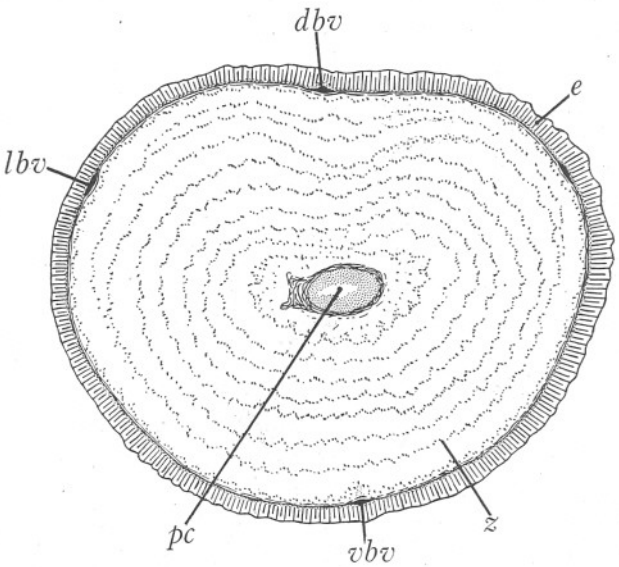

Fig. 2. Transverse section of contracted proboscis. ×24. *dbv*. dorsal blood vessel; *e*. epidermis; *lbv*. lateral blood vessel; *pc*. proboscis coelom; *vbv*. ventral blood vessel; *z*. concentric zone of longitudinal muscle fibres.

latter stain was suggested to us by Dr N.B. Eales and has proved to be especially useful for the definition it imparts to epidermal basement membranes and blood sinuses.

Specific characters

Externals

The total length of one complete specimen, but not a large one, was 20·5 cm. when narcotized. Another specimen, which was a very large one but was incomplete posteriorly, was 31·5 cm. long and it is estimated that it must have exceeded 35 cm. in length when complete. The partly contracted proboscis of large living specimens measured 1·3–1·8 cm. long and 4–5 mm. in diameter at the thickest part near the base. The proboscis of narcotized specimens, in which it appeared to be fully extended, was 2·8–3·2 cm. long. The collar in large narcotized specimens was 4·5–5·5 mm. long in the dorsal middle line and 3·0–4·0 mm. long ventrally; it was 3·5–4·0 mm. in diameter

anteriorly and 4·0–4·5 mm. in diameter posteriorly. Thus this species is slightly larger than *S. cambrensis*.

There is a very distinct dorsal groove reaching from the base to the tip of the proboscis. It is sufficiently marked posteriorly to render the base of the proboscis almost heart-shaped when viewed from above. There is also quite a distinct ventral groove reaching almost to the tip of the proboscis in the living animal. It is not apparent in material fixed with the proboscis contracted, but is distinguishable in specimens which were narcotized and in which the

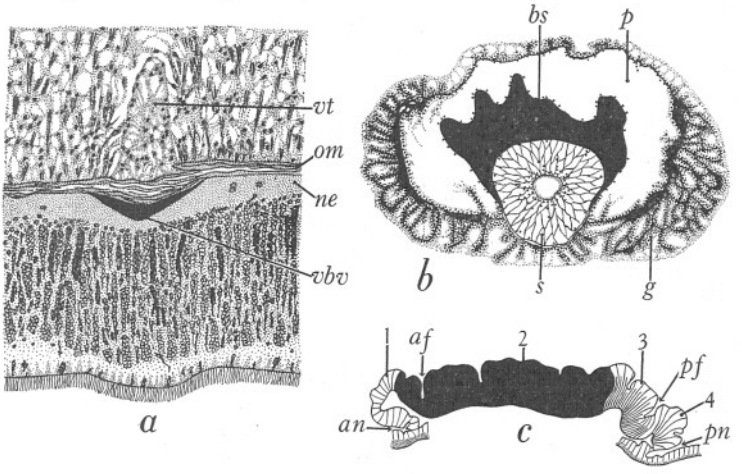

Fig. 3. *a*. Transverse section of ventral region of contracted proboscis shown in Fig. 2. ×150. *b*. Transverse section of the proboscis organs. ×60. *c*. Dorsal longitudinal sagittal section of the epidermis of the collar. The epidermal zones are numbered 1 to 4 from anterior to posterior. ×17. *af*. anterior furrow; *an*. anterior neuropore; *ne*. nerve fibre layer of epidermis; *pf*. posterior furrow; *pn*. posterior neuropore; *s*. stomochord; *vt*. ventral longitudinal tract overlying the ventral blood vessel; other guide letters as in previous figures.

proboscis was preserved in an expanded condition. A preoral ciliary organ, similar to that of *S. cambrensis* (Brambell & Cole, 1939*b*), but less apparent in the living animal, since its coloration is not distinctive, is present on the base of the proboscis.

The anterior border of the collar is slightly thickened and the posterior quarter is more definitely so. Posteriorly the margin of the collar overhangs the first two or three gill pores, forming a slight operculum.

The ventral muscle bands of the trunk project in the branchial and genital regions as a conspicuous rounded keel (Figs. 7, 8*b*). When the animal contracts this keel shortens more than the dorsal pharyngeal region. Consequently the muscular keel becomes more or less straight while the dorsal region coils sinuously around it. The gonads begin about 1 mm. behind the collar and extend a considerable distance behind the branchial region, overlapping the beginning of the hepatic region. They attain their greatest development at the anterior extremity of the oesophageal region, where they form

rounded projecting ridges on each side of the body (Fig. 7). The genital ridges and the muscle bands, though projecting distinctly, are never so pronounced as those of *S. kowalevskyi* (Spengel, 1893). The number of branchial pores varies from specimen to specimen, as is the rule in allied species. The number of gill slits in three adult specimens in which they were counted were 104 pairs, 115 pairs and, in one large specimen in which the series was not quite complete, 137 pairs. Thus they are more numerous than in any other known species of the genus.

The animal has two clearly distinguishable colour forms occurring in approximately equal numbers and intermixed in the same environment. The paler form has a yellowish white proboscis with a brown base and a rust-brown collar tinged with orange with a white ring round the posterior margin. The darker form has a salmon-pink proboscis with a brown base and with a brick-red collar with a white ring round its posterior margin. The colour of the trunk is the same in either variety. The branchial region is a rather transparent grey tinged with salmon-pink and there is no deep red patch such as is found in *S. cambrensis* just behind the collar on each side of the body. The pink gonads show through the body wall; in some specimens their colour is pronounced. The trunk immediately behind the branchial region is a rich dark brown and thereafter it gradually pales towards the posterior extremity, which is fawn. The whole of the trunk is sprinkled with numerous small raised spots that are paler in colour than their background. No carmine spots, like those in *S. cambrensis*, were observed. Throughout the trunk the dorsal nerve cord is sharply defined with a pale margin on each side.

Proboscis

The nerve-fibre layer of the epidermis is well developed throughout the proboscis, but is thickened over the basal region (Fig. 1), where the epidermis is less glandular and more strongly ciliated than elsewhere. A preoral ciliary organ, so similar to that described in detail in *S. cambrensis* that it does not merit separate description (Brambell & Cole, 1939*b*), is present. The dorsal groove of the proboscis is more pronounced than in *S. cambrensis* or *S. kowalevskyi* and the nerve-fibre layer beneath it is thickened, forming a longitudinal tract lying between the groove and the dorsal subneural blood vessel. The ventral longitudinal groove is apparent in transverse sections of the extended proboscis, but is not accompanied by any thickening of the nerve-fibre layer, which tends rather to be reduced in thickness where it overlies the ventral longitudinal blood vessel (Fig. 3*a*). The circular muscle layer is fairly well developed and is as thick as, or slightly thicker than, the nerve-fibre layer. It is not thickened in the form of a sphincter at the end of the proboscis. The longitudinal muscles are arranged in nine or more concentric rings (Fig. 2) which are apparent even at the centre, though more clearly seen at the periphery. This complete concentric arrangement of the longitudinal musculature resembles that of *S. kowalevskyi*, although it is not so clearly defined as in

that species, rather than that of *S. cambrensis*, and it distinguishes the species from the two remaining British species, *S. ruber*, Tattersall (1905) and *S. serpentinus*, Assheton (1908). The cavity of the proboscis is narrow, having a diameter at the narrowest part in the anterior half of the contracted proboscis of about one-tenth that of the whole organ. It is nearly filled with rounded coelomic cells, with very vacuolated cytoplasm and relatively small nuclei, which are either attached to its wall or floating freely in it. A thin layer of

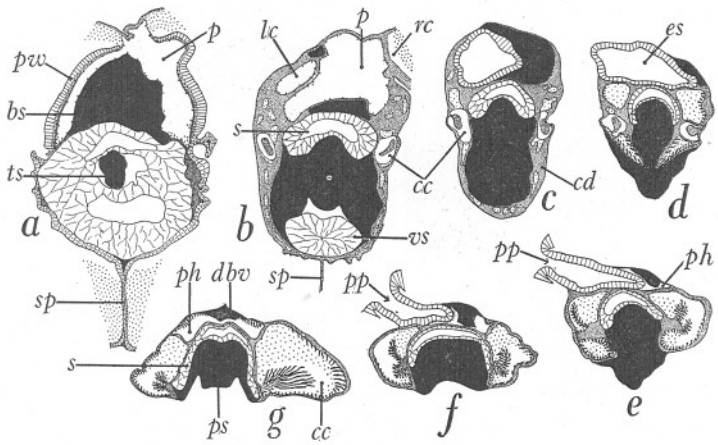

Fig. 4. Series of seven transverse sections at intervals of 100μ through the neck of the proboscis, showing the relations of the coelomic cavities, blood vessels, etc., and the shape of the body of the proboscis skeleton. Blood vessels and skeleton shown in solid black. The crura of the skeleton diverge within 50μ of the last section depicted in *g*. ×45. *cc*. extension of coelomic cavity of collar; *cd*. chondroid tissues; *lc*. left dorsal pouch of proboscis coelom; *ph*. perihaemal cavity; *pp*. proboscis pore; *pw*. pericardial wall, very thick in this region; *rc*. right dorsal pouch of proboscis coelom; *sp*. ventral septum of proboscis; *ts*. tip of proboscis skeleton; other guide letters as in previous figures.

circular muscle fibres bounds the cavity (Figs. 1 and 2), which is not enlarged at the anterior end. Within the outer circular muscle layer and overlying the ventral blood vessel the stromal tissue is slightly differentiated to form a longitudinal tract, conical in cross-section (Fig. 3*a*), which can be distinguished only in well-preserved material. This tract appears to be free from longitudinal muscle fibres. Posteriorly it is continuous with the ventral extremity of the ventral septum through which the ventral blood vessel runs. Its function is obscure, but its structure and position are suggestive of some sort of neuromotor organ. The ventral septum is very short (Figs. 4*a* and *b*), extending only about 100μ in front of the caudal ends of the shallow ventral coelomic sacs. The left dorsal coelomic sac is connected to the wide median end sac, from which the proboscis pore opens to the exterior on the left side of the neck of the proboscis (Fig. 4). The stomochord is straight with a wide and continuous lumen throughout (Fig. 1). It is provided with a single ventral diverticulum. The walls of that region which lies above the proboscis skeleton

are very thin (Fig. 4). Thus the stomochord is similar in shape to that of *S. kowalevskyi* and differs from that of *S. cambrensis*, in the absence of an S-shaped bend, in being shorter and stouter and in having thinner walls posteriorly. This character alone readily distinguishes the species from *S. cambrensis*. The large pericardium extends more than half-way round the stomochord (Fig. 3*b*). The glomerulus is continued around the stomochord

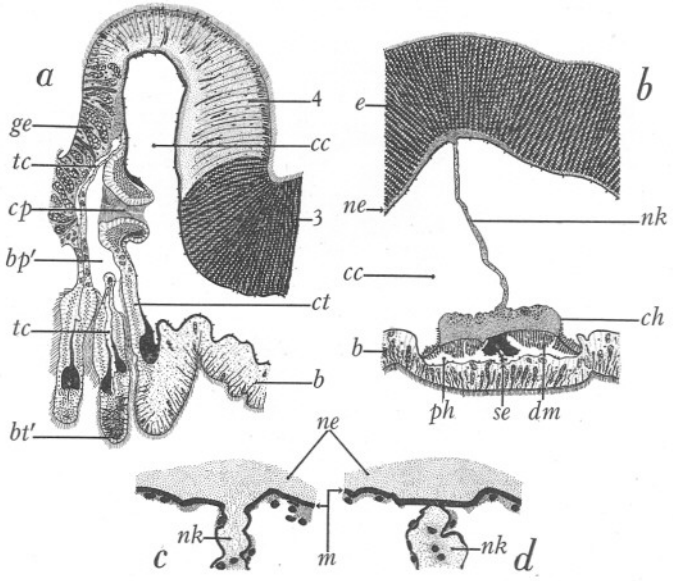

Fig. 5. *a*. Longitudinal section through the region of the collar pore. ×60. *b*. Transverse section through the dorsal region of the collar. ×45. *c* and *d*. Two transverse sections 30μ apart through the junction of the neural keel with the epidermis, showing an interruption in the epidermal basement membrane (*m*) in *c* and its continuity in *d*. ×325. *b*. epithelium of buccal cavity; *bp′*. first branchial pouch; *bt′*. first tongue; *cp*. collar pore; *ct*. collar-trunk septum; *ge*. branchial epidermis; *nk*. neural keel; *se*. septum between perihaemal cavities; *tc*. trunk coelom; other guide letters as in previous figures.

ventrally and the right and left halves extend considerably more than half-way towards the mid-dorsal line of the pericardium, though there is no dorsal glomerulus. The form of the body of the proboscis skeleton (Figs. 1 and 4) is similar to that of *S. cambrensis* and the crura embrace between one-half and two-thirds of the circumference of the buccal cavity, though they only extend backwards between one-quarter and one-third of the length of the collar from its anterior margin. No hard concretion, like that described in *S. cambrensis*, was found in the core of the proboscis skeleton of any specimen.

Collar

There are four epidermal zones in the collar (Fig. 3*c*). The first forms the anterior margin of the collar and is composed mainly of ciliated cells; the

second, in which basophil glandular cells are predominant and the epidermis is thickest, extends over the anterior two-thirds of the surface; the third does not stain so densely basophil as the second, but glandular elements are numerous in it and the epidermis is almost as thick; the fourth, which is composed mainly of ciliated cells with relatively few glandular elements, forms the posterior margin. The first, second and fourth zones evidently correspond to the first, second and fifth zones of the collar respectively in *S. cambrensis*, while the third zone probably represents both the third and fourth zones of the Welsh species. The number of epidermal folds in the collar varies according to the state of contraction, but one deep fold in the second zone near the anterior margin and another between the third and fourth zones appear to be constant and are responsible for the appearance of rims around the anterior and posterior margins.

The right and left coelomic cavities of the collar are separate from each other. The ventral mesentery is complete. Dorsally the coelomic cavities are separated by the nerve cord, joined throughout its length to the epidermis by a pronounced keel, by the perihaemal cavities, the stomochord and the proboscis skeleton. Anterior prolongations of the collar cavities extend throughout the neck of the proboscis, reaching almost to the level of the anterior extremity of the proboscis skeleton (Fig. 4). The epithelium of the collar pores is thick and the nuclei of the cells are arranged in many rows (Fig. 5*a*). The two perihaemal cavities in the collar are completely separated from each other posteriorly by the septum, in which the dorsal subneural blood vessel runs (Fig. 5*b*). They join at the level of the posterior extremities of the crura of the proboscis skeleton and the dorsal subneural blood vessel in front of this point runs in the dorsal wall of the single perihaemal cavity, between it and the nerve cord (Fig. 4). This median perihaemal cavity extends into the neck of the proboscis as far as the proboscis pore (Fig. 4*e*). The anterior extensions of the dorsal longitudinal muscles of the trunk extend throughout the length of the perihaemal cavities. The collar-trunk septum is deflected forwards on each side of the buccal cavity ventro-laterally, as in *S. cambrensis* and *S. kowalevskyi*, forming triangular diverticulae of the trunk cavities which taper anteriorly and end a short distance behind the extremities of the crura of the proboscis skeleton. Prolongations of the ventral longitudinal muscle bands of the trunk extend throughout the length of these cavities, the fibres being attached anteriorly to the posterior surface of the septum. These muscles, together with the longitudinal muscle fibres of the collar which are attached to the anterior wall of the septum and to the crura of the proboscis skeleton, form presumably the retractor mechanism of the collar and of the neck of the proboscis.

The nerve cord is solid throughout its length, the anterior and posterior neuropores, if the shallow depressions which represent them justify the name, ending blindly. The neural crest or keel, connecting the nerve cord with the epidermis throughout its length, is well developed (Fig. 5*b*). The tissues of this

keel are in direct continuity with the nerve-fibre layer of the epidermis at several separate points throughout the length of the collar, but in the intervening regions they are separated by the basement membrane of the epidermis (Fig. 5*c* and *d*). The points of continuity are in the nature of perforations in the basement membrane, perhaps 10μ in diameter, and are not associated with any local thickening of the keel. This is similar to the condition in *S. kowalevskyi* and is intermediate between that in some species, such as *S. cambrensis*, in which there is no continuity between the keel and epidermis, the continuous basement membrane intervening, and that in others, such as *S. inhacensis*, Kapelus (1936), and *S. pusillus* (Ritter) (van der Horst, 1930), in which the tissues of the keel are in uninterrupted continuity with the epidermis.

Circular muscle fibres in the collar cavities around the mouth form an oral sphincter (Fig. 1). The buccal cavity has no dorsal diverticulum, such as is found in *S. gurneyi* (Robinson, 1927) and *S. otagoensis* (Benham) (van der Horst, 1930).

Trunk

The epidermis of the branchial region surrounding the gill pores is characteristic (Fig. 8*a*). It is strongly ciliated and is packed with long narrow glandular elements, filled with homogeneous secretion which stains intensely with eosin. Consequently this epithelium can be distinguished in sections, even with the naked eye, by its eosinophility. Scattered among the eosinophil elements are a few basophil goblet cells, oval or pear-shaped in form with vacuolated cytoplasm. These are more numerous where the branchial epidermis joins that of the collar. The branchial epidermis thus differs widely from that of *S. cambrensis*, in which the dominant glandular elements are mucous-secreting goblet cells which appear vacuolated and partly disorganized with little affinity for stains, since large numbers of them discharge their secretions before fixation. The epidermis of the trunk behind the branchial region is thinner and is composed mainly of ciliated cells with relatively few glandular elements, except for numerous scattered patches of thicker, more glandular, epithelium.

The dorsal nerve cord is clearly defined in the branchial region, but there is no definite dorsal groove in any part of the trunk. The ventral nerve cord, which is much the larger, lies above a shallow ventral groove that extends throughout the branchial region, and gradually fades out in the oesophageal region. Otherwise the epidermal nerve-fibre layer is fairly well developed throughout the branchial region, but is very thin behind it.

The ventral longitudinal muscles of the trunk are well developed and the dorsal longitudinal muscles form two much smaller bundles extending throughout the branchial region (Fig. 6).

The dorsal blood vessel runs in the dorsal mesentery of the gut, which is continuous throughout the trunk. Anteriorly the epidermal basal membrane of the ventral nerve cord is in contact with the wall of the pharynx in the

mid-ventral line, there being no ventral mesentery in this region in consequence (Fig. 6). Throughout the remainder of the trunk there is a continuous ventral mesentery, containing the ventral blood vessel (Figs. 7, 8*b*,

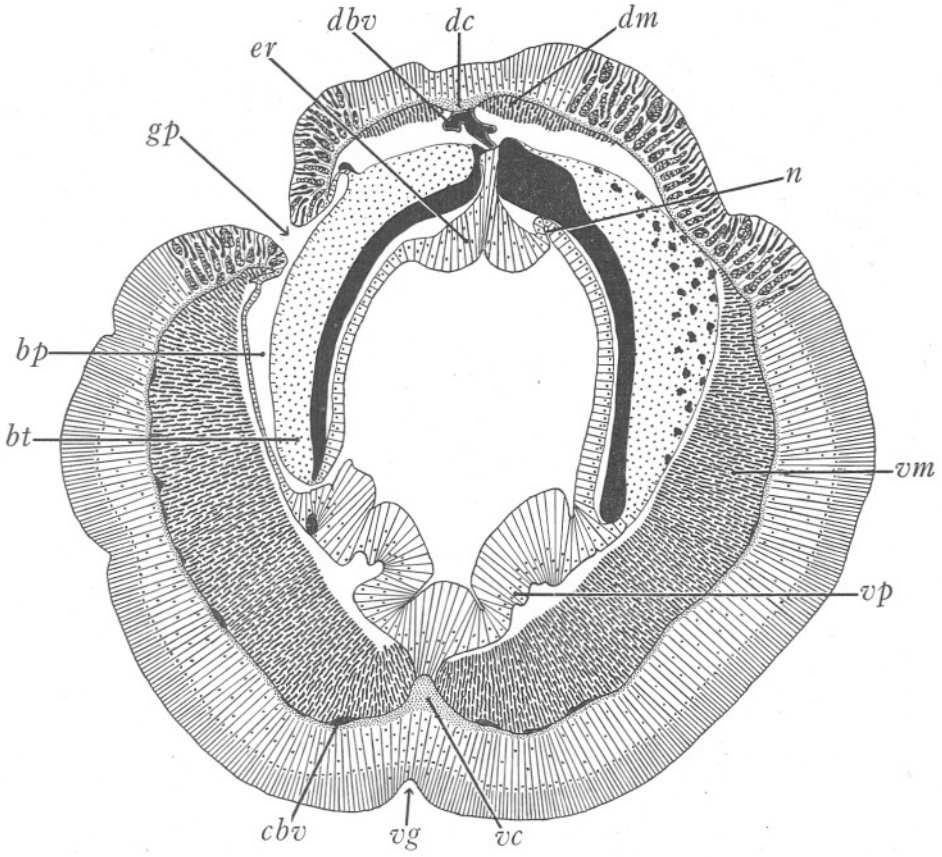

Fig. 6. Transverse section through the anterior part of the branchial region, passing through a branchial pore and tongue on the left and a septum on the right. ×46. *bp*. branchial pouch; *bt*. tongue; *cbv*. circular blood vessel; *dbv*. dorsal blood vessel; *dc*. dorsal nerve cord; *dm*. dorsal longitudinal muscle band of trunk; *er*. epibranchial ridge; *gp*. branchial pore; *n*. niche or recess between septum and epibranchial ridge; *vc*. ventral nerve cord; *vg*. ventral groove; *vm*. ventral longitudinal muscle band of trunk; *vp*. ventral non-branchial region of pharynx. Skeletal rods and blood vessels shown in solid black.

8*c* and 8*d*). Thus the two trunk cavities do not appear to communicate at any point.

The gill slits embrace the dorsal two-thirds of the pharynx (Fig. 6). At the posterior extremity of the series the last few gill slits decrease progressively in size but tongues are present even in the last and smallest. Concretions such as those described in *S. cambrensis* are not found in the skeletal bars of this

species. The tongues are broader and project farther into the lumen of the pharynx than do the septa (Fig. 8*a*). The ciliated epithelium covering the

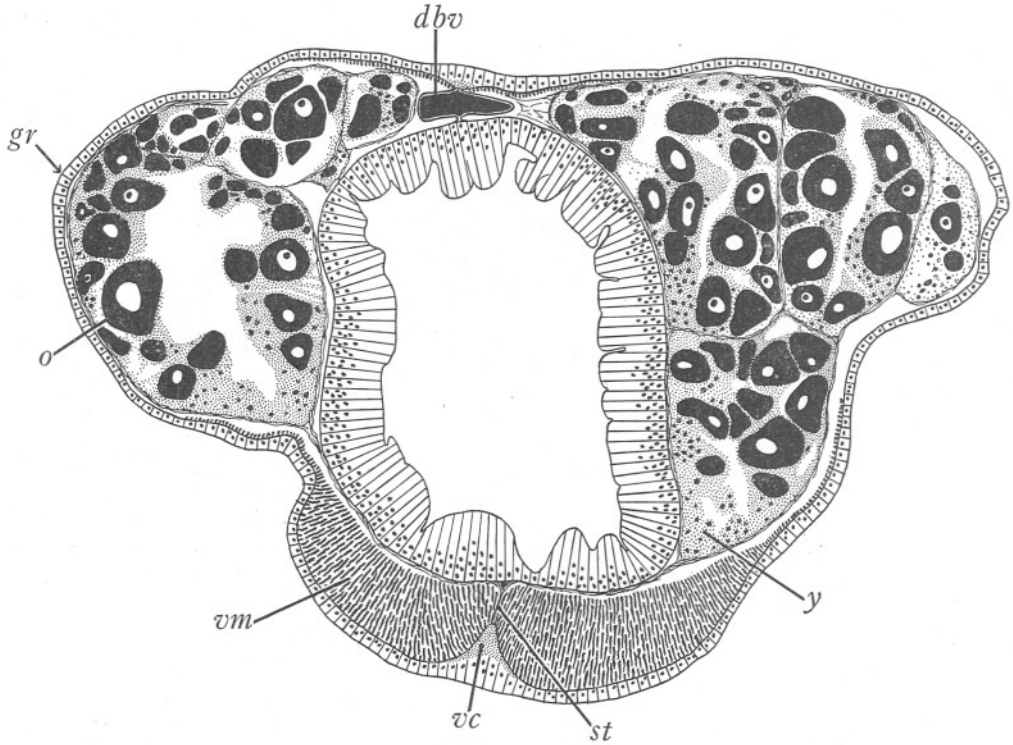

Fig. 7. Transverse section through the first region of the oesophagus showing the maximum development of the genital ridges. ×46. *gr*. genital ridge; *o*. oocyte in ovary; *st*. ventral septum of trunk; *y*. yolk-cells in ovary; other guide letters as in previous figures.

inner surface of the tongues and septa is similar, except that that on the tongues is thicker and contains many basophil glandular elements while that on the septa contains few. A thick epithelium, with long cilia and regularly arranged nuclei but devoid of glandular elements, covers the sides of both the tongues and septa. The epithelium lining the gill chambers and covering the outer surfaces of the tongues is not ciliated and the nuclei are not regularly arranged. Numerous large stellate pigment cells are scattered throughout this epithelium, except where it covers the tongues. The epibranchial ridge is grooved longitudinally in the mid-dorsal line and hence appears bilobed in transverse sections (Fig. 6). It differs in this respect from the epibranchial ridges in both *S. cambrensis* and *S. kowalevskyi*. The epithelium on the inner surfaces of the septa at their dorsal extremities, where they join the epibranchial ridge, is reduced in thickness, thus forming a niche

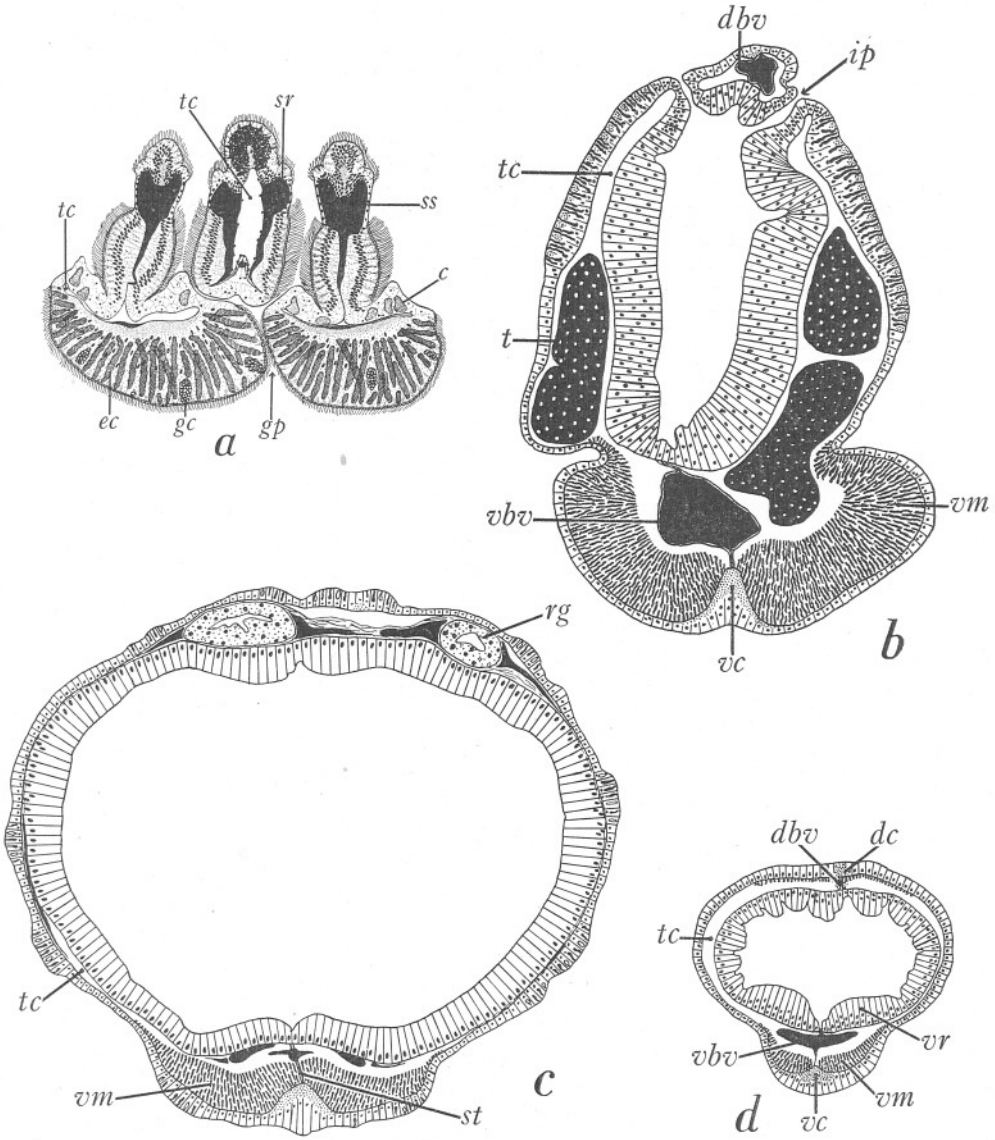

Fig. 8. *a*. Longitudinal horizontal section through two branchial septa, a tongue and a branchial pore. The character of the branchial epidermis is shown. × 127. *b*. Transverse section through the beginning of the second region of the oesophagus showing intestinal pores. × 46. *c*. Transverse section through the hepatic region. × 46. *d*. Transverse section through the intestinal region. × 46. *c*. pigment cell; *ec*. eosinophil glandular cell of epidermis; *gc*. goblet-cell; *ip*. intestinal pore; *rg*. rudimentary gonad; *sr*. skeletal rod in tongue; *ss*. skeletal rod in septum; *t*. testis; *vr*. ventrolateral ridge of intestinal epithelium; other guide letters as in previous figures.

or recess on each. No such recesses are found on the dorsal ends of the tongues. The epithelium of both the epibranchial ridge and the ventral non-branchial part of the pharynx resembles that of the buccal cavity. It is thick, strongly ciliated and contains a number of basophil glandular elements.

The oesophagus is relatively short, though, owing to contraction, its length could not be measured with precision. It is divided into three distinct regions. The epithelium lining the first (Fig. 7) resembles that of the ventral non-branchial part of the pharynx, but the glandular elements are rather more numerous and are regularly arranged, the oval bodies of the cells occupying the central zone of the epithelium and not extending into the basal zone, while their narrow necks traverse the peripheral zone to reach the surface. The second region has much thicker walls. A transverse section through the middle of this region reveals a cruciform lumen. The thickest epithelium lines the dorsal arm of the lumen. It is full of basophil glandular elements. The epithelium of the horizontal arms of the lumen is much thinner and is sharply differentiated from the rest by its lack of chromophility. The epithelium of the ventral arm is very thick, though not so thick as that of the dorsal arm, and it stains still more densely with haematoxylin. The horizontal arms of the lumen constitute two lateral longitudinal grooves in the wall which begin dorsally and slope backwards and downwards. The intestinal pores open into these grooves at their anterior and dorsal extremities (Fig. 8*b*). The epithelium lining these pores is similar to that lining the grooves into which they open internally. The number of pores varies from 4 to 8 in different specimens and indeed on the two sides of the same specimen. The numbers observed were 4+5, 5+5, 5+7 and 7+8. No rudimentary pores such as those described in *S. cambrensis* were observed. The third region of the oesophagus is lined by thinner epithelium, less crowded with glandular elements and distinguished by the oval nuclei being confined to the basal regions of the columnar cells.

The epithelium of the posterior part of the oesophagus grades into that of the long hepatic region, where it is somewhat thicker but otherwise very similar, without any sharply defined transition. The hepatic region of the gut (Fig. 8*c*) of this species is remarkable for the total absence of any trace of sacculation of its walls. The lumen is expanded and the gut nearly fills the trunk cavities, its outer surface being almost in contact with the body wall laterally and only separated from it by the gonads dorsally and the muscle bands ventrally.

The intestine is also expanded and has a wide lumen (Fig. 8*d*). The coelomic cavities in both the hepatic and intestinal regions are much narrower than in *S. cambrensis*. The epithelium of the intestine is somewhat thinner than that of the hepatic region, but is thickened ventrally to form two broad convex ridges, one on each side of a mid-ventral groove. The anal region was missing from all the specimens sectioned.

The gonads, both ovaries and testes, are simple unlobed sacs. The more posterior gonads of the series are rudimentary and do not display active gametogenesis. The genital ducts are extremely short, so that the gonads open almost directly at the genital pores. The ovaries (Fig. 7) contain yolk cells and the oocytes were observed to attain a size of 0·23 mm. long by 0·17 mm. broad. Thus, assuming that some of the specimens were mature, the oocytes are considerably smaller than those of *S. cambrensis* and the number present in a single ovary is correspondingly greater.

Comparison with other Species

S. horsti is a well-defined species. It is readily distinguishable from *S. ruber* and *S. serpentinus*, though neither has been described in detail, both by the large number of gill slits and by the concentric arrangement of the musculature of the proboscis. It resembles more closely both *S. cambrensis* and *S. kowalevskyi* and appears to occupy a systematic position intermediate between them, though probably more closely allied to the latter than to the former species. It differs from *S. cambrensis* in coloration, habitat and size; in the more complete concentric arrangement of the longitudinal musculature of the proboscis; the presence of a ventral groove on the proboscis; the straight stomochord; the presence of only four epidermal zones in the collar; the shorter crura of the proboscis skeleton; the slight operculum; the continuity at intervals between the tissues of the neural keel and the nerve-fibre layer of the epidermis; the larger number of gill slits; the projecting ventral muscle bands of the trunk; the genital ridges and extreme anterior extension of the gonads; the form of the epibranchial ridge; the number of intestinal pores. It differs from *S. kowalevskyi* in the presence of dorsal and ventral grooves on the proboscis; the complete separation of the collar cavities; the shorter crura of the proboscis skeleton; the larger number of gill slits; the fact that the tongues project further into the lumen of the pharynx than do the septa; the form of the epibranchial ridge; the less projecting ridges formed by the ventral muscle bands and the gonads.

The structural similarity of *S. horsti* and *S. kowalevskyi* raises the problem of whether Caullery & Mesnil's (1916) record from the coast of France should be ascribed to the British or American species. They had only one fragmentary specimen, which included part of the proboscis, the collar and the anterior part of the branchial region. It was found in very fine and compact grey sand at low-water mark of spring tides. The collar was 3 mm. in diameter and 4–5 mm. long with a marked posterior thickening. There were numerous gill pores which were not covered by lateral folds of the trunk. The proboscis was rosy white in colour and the predominant colour of the rest of the body was orange-salmon. They stated that this coloration was closely similar to that in Spengel's (1893) figure of *S. kowalevskyi*, though not so bright. The musculature of the proboscis was well developed and concentrically arranged.

A single proboscis pore was present on the left side. The proboscis skeleton was similar to that described by Spengel in *S. kowalevskyi*. The perihaemal spaces were fused anteriorly and the muscles in them were much less developed than those figured by Spengel. The branchial epithelium was of the same type as *S. kowalevskyi* and the ectoderm of the collar was very thick with an enormous development of mucus glands. This description is applicable equally to either species, except for the poor development of the muscles in the perihaemal cavities, which conforms with neither. The size, coloration and musculature of the proboscis serve to distinguish it from the other British species, *S. ruber*, *S. serpentinus* and *S. cambrensis*. Thus, while the characters permit of assigning the specimen to either *S. horsti* or *S. kowalevskyi* they do not allow of distinguishing to which it belongs. The geographical evidence points strongly to *S. horsti*, which occurs within 75 miles, whereas all other records of *S. kowalevskyi* are from the other side of the Atlantic Ocean. On this assumption *S. kowalevskyi* falls into line with all other members of the genus in being confined in its distribution.

The known species of the genus *Saccoglossus* now number fourteen. The characters of these, excepting *S. cambrensis* and *S. horsti*, are admirably summarized and a full bibliography of the original literature is provided by van der Horst (1927–39). Nevertheless it may be convenient to include herein a very brief summary in English of the more important and distinctive characters of the species other than that now described.

S. sulcatus (Spengel), from Japan. Deep dorsal sulcus on proboscis rendering it half-moon shaped in cross-section. Collar as broad as long. There appear to be only 10–11 pairs of gill pores. Other characters unknown.

S. otagoensis (Benham), from New Zealand. Deep dorsal groove on proboscis but not so pronounced as in last-named species. Collar as broad as long. 10–15 pairs of gill pores. Longitudinal muscle fibres of proboscis in concentric rings. Dorsal diverticulum of buccal cavity present. Dorsal and ventral mesenteries of collar absent. Gonads extending anteriorly to level of 4th gill pore. Ventral septum of proboscis short. One pair of intestinal pores.

S. pygmaeus Hinrichs and Jacobi, 1938, from Heligoland. Proboscis without pronounced dorsal groove. Collar broader than long. 9–22 pairs of gill pores. Longitudinal muscle fibres of proboscis not in concentric rings. No dorsal diverticulum of buccal cavity. Dorsal and ventral mesenteries of collar complete. Gonads begin at posterior extremity of branchial region. Ventral septum of proboscis very short. One pair of intestinal pores. Very small form, about 3 cm. long.

S. gurneyi (Robinson), from Suez. Collar nearly twice as broad as long. 40–60 pairs of gill pores. Ventral septum of proboscis very long, extending beyond tip of stomochord. Dorsal diverticulum of buccal cavity present. Median proboscis pore. Gonads beginning immediately behind collar. Dorsal and ventral mesenteries of collar complete throughout 4/5ths of length of collar.

S. caraibicus (van der Horst), from West Indies. Collar longer than broad. More than 50 pairs of gill pores. Ventral septum of proboscis long, extending to tip of stomochord. No dorsal diverticulum of buccal cavity. Median proboscis pore. Gonads beginning behind 4th gill pore. Dorsal and ventral mesenteries of collar complete. Perihaemal cavities separate throughout length and not extending into proboscis stalk. Dorsal glomerulus present.

S. bournei (Menon), from Madras. Collar broader than long. More than 62 pairs of gill pores. Ventral septum of proboscis very long, extending in front of tip of stomochord. No dorsal diverticulum of buccal cavity. Gonads beginning immediately behind collar. Dorsal mesentery of collar complete, ventral lacking in anterior half. Very deep anterior neuropore.

S. pusillus (Ritter), from California. Both gonads and ventral longitudinal muscle bands form projecting ridges on trunk. 60 pairs of gill pores. Longitudinal muscle fibres of proboscis in concentric rings. Ventral septum of proboscis short. Ventral mesentery of collar complete. Gonads beginning behind 12th–17th gill pore. One pair of intestinal pores.

S. mereschkowskii (Nic. Wagner), from Northern and Eastern Russia. Both gonads and ventral longitudinal muscle bands form projecting ridges on trunk. 50 pairs of gill pores. Longitudinal muscle fibres of proboscis in concentric rings. Ventral septum of proboscis short. Dorsal mesentery of collar complete, ventral incomplete anteriorly. Gonads beginning in middle of branchial region. Epidermis of collar very thick (0·5 mm.). Seven pairs of intestinal pores.

S. inhacensis Kapelus, 1936, from S. Africa. Both gonads and ventral longitudinal muscle bands form projecting ridges on trunk. 82 or more pairs of gill pores. Longitudinal muscle fibres of proboscis not in concentric rings. Ventral septum of proboscis short. Ventral mesentery of collar complete. Gonads beginning at level of 4th gill pore. Four pairs of intestinal pores, the first with several internal openings each.

S. kowalevskyi (A. Agassiz), from Atlantic coast of U.S.A. Both gonads and ventral longitudinal muscle bands form projecting ridges on trunk. 100 pairs of gill pores. Longitudinal muscle fibres of proboscis in concentric rings. Ventral septum of proboscis short. Dorsal and ventral mesenteries of collar incomplete. Gonads beginning within $\frac{1}{2}$ mm. of collar. 4 to 6 pairs of intestinal pores.

S. cambrensis Brambell and Cole, 1939, from Wales. Trunk circular in cross-section, without genital or muscular ridges. 60–90 pairs of gill pores. Longitudinal muscle fibres of proboscis in concentric rings. Ventral septum of proboscis short. Dorsal and ventral mesenteries of collar complete. Gonads beginning in middle of branchial region. S-shaped bend on stomochord in front of ventral diverticulum. 8-12 pairs of intestinal pores, the first 3–5 pairs being rudimentary.

S. ruber (Tattersall), from Ireland. Trunk circular in cross-section, without genital or muscular ridges. 56–64 pairs of gill pores. Longitudinal muscle fibres of proboscis not in concentric rings. S-shaped bend on stomochord in

front of ventral diverticulum. Ventral septum of proboscis short. Wide proboscis coelom.

S. serpentinus (Assheton), from Scotland. Trunk circular in cross-section, without genital or muscular ridges. 60 pairs of gill pores. Longitudinal muscle fibres of proboscis not in concentric rings. Narrow proboscis coelom. Very elongated form, proboscis at least 25 mm. long.

Summary

1. *S. horsti* occurs on the Hampshire coast of the Solent near the mouth of the Lymington River, in glutinous grey mud associated with *Corophium volutator*.

2. The species is distinguished by the following characters: coloration; dorsal and ventral grooves present on the proboscis throughout its length; collar forming a slight operculum posteriorly; ventral muscle bands of the trunk forming projecting ridges; rounded genital ridges present, the gonads beginning within 1 mm. of the collar; 100–140 pairs of gill slits; longitudinal muscle fibres of the proboscis arranged in nine or more concentric rings; ventral septum of the proboscis short; stomochord straight, with ventral diverticulum and wide lumen throughout; no dorsal glomerulus present; single proboscis pore on the left side; proboscis skeleton embracing half to two-thirds of the circumference of the buccal cavity and extending one-fourth to one-third of the length of the collar; four epidermal zones present in the collar; collar cavities completely separated; anterior extensions of the collar cavities extending into the neck of the proboscis; perihaemal cavities separate posteriorly but confluent in front of the level of the tips of the crura, and extending into the neck of the proboscis as far as the proboscis pore; neural keel well developed and in continuity with the epidermal nerve layer at intervals; no dorsal diverticulum of the buccal cavity; branchial epidermis characterized by the predominant intensely eosinophil glandular elements; tongues broader and projecting further into the lumen of the pharynx than the septa; epibranchial ridge formed by two convex ridges bounding a median groove; oesophagus divided into three regions; 4–8 intestinal pores; hepatic region of gut not sacculated and with an expanded lumen; gonads not lobed; oocytes attain a size of $0{\cdot}23 \times 0{\cdot}17$ mm.; yolk cells present in the ovaries.

3. The probable identity of the specimen of *Saccoglossus* from the French coast of the English Channel, recorded by Caullery & Mesnil (1916) as *S. kowalevskyi*, with this species is discussed.

APPENDIX

Analysis of sample of mud, in which *S. horsti* occurs, for which we are indebted to Prof. G.W. Robinson:

Coarse sand (particles 2·0–0·2 mm. in diameter)	0·0
Fine sand (particles 0·2–0·02 mm. in diameter)	39·0
Silt (particles 0·02–0·002 mm. in diameter)	19·7
Clay (particles less than 0·002 mm. in diameter)	30·1
Moisture in air-dry sample	3·2
Calcium carbonate	1·6
Organic carbon	2·5

The sample was oven dried at 105° C. before analysis. Comparison with the analyses of sand in which *S. cambrensis* occurs (Brambell & Cole, 1939*a*) shows that this sample contains a much higher proportion of silt and clay and that the organic content is considerably greater.

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THE MALE OF THE AMPHIPOD *HAUSTORIUS ARENARIUS* SLABBER

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The amphipod *Haustorius arenarius* has been recorded by several workers as an inhabitant of the intertidal and shallow-water sands of the coasts of Britain, its characteristic shape being regarded as an adaptation to its habit of burrowing in the sand. Sars (1895) describes the female only, and Stebbing (1906) summarizes the description given by Sars. Chevreux & Fage (1925) record the species from numerous localities on the coast of France and give in addition the Kattegat, Holland, Great Britain and the east coast of North America as the extent of its distribution. They state that the male is unknown. This apparent absence of the male of the species formed a notable exception amongst the Amphipoda, a group in which the males may usually be distinguished from the females by a number of secondary sexual characters of which the brood lamellae of the adult female is the most characteristic.

Sexton (1925), in her study of the growth, moulting and mating habits of species in the genus *Gammarus*, has shown that the brood lamellae develop gradually, increasing in size through a series of moults and finally becoming fully formed by the development of long fringing hairs. Once formed the lamellae remain a constant feature at each moult. Hart (1930) shows that such a mode of development also occurs in *Corophium volutator*. Unwin (1920) has shown that in the isopod *Asellus aquaticus* the brood lamellae appear suddenly at the ecdysis accompanying fertilization and disappear at the moult which succeeds the release of the young from the pouch. Sheppard (1927) draws attention to this distinction between *Asellus* and *Gammarus* as one which may prove to be constant throughout the two groups.

Dennell (1934), in his study of the habits and feeding mechanism of *Haustorius arenarius*, states (p. 374): 'Of the 120 specimens examined all were females possessing oostegites, or lacking them, but resembling those so provided in all other respects'; and also p. 375, 'Whether reproduction takes place parthenogenetically or whether there may be a male of the species is unsettled'.

These remarks of Dennell suggested to me that the 'females lacking oostegites' were males. To test this assumption a collection of *H. arenarius* from the estuary of the river Dovey was examined and grouped on the presence or absence of brood lamellae. Of the fifty individuals which formed the sample twenty-five showed brood lamellae in various stages of develop-

ment as described by Sexton in the genus *Gammarus*. This group varied in total length from 5 to 14 mm., and a number of those 11 mm. and over carried eggs in the brood pouch. Of the remaining twenty-five, varying in length from 5 to 10 mm., a number were dissected and sectioned. All showed a male reproductive system which, as is usual in amphipods, lay dorsal to the alimentary canal and its associated diverticulae. The system consists of an anterior testis portion which narrows to a vas deferens on which a swollen receptaculum seminis occurs which opens at the base of the seventh segment of the mesosome.

This sample was collected in July. A further sample collected in December showed the same general features except that no egg-bearing females were obtained and the brood lamellae were without the fringing hairs. In each case no individual less than 5 mm. in length was obtained. A collection of *Haustorius arenarius* from Kames Bay gave individuals of 3 and 4 mm. total length, none of which size bore developing brood lamellae. It thus appears that the brood lamellae begin to form at a size of about 5 mm.

A comparison of the appendages of the adult males and females, particularly of those which show secondary sex differences in other amphipods, namely, the antennules and antennae, the first and second gnathopods and the third uropods, failed to show a single morphological difference. The related genera in the family Haustoriidae, *Bathyporeia* and *Urothoë*, show a secondary sex difference in the length of the flagellum of the antenna which is long and bears calceoli in the male and is short and without calceoli in the female. No such distinction occurs in *Haustorius*. Sexton & Spooner (1940) have shown that in the genus *Marinogammarus* the males bear special sensory setae, particularly on the antenna. I was unable to distinguish any differences in the setal armature of the male and female of *Haustorius*.

Summary

The male of *Haustorius arenarius* is identical with the female in all external morphological characters apart from the absence of brood lamellae. The females develop brood lamellae at 5 mm. total length, eggs appear in the brood pouch at 11 mm., and they may reach a length of 14 mm. The largest male obtained measured 10 mm.

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ON THE LIFE HISTORY AND DEVELOPMENTAL STAGES OF THE MEDUSA *PODOCORYNE BOREALIS*

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(Text-fig. 1)

CONTENTS

INTRODUCTION

It has been shown in a recent paper by Russell (1940) that in addition to *Podocoryne carnea* M. Sars, 1846, and the so-called *P. areolata*, two other *Podocoryne* medusae, viz. *P. hartlaubi* Neppi & Stiasny, 1913, and *P. minima* (Trinci), also occur in the plankton in British waters. The identity of both hydroid and medusa of the European[1] *P. carnea* is well established and the medusa has never been known to mature with more than 8 tentacles (Kramp, 1927; Russell, 1940). *P. minima* is a small medusa with only 4 tentacles and need not be considered further in this paper.

As there were only two species of medusae of the genus *Podocoryne* previously known from British waters, it was generally assumed that the newly liberated medusa of the hydroid *P. areolata* (liberated with 16 tentacles) was the earliest stage of the medusa found in the plankton with 16–32 tentacles. The discovery by Russell of a second species in the plankton (*P. hartlaubi* with 24–49 tentacles) indicated that the hydroid *P. areolata* might belong to either species.

While at Millport during 1940 I obtained a fine series of all stages of the so-called *P. areolata* in the plankton. The earliest stage of the medusa has only 8 tentacles, so that it cannot belong to the hydroid *P. areolata*. As will be shown

[1] The supposed occurrence of *P. carnea* in North American waters with more than 8 tentacles is discussed on p. 315.

below, the youngest stages may have been confused in the past with *P. carnea*. Kramp & Damas (1925) have regarded the mature medusa as identical with *Lymnorea borealis* Mayer, 1900. They have also re-examined the species described by Broch (1905) as *Limnorea norvegica* and identified it as *P. areolata*. Since this last specific name can no longer be used for this medusa, I propose to refer to the species as *P. borealis*. There is some evidence to suggest that a *Podocoryne* hydroid which I found in the Clyde belongs to this species.

The first certain records of this medusa from British waters are those of Browne (1895, 1897) as ?*Cytaeandra areolata* from Port Erin and Valentia. Hartlaub (1911) gives a full list of British and northern records of the medusa, but his records of the hydroid are those of *P. areolata* (Alder). Previous records of the medusa from Millport are given by Browne (1905), who states that in 1901 it occurred in April and July; in 1902, young stages were found in March and adult medusae in May. During 1940, young stages were found occasionally in the plankton from the beginning of March to the end of September, while the earliest mature medusae were present on 2 May. The young stages obtained throughout this period indicate that the hydroid is fertile during the spring, summer and autumn.

All the material of *P. borealis* was collected at Millport while I was holding a grant from the Royal Society. I wish to thank Dr Stanley Kemp, F.R.S., for facilities at Plymouth where the paper was completed.

The Hydroid

A well-developed colony of a hydroid, which appeared to be identical with, and was at first thought to be, *P. carnea*, was found on a dead *Buccinum* shell dredged off the North End of Cumbrae on 6 March 1940. When found, all the polyps were large and particularly well developed as in mature colonies of *Hydractinia echinata*, and some of them bore medusa buds on the body of the hydranth. On the fertile polyps, which possessed 9–14 filiform tentacles, there were from three to eight medusa buds. The polyps arose from a creeping network of stolons and there was no encrusting base or spines; the absence of the latter, however, might depend on the age of the colony. Medusae were liberated on the following day in the laboratory and these possessed better developed interradial tentacles than I had ever seen in specimens liberated from the true *P. carnea*. In all the young specimens liberated all 4 interradial tentacles were present. After many days of rearing the young medusae had developed no gonads on the stomach. Suspicions that this hydroid was distinct from *P. carnea* were confirmed when I found a complete series of twenty medusae, from the newly liberated to the sexually mature with 20–31 tentacles, in the plankton on 2 May 1940 (see Table I). The youngest specimens were obviously newly liberated and agreed in all points with my specimens from the hydroid. The hydroid had been many days in captivity before it was realized that it was distinct from *P. carnea*, and by this

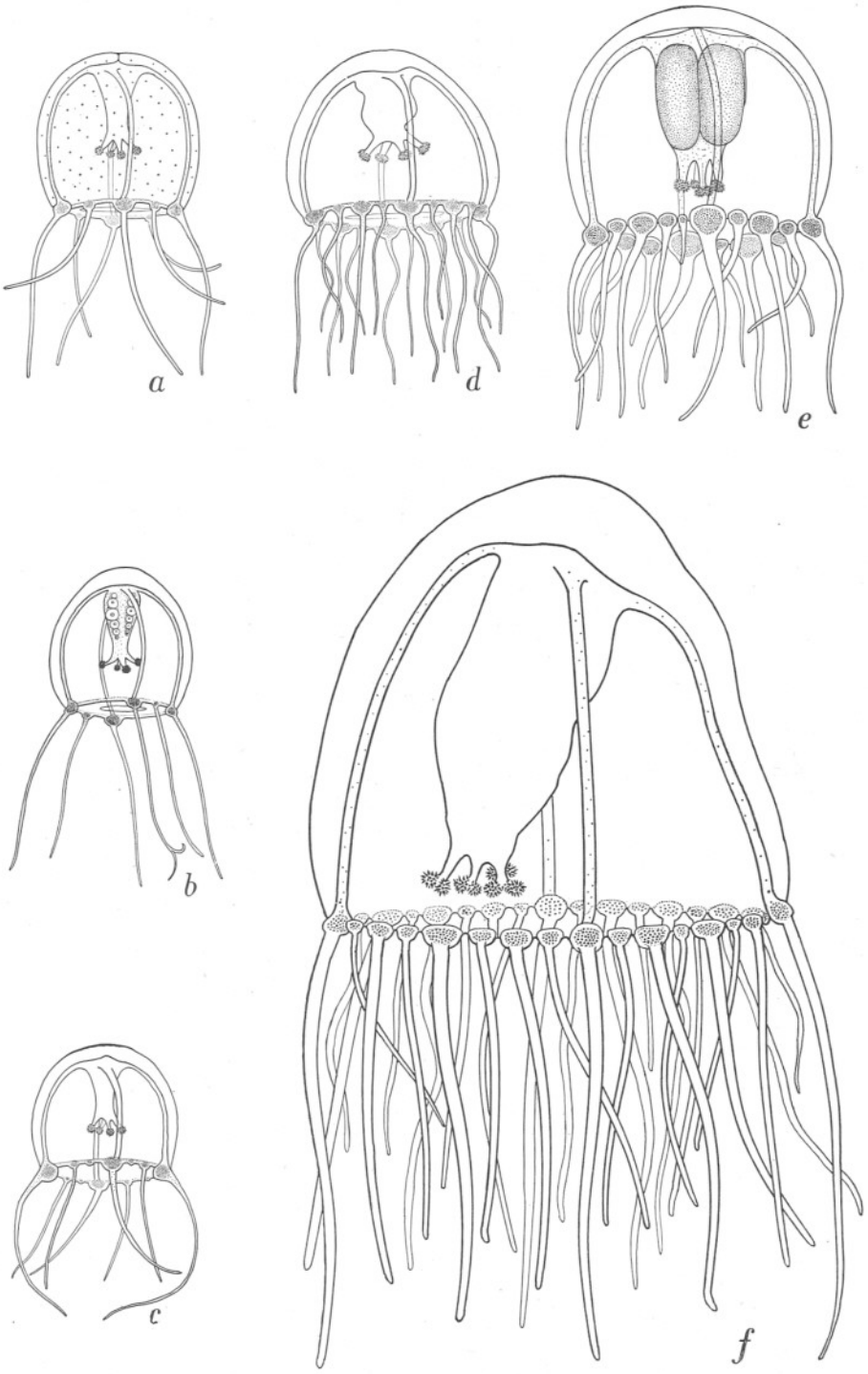

Fig. 1. *a*, *Podocoryne borealis*; medusa newly liberated from the hydroid, Millport, 7. iii. 40. *b*, *P. carnea*; mature female medusa reared from the hydroid, Plymouth, 2. x. 36. *c*, *P. borealis*; medusa, 8 tentacle stage, Millport, 6. v. 40. *d*, *P. borealis*; medusa, 16 tentacle stage, Millport, 6. v. 40. *e*, *P. borealis*; medusa, with 17 tentacles, mature male, Millport, 6. v. 40. *f*, *P. borealis*; mature female medusa with 31 tentacles, Millport, 2. v. 40.

time all the fertile and sterile polyps had become considerably reduced by the reproductive activity of the hydroid. A full description of the colony at this stage might give a misleading picture of the hydroid and it is hoped later to re-describe the species from fresh material.

The Newly Liberated Medusa (Fig. 1*a*)

Medusae newly liberated from the hydroid in the laboratory were 0·8–1·0 mm. high and 0·9–1·2 mm. in diameter. They were of a deep bell-shape with a uniformly thin umbrella with ex-umbrellar nematocysts and a well-developed velum. The stomach was tubular and in length about half the height of the sub-umbrellar cavity. There were four distinct mouth lips, each terminating in a knob of nematocysts. The radial canals were prominent and united around the margin by a ring canal. There were four large perradial tentacular bulbs, 0·1 mm. in diameter, each with a well-developed tentacle. Interradially, there were four smaller bulbs each with a shorter tentacle than the perradial tentacle. The base of the stomach and the tentacle bulbs were of a brownish colour. Of twenty-eight medusae liberated from the hydroid described on p. 308, all possessed 8 tentacles, 4 of which, the perradial tentacles, were long and of equal length, while the interradial tentacles were a little shorter. There was no trace of gonad in any of the medusae even after many days of rearing.

These medusae may be compared with the newly liberated ones obtained from *P. carnea* which I found on a shell of *Nassarius reticulatus* dredged in Cawsand Bay, Plymouth, on 14 September 1936. Those liberated between 21 and 26 September differed but little in size, colour and the diameter of the tentacle bulbs from *P. borealis*, but there was considerable variation in the development and number of the interradial tentacles. Some possessed none. Browne (1896) also noticed the variation in the number of interradial tentacles and states that he did not see a specimen with 8 tentacles amongst those reared from the hydroid in his jar. Forty of the fifty-one specimens examined possessed immature gonads. The variation in the development of the interradial tentacles is tabulated below.

	No. of medusae	No. of perradial bulbs	No. of interradial bulbs
21. ix. 36	1	4	2 less developed, 1 rudiment
23. ix. 36	1	4	4 less developed
25. ix. 36	7	4	4 less developed
26. ix. 36	10	4	None
	10	4	2 less developed
	10	4	2 fully and 2 less developed
	12	4	4 fully developed

A number of these were kept for rearing, and after a few days some of them became sexually mature (Fig. 1*b*). The specimen figured matured in a few days with 4 perradial tentacles and 2 interradial tentacles. The perradial tentacular bulbs of this specimen had a diameter of 0·18 mm.

Stages from the Plankton

The earliest stages from the plankton differed in no way from the medusae obtained in the laboratory and were 0·65–1·0 mm. high and 0·65–1·1 mm. in diameter. Here the tentacles are still unequal in length and may be referred to as 4+4. In the descriptions below, the smallest tentacles are indicated in this way. Rudimentary bulbs from which no rudiment of a tentacle has developed are not included in the total. The succeeding stages can be roughly grouped into 16, 24 and 32 tentacle stages, but these are not very well defined. The tentacles themselves vary in length; for example, in the 8 tentacle stage described below, the interradial bulbs and tentacles are smaller than the perradial ones, while the rudimentary tentacular bulbs of an additional 8 tentacles are already appearing. Thus new tentacles are appearing even before those formed earlier reach full size. Even at the 16 tentacle stage and in older specimens the interradial tentacular bulbs have a slightly smaller diameter than the perradial bulbs. The differences in the sizes of the tentacular bulbs, however, do not appear to be as well marked as in *P. hartlaubi*.

The 8 *Tentacle Stage* (Fig. 1*c*)

A medusa typical of this stage was captured on 6 May. The umbrella was 1·1 mm. high and 1·2 mm. in diameter. It had scattered nematocysts on the ex-umbrella. The stomach had a length of 0·8 mm., reaching a little more than half-way to the margin of the bell. The mouth had four simple lips each terminating in a round cluster of nematocysts. There were 8 tentacles, the 4 interradial ones being well developed. The perradial and interradial tentacular bulbs had a diameter of 0·2 and 0·4 mm. respectively. Eight additional bulbs were developing on the margin of the bell, but these were rudimentary bulbs without tentacles. All the bulbs were of a brownish colour. There was no trace of developing gonads. Kramp & Damas (1925) describe a small specimen 1·25 mm. in height which appears to belong to this stage. It has 8 tentacles (4+4) and 8 adradial tentacular rudiments. An apical canal was present and on account of this they regarded it as newly liberated. In my specimens an apical canal was rare, but when present it persisted for a long time. Kramp & Damas also describe the oral lips as undivided in young examples.

The 16 *Tentacle Stage* (Fig. 1*d*)

I have many intermediate stages between the 8 tentacle stage and the 16 tentacle stage. It is at this latter stage that the rudiments of gonads begin to appear. The following description is of an immature female specimen obtained on 6 May (Fig. 1*d*). The umbrella was 1·7 mm. high and 1·8 mm. in diameter. There were 16 tentacles, the perradial and interradial bulbs being much better developed than the adradial bulbs. The perradial, interradial and adradial bulbs had diameters of 0·28–0·3, 0·2–0·22 and 0·1 mm. respectively.

There were no rudimentary bulbs and the tentacles were more uniform in length than in many similar stages. The stomach was a little contracted and had a slight peduncle. The batteries of nematocysts on the four lips of the mouth were oval in shape, 0·08 × 0·12 mm.

Some medusae at this stage possess immature gonads and frequently become mature by the time a few more tentacles are added. Fig. 1*e* is a drawing of a mature male medusa with 17 tentacles (16 + 1). The majority of the sexually mature specimens that I collected, however, possessed 20–24 tentacles, while a few large specimens had 30–32 tentacles.

A 16 tentacle stage has been figured by Browne (1897, Pl. xlviii, fig. 1) as ?*Cytaeandra areolata* Haeckel. It is an earlier stage than Fig. 1*e* and is immature. The constriction at the base of the stomach in Browne's figure is more pronounced than in my specimens. Mayer (1910, Pl. 14, figs. 4, 5) figures two 16 tentacle stages similar to mine as *P. carnea*; there is no doubt that they belong to the present species.

The 24 *Tentacle Stage*

Mature and immature medusae were found at this stage and at intermediate stages between this and the 16 tentacle stage. In all the 24 tentacle stages found the mouth lips on the stomach were divided, possessing eight nematocyst knobs. An immature medusa belonging to this stage has been described and figured by Browne (1897, Pl. xlviii, fig. 2) from material obtained at Valentia, Co. Kerry. He also notes the occurrence of a female medusa with 24 tentacles and bearing ova on the stomach.

The 30–32 *Tentacle Stage* (Fig. 1*f*)

The biggest medusa caught in the series obtained on 2 May 1940 belonged to this stage. This specimen (Fig. 1*f*) was a ripe female medusa having a height of 3·55 mm. and a diameter of 4·0 mm. The umbrella was thickened at the apex and gradually thinned towards the margin of the bell. There were 31 tentacles (30 + 1) and one tentacular bulb without a tentacle. The perradial, interradial, and adradial tentacles were better developed than later developed tentacles. The perradial, interradial and adradial tentacular bulbs had diameters of 0·35, 0·28–0·3 and 0·3 mm. The tentacles, too, showed much variation in length. The radial canals had a diameter of 0·1 mm. The stomach was long and tubular, reaching nearly to the margin of the bell. The four mouth lips and the nematocyst knobs had become completely divided, so that there were eight distinct nematocyst knobs: in the preserved specimen as drawn the lips are contracted and do not show this well. These branched lips during life had the same appearance as those figured by Browne (1897, Pl. xlviii, fig. 2) in a specimen with 25 tentacles. In this and other well-developed specimens, the nematocysts formed a distinct continuous band along the edges of the lips from knob to knob. The nematocysts were of the spindle shape

figured by Mayer (1910) and Allman (1871–2). The four gonads were interradial in position and occupied the greater part of the length of the stomach and extending to meet each other in the perradii. The mature eggs on the interradii were dark and granular in appearance, while the more immature towards the edge of the gonad were transparent, giving, as Mayer states in his description of *Lymnorea borealis*, a reticulate appearance to the surface of the gonad. In the immature eggs the nuclei, each with a nucleolus, were plainly visible under the low power of the microscope.

The stomach, radial canals and ring canal were a delicate pinkish red in colour, while the endoderm of the tentacle bulbs was of a deep reddish-brown colour giving the living medusa a handsome appearance. During life, when the medusa was propelling itself through the water, the tentacles were frequently coiled in tight spirals.

Another mature stage with 29 tentacles, caught on 3 October 1940, had more fully branched oral lips which were twice dichotomously branched, so that there were sixteen nematocyst knobs. This is the condition figured by Mayer (1910, Pl. 15, figs. 1–3) for *Lymnorea borealis*.

Additional data and measurements of a fine series of medusae caught in a single haul from Keppel Pier on 2 May 1940 are given in Table I. The youngest specimen was a newly liberated specimen, while all the specimens with more than 20 tentacles were sexually mature.

Discussion

The discovery of the various stages in the life history raises the question of what name the species should bear, since it can no longer be called *Podocoryne areolata*. Mayer stated his belief that the *P. areolata* of British waters might prove to be identical with his *Lymnorea borealis*. It is well known that the oral lips of the manubrium are branched in *P. areolata*, and it has been shown in this paper that the lips may branch in the larger specimens, so there is now no doubt that my species from Millport is identical with *L. borealis*. Mayer distinguished the genus *Podocoryne* from *Lymnorea* by its simple oral lips, but in this species the distinction only holds good for the youngest stages, and I have therefore referred the species to *Podocoryne* as *P. borealis*.

It will be seen from the foregoing description that three species are associated with the history of this species and may have been confused with it in the past. These are *P. carnea* M. Sars, 1846, *P. areolata* (Alder, 1862) and *P. hartlaubi* Neppi & Stiasny, 1913.

The European *P. carnea* is well known and the first description of the hydroid and young medusa by Michael Sars undoubtedly refers to the species now generally recognized under that name, that is, with the medusa maturing with 8 tentacles. Lovén (1857) also recorded this medusa from Bohuslans in Norway and was able to obtain sexually mature medusae with 8 tentacles. Kramp (1927) states that 'no specimens of this medusa have ever been taken

TABLE I. *PODOCORYNE BOREALIS* (MAYER). SERIES OF MEDUSAE CAUGHT AT KEPPEL PIER, MILLPORT, 2 MAY 1940. MEASUREMENTS IN MM.

Specimen no.	Diameter	Height	No. of tentacles	Length of stomach	Diameter of per-radial bulbs	Sex	No. of nematocyst knobs on oral lips	Remarks
1	0·85	0·95	8 (4+4)	0·4	0·13	—	4, round	Newly liberated
2	1·2	1·5	16 (8+8)	0·6	0·22	—	4, round	Young specimen
3	1·7	1·45	16 (8+8)	0·8	0·2	—	4, round	Fairly young specimen
4	1·1	1·3	16 (8+8)	1·0	0·2	—	4, round	Stomach full
5	1·7	1·6	16 (12+4)	1·2	0·25	Imm. male	4, oval	Stomach reaching bell margin
6	1·9	1·6	16 (8+8)	1·0	0·26	?Imm. female	4, oval dividing	Gonads just appearing
7	2·0	1·6	16 (8+8)	1·4	0·2	Imm. male	4, oval	Stomach reaching bell margin
8	2·2	2·3	16 (8+8)	—	0·26	Imm. male	—	Mouth and part stomach missing
9	2·1	2·0	16 (12+4)	1·45	0·24	Imm. male	4, oval	Stomach nearly reaching margin
10	2·1	2·1	19 (16+3)	1·05	0·25	Imm. female	4, all dividing	Stomach reaching half-way to margin
11	2·1	1·8	16	1·45	0·3	Male	4, 2 oval, 2 dividing	Stomach reaching bell margin
12	2·15	2·0	19 (16+3)	1·3	0·25	Imm. female	4, 2 oval, 2 dividing	Gonads just appearing
13	2·6	2·15	21 (17+4)	—	0·22	Male	—	Mouth and part stomach missing
14	2·8	2·2	20 (16+4)	1·3	0·3	Imm. female	4, all dividing	The 4 small tentacles are very small
15	2·65	3·0	20 (16+4)	1·5	0·33	Male	4, all dividing	Gonads mature
16	2·4	2·0	20 (16+4)	1·45	0·25	Male	8 distinct knobs	Gonads mature
17	3·0	2·7	25 (22+3)	1·85	0·32	Male	8 distinct knobs	Gonads mature
18	3·0	3·0	24 (19+5)	2·2	0·3	Male	8 distinct knobs	Gonads mature
19	3·4	2·6	26 (22+4)	1·8	0·3	Male	8 distinct knobs	Gonads mature
20	4·0	3·55	31 (30+1)	3·0	0·35	Female	8 distinct knobs	Gonads mature

in north European waters with more than eight marginal tentacles'. Russell (1940) makes a similar statement. There is, however, a very doubtful record of a specimen with 16 tentacles from Trieste by Graeffe (1884). Mayer (1910) has described *P. carnea* from North American waters maturing with 8, 16, 24 or 32 tentacles, and because of the numerous tentacles has referred to his species as *P. carnea* var. *americana*.

If we were to accept the view that Mayer was dealing with a single species, then my specimens from the Clyde would have to be referred to *P. carnea*, and we would expect to find mature medusae with 10, 12, 14 or 15 tentacles in the plankton. However, none has ever been recorded in north European waters and we are left with the view that there are two separate species—one medusa maturing with 8 tentacles and the other maturing with 16–32 tentacles. The close similarity of the two hydroids and an apparently continuous series of mature medusae at the 8, 16, 24 and 32 tentacle stages may have led Mayer to assume that he was dealing with a single species. There is nothing in Mayer's account to suggest that he obtained mature medusae at intermediate stages between the 8 and 16 tentacle stages. A careful study of Mayer's description of the hydroid shows that in all probability he was dealing with two distinct species—one of which was identical with the European *P. carnea* (Mayer, 1910, text-fig. 74) and the other was *P. borealis* (Pl. 14, fig. 2). He states that 'the reproductive polypites, or gonostyles, are frequently exactly similar in size and shape to the feeding-polypites, and, in fact, are probably merely feeding-polypites which have developed medusa buds (see Pl. 14, fig. 2, *g*). In other instances the gonostyles are smaller and more slender, and possess not more than 4 to 8 tentacles.' In the hydroids of *P. carnea* which I have been able to collect at Plymouth the reproductive polyps have always been small with only 4 or 5 tentacles. In the hydroid from Millport, however, the reproductive polyps were as large as the sterile ones and only later diminished in size after liberating numerous medusae. Allman (1871–2) and Hincks (1868) have probably described the two species under the name *P. carnea*, and a careful redescription of both hydroids from ample material is much to be desired. It is thus clear that some at least of the records of the hydroid and young medusa of *P. carnea* are records of *P. borealis*.

The hydroid *P. areolata* (Alder) may be distinguished from other known British species of the genus by the irregular grouping of the chitinous spines on the basal crust and by the position of the medusa buds; these are sessile on the encrusting base. Further, when the medusa is liberated it has 4 large perradial tentacles and 12 smaller intermediate ones, making a total of 16 tentacles. It is, perhaps, because this species has always been associated with the medusa *P. borealis* in British waters that all hydroids of this kind in which the medusa buds originate on the body of the polyps have been assigned to *P. carnea*. Russell (1940) has suggested that *P. hartlaubi* may be the medusa of Alder's species.

Russell has also indicated that there is a possibility that *P. hartlaubi* may

have been previously recorded from British waters as '*Podocoryne areolata*' because of its superficial similarity to that medusa.

It is suggested that the synonymy of *P. borealis* and *P. areolata* becomes:

Podocoryne areolata (Alder, 1862):
Hydractinia areolata, Alder, 1862.
Rhizocline areolata, Allman, 1864.
Podocoryne areolata, Hincks, 1868.
Cytaeandra areolata, Haeckel, 1879.

Podocoryne borealis (Mayer, 1900):
Cytaeandra areolata, Browne, 1895.
?*Cytaeandra areolata*, Browne, 1897.
Podocoryne areolata, Mayer, 1910; Hartlaub, 1911 (medusa only).
Lymnorea borealis, Mayer, 1900.
Limnorea norvegica, Broch, 1905.

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OSMOREGULATION IN SOME PALAEMONID PRAWNS

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(Text figs. 1–8)

CONTENTS

INTRODUCTION

In recent years much progress has been made in our knowledge of osmotic changes in aquatic organisms, chiefly in marine animals that can successfully withstand a certain amount of dilution of the external medium. In addition to confirming the classical findings of Bottazzi (1897, 1908) that, unlike the teleosts, the body fluids of marine invertebrates and elasmobranchs are isotonic to the sea water in which they live, investigations by numerous workers have shown that the colonization of fresh and brackish waters by marine animals is closely dependent upon the development of osmoregulatory powers which attain a high degree of perfection in freshwater organisms. Schlieper and his school (Schlieper, 1929, 1930, 1935; Schlieper & Herrmann, 1930; Schwabe, 1933; Scholles, 1933; Peters, 1935) brought evidence of the relative importance of the excretory organs in osmoregulation; this has been confirmed by other investigators who have also emphasized the question of differences

in permeability. Finally, the discovery of Krogh (1937, 1938) that many animals in fresh water are capable of actively absorbing ions from their surroundings, even when the latter are present only in exceedingly minute quantities, has introduced a new aspect to the problem of adaptation of marine animals to fresh and brackish waters.

One would normally expect a marine invertebrate to be isotonic with its medium when in sea water, and it was therefore of unusual interest to find two crustaceans in the sea-water tanks of the Plymouth Laboratory showing a blood concentration markedly hypotonic to the external medium. One is the widely distributed and well-known brackish-water prawn *Palaemonetes varians* (Leach) (Panikkar, 1939) and the other the common marine prawn *Leander serratus* (Pennant) (Panikkar, 1940*a*). A similar behaviour was also later discovered in *Leander squilla* (L.). The difficulty these animals have to overcome is not that of maintaining a higher concentration of blood in an external medium of low concentration, which we find in most estuarine animals, but of keeping the blood dilute in defiance of the more concentrated external medium and the consequent prevention of loss of water from the tissues. This condition, however, changes when these same species are in brackish or fresh water, where the body fluids have to remain in a state of definite hypertonicity. The mechanism of osmoregulation needs to be equally efficient in maintaining a stable hypotonicity when in sea water and hypertonicity when in fresh or brackish water.

Among marine invertebrates, the few known instances of slight hypotonicity when in sea water are some of the grapsoid and ocypodid crabs (Edmonds, 1935; Baumberger & Olmsted, 1928; Schwabe, 1933; Conklin & Krogh, 1938; Pora, 1939; Pearse, 1932) which belong to groups that have become semi-terrestrial in habits (Table I). Other parallel instances are the highly specialized brine shrimp *Artemia* (Medwedewa, 1927; Kuenen, 1939), the insects that can inhabit salt water (Beadle, 1939; Claus, 1937; Wigglesworth, 1933, 1938), and certain permanently attached parasites that feed entirely on hypotonic blood (Panikkar & Sproston, 1941).

The general condition observed in these prawns is similar to that of the marine teleosts, which are always hypotonic in sea water and hypertonic in fresh. The peculiar osmotic behaviour in the prawns here described becomes still more significant when the habits and distribution of the group to which they belong are considered. *Palaemonetes varians* is known to exist in two distinct varieties, a freshwater and a brackish-water one, confined respectively to the southern and north-western parts of Europe; the widely distributed genus *Leander* includes marine, estuarine and freshwater species. Can we correlate their osmotic behaviour with their plasticity of habits and the ability of certain palaemonid prawns to migrate into fresh water? It seems reasonable to assume that the osmotic independence shown by *L. serratus* and *L. squilla* must have been acquired in fresh water at some period in their evolutionary history, and, if this assumption is correct, we are dealing with a remarkable

Table I. Crustacea which are Hypotonic in Sea Water or Concentrated Salt Water*

Species	Blood			External medium			Author	Date	Remarks
	Δ − ° C.	mM.	% NaCl	Δ − ° C.	mM.	% NaCl			
Crustacea: Phyllopoda									
Artemia salina	0·70	205	1·20	2·75	804	4·70	Medwedewa	1927	Barger's method. Values for external medium only approximate
	0·76	222	1·30	4·85	1420	8·30			
	0·70–0·82	205–240	1·2–1·4	3·39	992	5·80	Kuenen	1939	Barger's method
	1·28–1·52	376–445	2·2–2·6	10·16	2976	17·40			
Crustacea: Copepoda									
Lernaeocera branchialis	1·17–1·63	342–479	2·0–2·8	1·97–2·04	577–599	3·4–3·5	Panikkar & Sproston	1941	Vapour pressure of blood of ♀ which is permanently attached to fish. Subject to considerable variation
Crustacea: Decapoda									
Palaemonetes varians	1·28–1·40	376–411	2·2–2·4	1·93–2·04	565–599	3·3–3·5	Panikkar	1939	Vapour pressure method. Details in this paper
	1·53	450	2·63	2·79	817	4·78			
Leander serratus	1·52–1·70	445–498	2·6–2·9	1·93–2·04	565–599	3·3–3·5	Panikkar	1940	Vapour pressure method. Details in this paper. Also confirmed by freezing-point method
Leander squilla	1·46–1·52	428–445	2·5–2·6	1·93–2·04	565–599	3·3–3·5	Panikkar	1941	Vapour pressure method. Details in this paper
Grapsus grapsus	1·92	563	3·29	2·04	599	3·5	Pearse	1932	Freezing-point
Pachygrapsus marmoratus	2·01–2·11	589–618	3·4–3·6	2·20–2·33	645–683	3·8–4·0	Schwabe	1933	Freezing-point. Also confirmed by Pora (1939)
Pachygrapsus crassipes	1·32	387	2·3	1·97	577	3·4	Baumberger & Olmsted	1928	Freezing-point. Hypotonicity disappears in certain stages of moult cycle
Leptograpsus variegatus	1·95	571	3·3	2·13	624	3·6	Edmonds	1935	Freezing-point
Heloecius cordiformis	2·85	835	4·9	3·40	996	5·8	Edmonds	1935	Freezing-point
	1·95	571	3·3	2·17	636	3·7			
Eriocheir sinensis	1·85–2·05	541–600	3·2–3·5	2·20–2·28	659–667	3·8–3·9	Conklin & Krogh	1938	Vapour pressure method. Hypotonicity apparent only in concentrated sea water
	2·32	679	4·0	2·54	744	4·4			
Ocypoda albicans	1·70	498	2·9	2·04	599	3·5	Pearse	1932	Land crabs. Freezing-point method
Cardisoma guanhumi	1·66	486	2·8	2·04	599	3·5	Pearse	1932	
Gecarcinus lateralis	1·65	483	2·8	2·04	599	3·5	Pearse	1932	

* Values of Δ and mM. given in this table are based on the relationship that 0·293 mol. Na (or Cl) gives a depression of freezing-point of − 1·0° C.

group of crustaceans which had at one time become accommodated to life in fresh water and has since returned to a marine habitat. The osmotic adaptation involved is of interest because its object is the converse of what is seen in euryhaline animals. The present paper is an attempt to elucidate this osmoregulatory mechanism as studied by changes in total osmotic pressure of blood and urine, and deals with the problem as viewed from the general biological standpoint; the question of ionic regulation will be dealt with in a subsequent paper.

Material and Methods

The prawns used in this investigation were obtained at Plymouth, *Leander serratus* from the Sound, *L. squilla* from Laira, and *Palaemonetes varians* from Chelson Meadow. They were stocked in circulation tanks. No special attempt was made to feed *Palaemonetes*, but *Leander* was now and then fed with bits of *Sepia* and mussels. Judged by the comparatively low mortality among prawns kept in tanks it may be concluded that they remain in a healthy state under circulation. For experimental purposes they were transferred to different media prepared from clean sea water, aerated and kept in large glass jars. The tank water is more saline than outside sea water and has an osmotic pressure of 3·3–3·5 % NaCl; it has a slightly higher calcium content (0·015 *M* as against 0·0098 *M* for outside sea water (Cooper, 1932)). The *p*H is about 8·0, but is subject to slight variation (Atkins, 1922). Dilutions of sea water were always made with Plymouth tap water, which is a very soft water with a slight trace of chloride and about 3 parts per million of silica. Experimental animals were not fed; nor were the jars aerated after the animals had been put into them. Artificial sea water was made with 0·6 *M* NaCl, 0·6 *M* KCl, 0·4 *M* $CaCl_2$ and 0·4 *M* $MgCl_2$ solutions mixed in the proportion of 801·9, 17·2, 27·2 and 139·6 ml. respectively, along with 2 drops of *M* $NaHCO_3$ for every 100 ml. of the mixture, and brought to *p*H 8 with NaOH. Concentrated sea water was prepared by the addition of Tidmann's sea salt to clean sea water, care being taken to bring the *p*H to normal by appropriate buffering.

Estimations of osmotic pressure were made by Baldes's modification of the Hill thermo-electric technique (Baldes, 1934; Hill, 1930, 1931). The standard solutions employed for comparing the vapour pressures were over a fairly wide range, i.e. between 1·8 and 2·5 % NaCl for the blood of *Palaemonetes* and between 2·0 and 3·0 % NaCl for that of *Leander*. After mounting the solutions on the thermocouples in a moist chamber, they were equilibrated in a thermostat kept constant at 20·8° C. ± 0·001, and the deflexions taken from a Downing (moving-coil) galvanometer of sensitivity $1{\cdot}28 \times 10^{-8}$ V./mm. at a distance of 3 m. Using tested thermocouples, 1 mm. deflexion on the scale was equivalent to an e.m.f. caused by solutions differing in strength by 0·005 % NaCl, so that, using a magnifying glass, differences up to half the above figure could be read on the scale. All estimations were made in duplicate

on two separate instruments, and most of the readings were also taken in the same instrument with the unknown sample placed in a 'normal' and a 'reversed' position. The data given in the tables and graphs are the mean values of the readings taken with each sample. The values of osmotic pressure are throughout expressed as percentage of sodium chloride, i.e. the grams of NaCl dissolved in 100 g. of distilled water to make a solution having the same vapour pressure as the unknown at the same temperature and atmospheric pressure. Owing to variations in the salinity of the tank water, parallel estimations of osmotic pressure of tank water were made in all cases where the animals were taken from the circulation. The experimental solutions used as external media were measured in the same manner as blood and urine, but the standard solutions employed ranged from distilled water to 3·5 % NaCl; special attention was paid to having the filter paper on the walls of the couple chamber moistened with the solutions being compared, to ensure greater accuracy (cf. Hill, 1931). In all experiments the thermocouples were calibrated both before and after a series of estimations, and the mean values taken for final calculations.

The following uniform procedure was adopted for the removal of blood from prawns. The animals were wiped with a dry towel, and the water in the gill chambers and between the cephalothoracic shield and the first abdominal segment was completely removed with strips of filter paper. A capillary pipette (specially drawn out from glass tubing cleaned in bichromate sulphuric mixture and distilled water, and kept in readiness in a desiccator) was introduced into the heart at the point where it is easily accessible from outside, i.e. under the hind-margin of the cephalothoracic shield. The blood collects in the tube because of the hydrostatic pressure and capillarity, but if larger quantities were required, the blood could be sucked in by an attached rubber tube. Blood from the pipette was transferred direct to the thermocouples (kept in the moist chamber) almost immediately after removal because of its tendency to clot, especially in *Leander*. When drops of blood were mounted, the first drop from the pipette was not used, nor was the last, so as to reduce the errors due to evaporation to a minimum. No animal from which blood was removed once was ever again used except for the removal of urine.

The obtaining of samples of urine was considerably more difficult. Repeated trials showed that the following method gave the best results. The prawn, after the adhering water has been wiped off, is laid on its back on a glass plate under a binocular microscope; if this proves difficult it may be kept in position by a rubber band running round the plate. By careful manipulation, the opening of the antennary gland at the base of the antenna may now be located and its outside carefully wiped with strips of filter paper. The tip of a micropipette, with an attached rubber tube, is introduced into the pore of the antennary gland and the contents sucked into the pipette. In many experiments the hydrostatic pressure of the urine inside the bladder was sufficiently high to fill the pipette without much effort. While this procedure worked well with *Leander*, success

was only partial with *Palaemonetes* owing to its small size. Some samples were obtained from larger individuals by the same method as the above. In others, only the contents of the nephroperitoneal sac were taken for estimation of osmotic pressure. In *Leander*, the contents of this sac (which is in direct communication with the bladder, Fig. 5, p. 332), as obtained by dissection, had nearly the same osmotic pressure as the urine collected at the base of the antenna (Table III). Applying this information to *Palaemonetes*, the contents of the nephroperitoneal sac may be expected to give an indication of the properties of the urine; the source of the samples is indicated separately in the tables.

The dry weight of specimens used for estimation of water content was taken after the animals had been crushed and kept in a hot-air oven at a temperature of 105° C. for 24–36 hr. which was about the time required to get constant weights. The weighings were to within 2·5 mg.

The freezing-point of the blood of *Leander serratus* was determined by a cryoscope of the Burian and Drucker model; the Beckmann thermometer was graduated to 0·02° C. and the readings taken were correct to about 0·01° C.

Osmotic Changes in the Blood of *Palaemonetes*

Palaemonetes varians inhabits brackish waters in the vicinity of Plymouth and adjoining places; though it is common in waters of varying salinity on the English and Irish coasts, it is rarely, if ever, met with in the open sea. In northern and western Europe the species is known only from water with some admixture of salt, whereas in the Mediterranean it is a freshwater species; but even there it is not exclusively fluviatile as shown by Gourret's record of it from salt water in the south of France along with strictly marine species (Gurney, 1923). Physiological varieties of the species have been distinguished (vide Boas, 1898, for summary and literature) in accordance with the size of the egg and reproductive habits; the brackish-water form with smaller eggs and full complement of larval stages is called var. *microgenitor* (=var. *occidentalis*, Sollaud, 1923) and the freshwater variety with larger and fewer eggs and a reduced development is designated var. *macrogenitor* (=var. *lacustris* von Martens). Sollaud added a third variety *mesogenitor* from Tunisia, but this is probably a distinct species (Gurney, 1923). It may be pointed out that there is no important structural difference between these varieties. In a later paper, Sollaud (1932) has suggested that the four forms of *Palaemonetes* found around the Mediterranean which are usually considered as different varieties of *P. varians* are specifically distinct, though they form a natural group of their own ('clan') within the genus. Of these, *P. varians* has small eggs (microgenitor), *P. antennarius* and *P. mesopotamicus* have large eggs (macrogenitor), while *P. mesogenitor* shows the intermediate condition.

The locality from which specimens were obtained for this study is subject to wide fluctuations in salinity during the year. The Chelson Meadow consists

of a large tract of unreclaimed land near the mouth of the Plym, from which it is separated by an embankment. Salt water enters during high tide and the excess of water drains off into the river through sluice gates. The animals that are commonly obtained with *Palaemonetes* in the ditches at Chelson Meadow are *Potamopyrgus*[1] *jenkinsi* and *Gammarus chevreuxii*, both of which are well known for their high degree of tolerance to salinity changes (Sexton & Matthews, 1913). During the course of this work the highest salinity observed was in the summer and autumn, and the lowest during the winter (Fig. 1).

The blood of *Palaemonetes* obtained from its natural habitats has an osmotic pressure varying between 2·0 and 2·4 % NaCl according to changes in the environment at different times of the year as shown in Fig. 1. The results plotted indicate an almost straight-line correlation except for the values obtained during February, which are comparatively higher, and of May (summer) which are lower. The blood is hypotonic in concentrations up to about 2·25 % when isotonicity is established. The blood is hypertonic in further dilutions of the environment, and the fall in osmotic pressure compared with that of the external medium is very limited, the lowest value being about 2·0 % NaCl. The blood of prawns that were living in the sea-water tanks for some months gave values of about 2·30 % NaCl. Thus in prawns taken from the natural habitats the maximum difference between the highest and lowest values for blood is only 0·40 % NaCl for a difference of nearly 3·20 % NaCl in the external medium, while under experimental conditions the corresponding value is nearly 0·50 % NaCl.

The osmotic changes in the blood of *Palaemonetes* when transferred from sea water to different experimental media are shown in Fig. 2A. The curve obtained for these values does not exactly correspond with the values for specimens from Chelson Meadow (Fig. 2). The difference seen is no doubt due to the slow and prolonged acclimatization which the experimental animals are denied. But this is not explained entirely on the basis of the time factor, since experimental animals have been observed to attain a more or less steady state after some time.

Palaemonetes is able to survive sudden transfer from sea water to water of very low salinity, i.e. about 0·5 % NaCl. The prawns remain active and healthy in spite of sudden changes in external medium and, by slow acclimatization, the animals could be brought down to water of as low an osmotic pressure as 0·01 % NaCl. If directly transferred to distilled water from sea water they survive for about 2–3 hr.; but in distilled water with a trace of sodium chloride (osmotic pressure 0·005–0·01 % NaCl) they live for 24–30 hr. Six specimens acclimatized to 0·45 % NaCl lived for 18–22 hr. in Plymouth tap water, and their survival period could be prolonged to several days by the addition of a small quantity of NaCl solution so as to raise the osmotic pressure of the medium to about 0·005–0·01 % NaCl.

Values of osmotic pressure of specimens transferred from sea water to

[1] Also known under the names *Hydrobia* and *Paludestrina*.

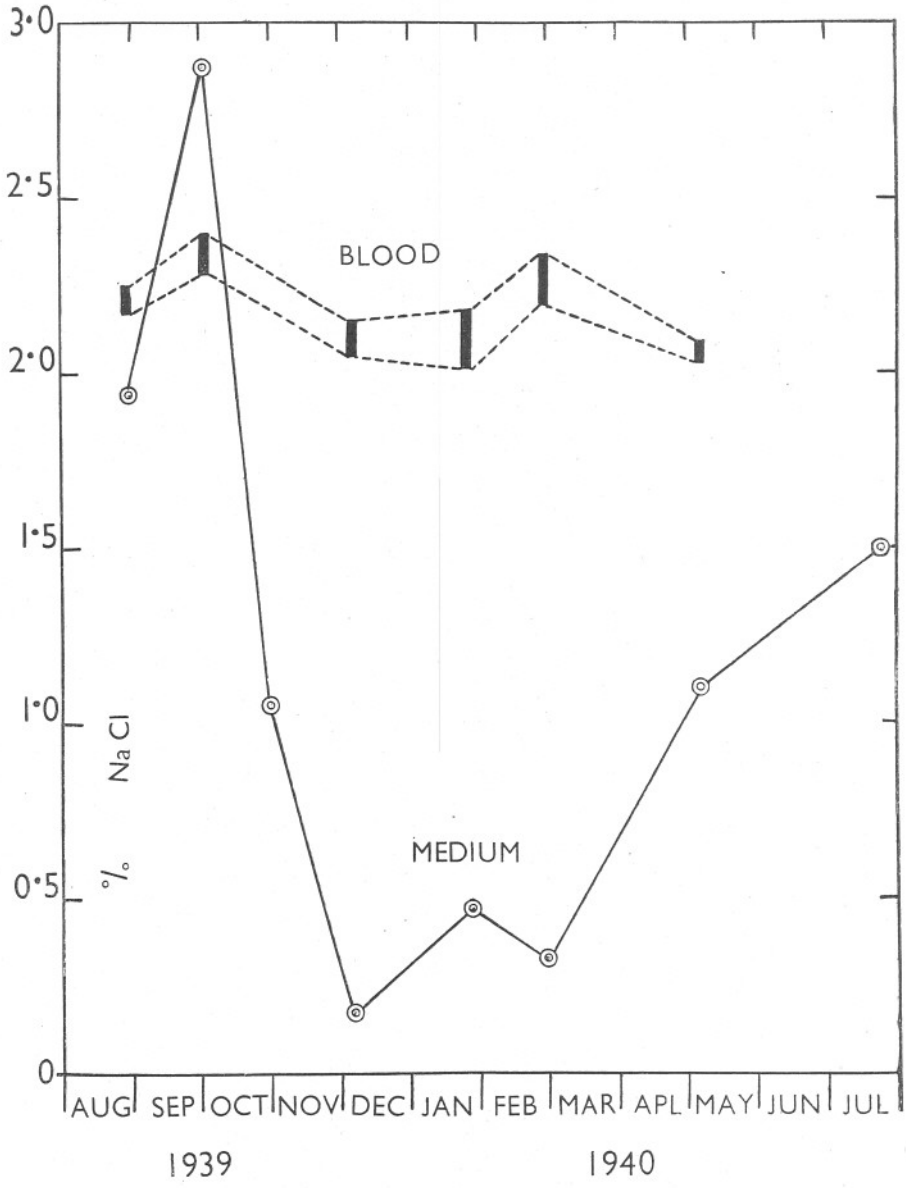

Fig. 1. Seasonal changes in the osmotic concentration of the blood of *Palaemonetes varians* in its natural habitat at Chelson Meadow. Ordinates represent osmotic pressure of blood (% NaCl) and the salinity of the external medium (expressed for convenience in % NaCl). The range of values for blood falls between the dotted lines.

water of low salinity and from water of low salinity to sea water are given in Figs. 3 and 4. In the first transfer (Fig. 3*a*) there is a steady fall in values from about 2·4 to nearly 2·0 % within the course of 8–10 hr., and an almost steady state is thereafter attained. The curve, however, is by no means smooth, which may be partly due to the differences in adaptive powers among the individuals taken for experiment. There is also an indication that the osmotic pressure fluctuates before a final steady state is reached, and this is also seen in animals transferred to sea water (Fig. 4*a*). In both experiments the animals show a direct and immediate response to changes in external osmotic pressure and the attainment of a steady state by later adjustment.

In 1925 Vialli made some measurements of the osmotic pressure of the freshwater variety of *Palaemonetes varians* from Italy by employing Monti's method. From thermoelectric readings of what he calls the coelomic cavity, he found that the prawns showed a Δ of $-0{\cdot}54^{\circ}$ C. which is equivalent to about 0·92 % NaCl. Even the lowest value I have obtained in specimens acclimatized to nearly freshwater is considerably higher than Vialli's. Confirmation of his results would be of great interest, since it may indicate another physiological difference between the Mediterranean and Western European varieties.

By allowing the water in which they are kept to evaporate, or by the addition of small quantities of Tidmann's sea salt, specimens of *Palaemonetes* have been acclimatized to concentrations as high as 5·2 % NaCl. From sea water the prawns can survive direct transfer to concentrated sea water having a value of 5·0 % NaCl; but animals acclimatized to hypotonic media were unable to live long in water of 4·5 % NaCl after direct transfer.

Osmotic changes of *Palaemonetes* consequent upon direct transfer from sea water to concentrated sea water of 5·00 % NaCl are given in graphs *b* and *c*, Fig. 4. There is a steady rise in the values for blood to about 3·0 % within the first 4 hr., but afterwards there is great fluctuation in values. It is possible that some of the specimens which showed abnormally high values were those which in due course might have died owing to inefficient regulation, but at the time when estimations were made they were alive and active. The critical stage in adaptation is evidently after about 4–5 hr. when the osmotic pressure is already 3·0 % NaCl, since the high degree of scatter in the values is observed only after this period. It is noteworthy that prawns that lived in the medium for 3 days and had attained a steady state also show a value of nearly 3·0 %. In 4·78 % NaCl, the mean value for blood came down to about 2·6 % in a week's time (Table II).

The figures obtained when animals are subjected to sudden change of environment show definitely that there is an initial rise or fall in osmotic pressure which is considerably higher or lower than the final steady value. Thus the upset of osmotic equilibrium is not avoided by the animals by passive control, but adjustment is later effected by osmotic work done by them. The fluctuations in the values may largely be due to individual differences in

adaptive capacity owing to differences in size and moult stage, but this cannot account for the rise and fall after 4 hr. in experiments with concentrated sea water. Possibly the entry of salts into the prawns may take place discontinuously, as for example when they occasionally drink water to make up for water loss, and this is partly responsible for the wide fluctuations.

It has already been mentioned that the osmotic pressure of the blood of animals taken from the natural habitat during February was slightly higher that one would expect from the curve. Similarly there is a steady fall from February to May in spite of the rise in salinity of the medium (Fig. 1). This would indicate a lower value in summer and a higher one in winter. A similar seasonal cycle has been observed by Widmann (1935) and Otto (1937) working with other Crustacea. According to their observations as well as mine, higher temperature seems to give lower values of osmotic pressure. The possible significance of this in certain problems of animal distribution has been discussed elsewhere (Panikkar, 1940*b*).

Palaemonetes is able to take in salt when in hypotonic media. Prawns collected from Chelson Meadow, found living in a medium of 0·175 % NaCl and having an average osmotic pressure of 2·108 % NaCl, were transferred to dilute sea water of 1·450 % NaCl. Osmotic changes cannot be expected since the new medium is also hypotonic to the blood, but after 48 hr. the average osmotic pressure of the prawns rose to 2·290 % NaCl. Judging from the analogy of freshwater and brackish-water organisms investigated by Krogh (1938) the gills would seem to be the primary site of absorption, though a small amount of salt may also be absorbed through the gut.

Osmotic Changes in the Blood of *Leander*

Leander serratus

Leander serratus, the so-called 'common prawn', is a littoral marine species abundant in the southern parts of the North Sea and in the Mediterranean. Details of its distribution are given by Gurney (1923). Though it is able to withstand a certain amount of dilution of the environment, this prawn is essentially marine in its habits, but it is not uncommon to find it among sea weeds on the coastal regions where slight dilution of the sea water may take place from rain or river water. Unlike the closely related species *L. longirostris*, *L. serratus* does not seem to migrate much into rivers; nor does it show any preference for brackish water or estuarine habitats as do many other *Leander*.

The osmotic pressure of the blood of *L. serratus* when in sea water has a value of 2·6–2·9 % NaCl. The variations of osmotic pressure among individuals of the same batch is higher in this prawn than in *Palaemonetes*, and, as will be shown later, much of this variation may be expected on the basis of differences in moult stages. It is worth mentioning that my records show that, as in *Palaemonetes*, the blood shows a higher value in winter than in summer. The mean value for the blood of twelve prawns of about the same intermoult

period, as judged by appearance, taken from sea water of 3·45 % NaCl in summer was 2·60 % NaCl, but the corresponding value for winter was 2·85 %. While there is no perceptible difference between males and females not in berry, the ovigerous females on the whole show a slightly higher value of 2·95 % (winter). I have not been able to find any definite correlation between size and osmotic pressure of blood among individuals averaging from

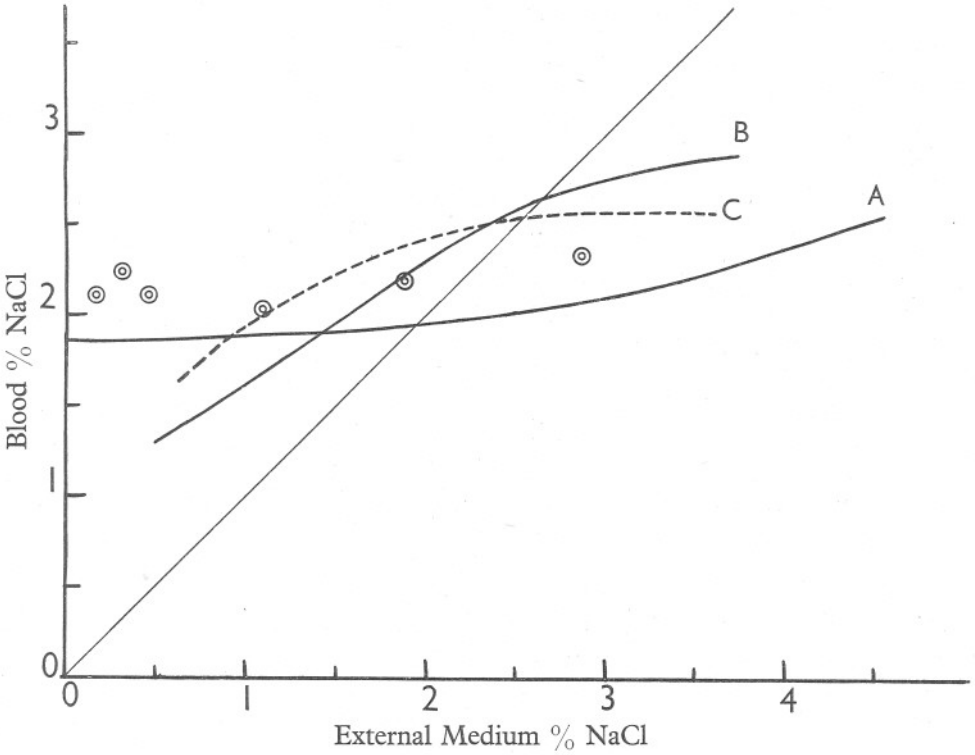

Fig. 2. Osmotic pressure (in % NaCl) of the blood of (A) *Palaemonetes varians*, (B) *Leander serratus* and (C) *Leander squilla* (dotted line) in different concentrations of the external medium measured under experimental conditions. Abscissae, external medium; ordinates, blood. Straight line indicates where points would fall if external and internal media were isotonic. Circles represent osmotic pressures (mean values only) of *Palaemonetes* from water of different salinities in its natural habitat.

35 to 80 mm. in length, but the larger prawns of 96–100 mm. usually show higher values than smaller individuals of the same batch. In the absence of data on the moult stage of each prawn investigated, I am not inclined to emphasize these differences, especially when we consider that the larger prawns have a longer intermoult period.

Osmotic pressure of the blood of *Leander serratus* was also measured by the freezing-point method. Blood from about twenty-five specimens was collected for this purpose and allowed to stand for 6 hr., after which the clear

bluish serum was separated and poured into the cryoscope. In two series of estimations with two sets of prawns taken from circulation in January 1941, Δ values of $-1{\cdot}70$ and $-1{\cdot}66$ °C. were obtained for the blood serum and of $-2{\cdot}06$° C. for sea water. These values are in fairly close agreement with the results obtained by the vapour-pressure method.

Table III and the graph B in Fig. 2 show the osmotic relations of *L. serratus* in different dilutions of the environment. The prawn is homoiosmotic in normal sea water down to a dilution of sea water equivalent to 2·5 % NaCl, which is the approximate point of isotonicity. There is a steady decline in value after this, but even in lower dilutions the ability of the animal to maintain a hypertonic blood concentration is well marked. The curve has thus the essential features of the *Palaemonetes* curve, the differences observed being caused by the lower osmotic deficit when in sea water, and absence of efficient regulation in very dilute sea water. I have not been able to acclimatize *Leander serratus* to live in water below 0·6 % NaCl; the mortality was very high in experiments with water of 0·6–1·0 % NaCl, and no prawn which lived for a few days in these dilute media showed an osmotic pressure less than about 1·6 % NaCl.

Acclimatization experiments show that the degree of tolerance to changes in the external medium is very limited in *Leander* as compared with *Palaemonetes*, but certainly much better developed than in most marine invertebrates. It can survive direct transfer to media as low in concentration as 1·6 % NaCl. Even in lower dilutions the prawns live for 3–4 hr. after direct transfer and may recover if taken back to sea water before they have become comatose. In all these the size of the individual and the moult stage seem to have a close bearing on the ability to survive sudden salinity changes. Smaller juvenile prawns have a greater degree of tolerance than larger ones, and the ovigerous females have the minimum amount of tolerance to diluted media. Similarly, the freshly moulted individuals die in a few hours if transferred to sea water of even moderate dilutions, such as 2·0 % NaCl. The period of survival after such changes is longer the longer the time that has elapsed after moult.

Slow acclimatization to concentrated sea water is possible, the maximum upper limit being about 3·9 % NaCl, the osmotic pressure of blood rising in the meantime to 3·1 % NaCl. This value is not represented in curve B, Fig. 2, since the observations have not been extensive.

Osmotic changes of prawns when they are directly transferred to dilute sea water are shown in Fig. 3. As may be expected, the time taken to reach the lowest value varies according to the strength of the experimental medium and the sizes of individuals, the small prawns showing a higher rate of change. The dilution curve is steady until the final equalization, followed by a slight rise, the curve having the same shape as the one obtained for *Palaemonetes*.

Like *Palaemonetes*, *Leander serratus* is able to absorb salt from hypotonic surroundings. Prawns acclimatized to 50 % sea water (osmotic pressure

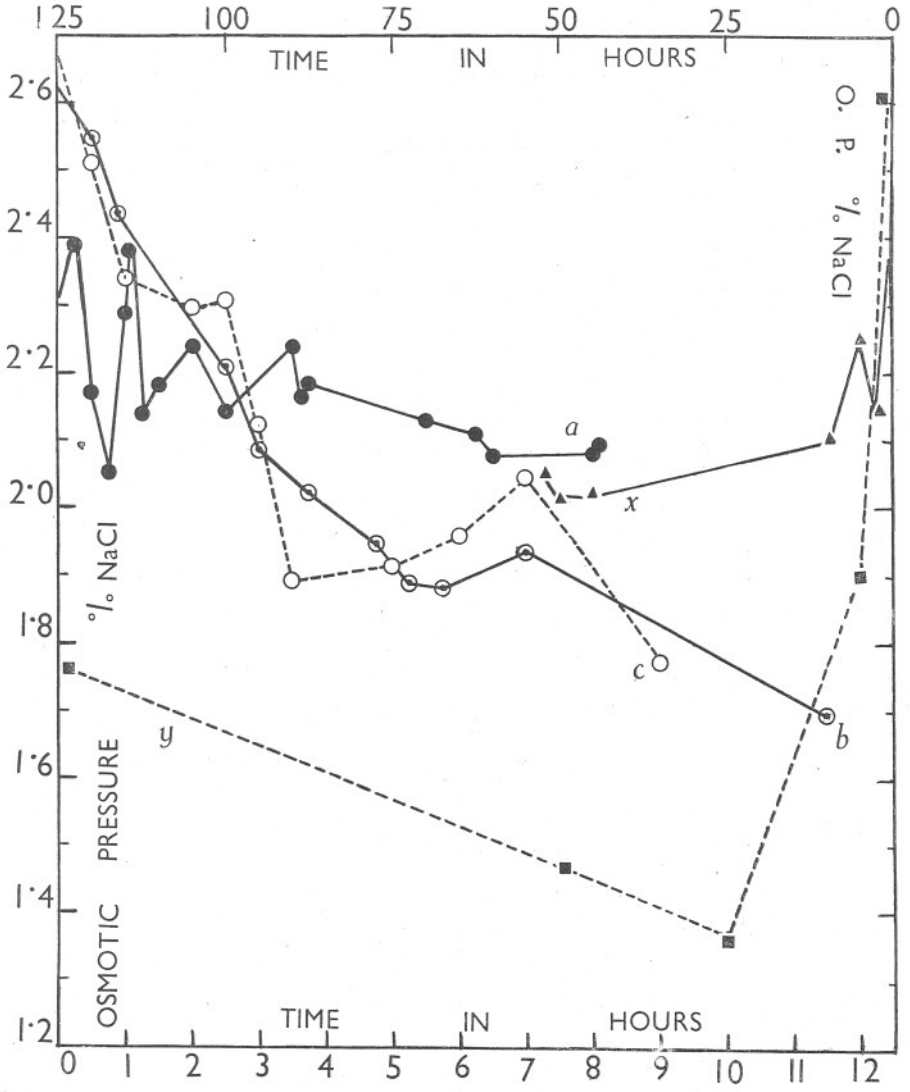

Fig. 3. Osmotic changes of *Palaemonetes varians* and *Leander serratus* when transferred directly from normal sea water to dilute sea water. Abscissae, time in hours; ordinates, osmotic pressure of blood in % NaCl. (*a*) *Palaemonetes* from sea water to dilute sea water of 0·565 % NaCl (9. xii. 39); (*b*) *Leander* from normal sea water to dilute sea water of 0·616 % NaCl (29. xi. 39); (*c*) *Leander* from sea water to dilute sea water of 0·65 % NaCl (dotted line, 6. xii. 39); (*x*) same experiment as (*a*) but continued for 54 hr.; (*y*) *Leander*, same experiment as (*b*) but carried on for 124 hr. (dotted line). Lower time scale for (*a*), (*b*) and (*c*); upper scale for (*x*) and (*y*).

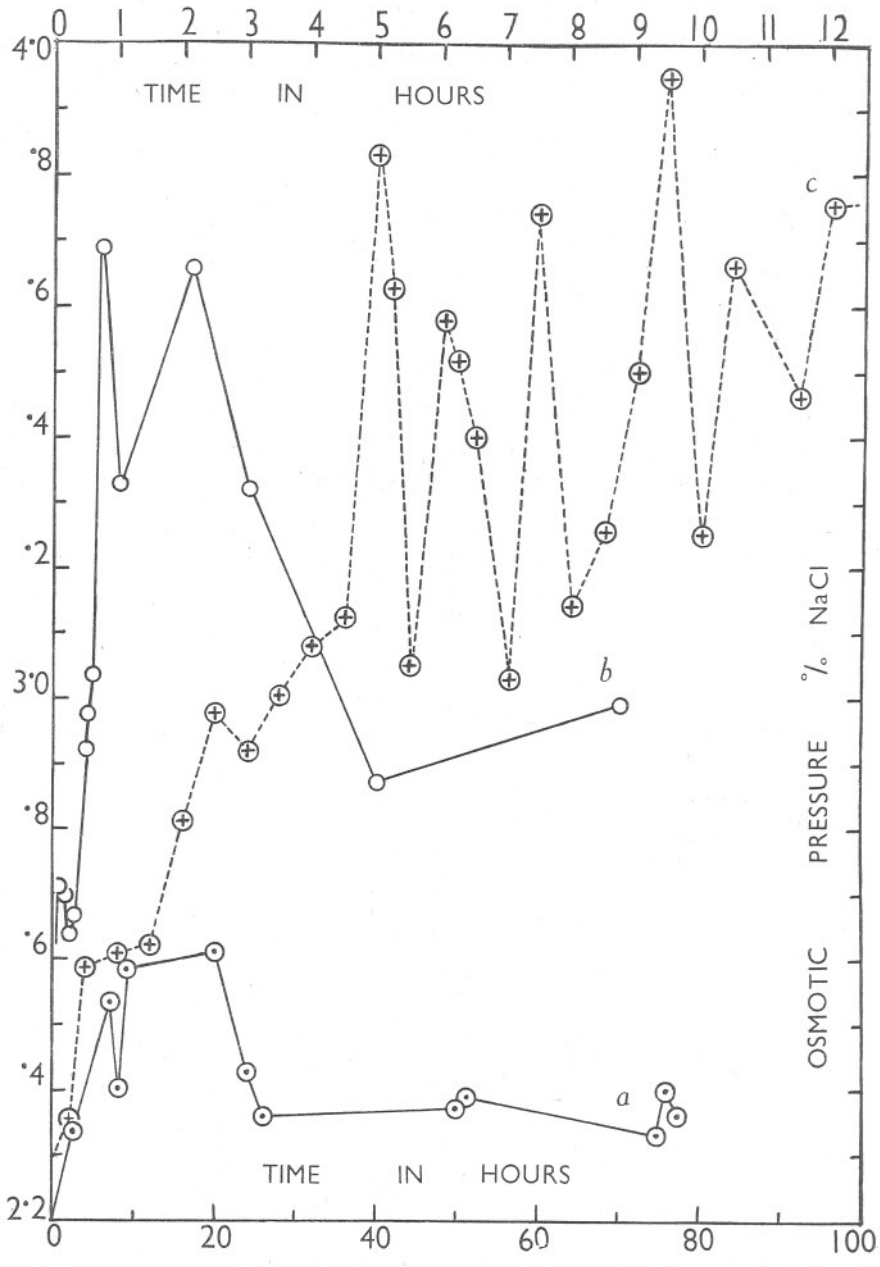

Fig. 4. Osmotic changes of *Palaemonetes varians* on direct transfer to concentrated media. Abscissae, time in hours; ordinates, osmotic pressure of blood in % NaCl. (*a*) From water of 0·325 % NaCl (natural habitat) to circulating sea water of 3·341 % NaCl (1. iii. 40); (*b*) from sea water of 3·4 % NaCl to concentrated sea water of 5·0 % NaCl (3. iii. 40); (*c*) from sea water of 3·4 % NaCl to 5·5 % NaCl (dotted line) the changes observed during the first 12 hr. Lower time scale for (*a*) and (*b*); upper for (*c*).

1·720 % NaCl) and having a mean osmotic pressure of 2·3 % NaCl showed an increase in value up to 2·5 % NaCl in 14 hr. when transferred to sea water of 2·2 % NaCl.

Leander squilla

Leander squilla (Linn.) is littoral in habits; the species enjoys a wide distribution, being common in the Baltic Sea, Scandinavian, Dutch, British and French coasts, the Mediterranean and the Black Sea (Gurney, 1923). It is frequently encountered in brackish water in the Black Sea and in pools in salt marshes on the English coasts along with *Palaemonetes varians*. In addition to occurring in the sea at Plymouth, the species has been recorded a few miles up the River Lynher, River Tamar and at Laira; in the spring of 1940 a few juvenile individuals were obtained along with *P. varians* at the Chelson Meadow. Gurney mentions that there can be no doubt that the whole larval life is spent in the sea. It was difficult to obtain a plentiful supply of this prawn during the period of study, and the observations on it are therefore not very extensive.

Fig. 2C gives the values of osmotic pressure of prawns from different salinities. In normal sea water the blood is equivalent to about 2·6 % NaCl; approximate isotonicity is established in a medium equivalent to about 2·5 % NaCl, and in lower dilutions the blood is hypertonic to an appreciable extent indicating effective regulation. The curve obtained when the results are plotted is very similar to that for *Leander serratus*. It is noteworthy, however, that the optimum value in sea water is slightly lower than in *L. serratus* (2·6 as against 2·8 %), and that the homoiosmotic behaviour is even better developed than in the former species. The osmotic adaptation is therefore intermediate between that of *Palaemonetes varians* and *Leander serratus*, and this is precisely what one would expect from its habits and distribution, and from the results obtained by Mathias (1938) and Pora (1938) in regard to its ability to withstand experimental changes in the external medium.

Osmotic Properties of the Urine of Palaemonids

Description of the Excretory Organs

The main excretory organs of prawns are the green glands or antennary glands which become functionally active as soon as the shell glands or maxillary glands disappear at the end of larval life. The green glands of the Palaemoninae have been described by Grobben (1880), Weldon (1889, 1891), Marchal (1892), Allen (1892*b*), Cuénot (1895) and Patwardhan (1937). The excretory pore, found at the base of the antennary peduncle in close proximity with the labrum, leads through the ureter into the bladder, to one side of which is attached the end-sac and the convoluted tubules of the labyrinth Fig. 5). The end-sac is a small, compact, specialized structure composed of

an outer layer of connective tissue containing blood spaces and an inner layer, thrown into folds projecting into the lumen of the sac, which is lined with large excretory epithelial cells having conspicuous nuclei and granular cytoplasm. The excretory tubules forming the glandular plexus or the labyrinth anastomose freely with one another, but they communicate by means of a common opening with the end-sac and by several openings with the bladder. The walls of these tubules are formed by a single layer of excretory epithelial cells, while the intervening spaces between the tubules are filled with loose connective tissue and numerous blood lacunae. The bladder wall is extremely thin and lined with epithelial cells resembling those seen in the tubules.

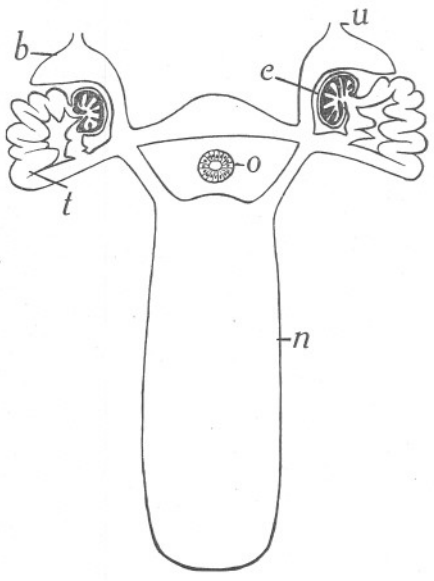

Fig. 5. Diagram of the palaemonid excretory system (after Weldon). *b.* bladder; *e.* end-sac; *n.* nephroperitoneal sac; *o.* oesophagus; *t.* tubule; and *u.* ureter.

Of special significance in the excretory system of the Palaemoninae is the large median renal or nephroperitoneal sac which is an unpaired structure lying in the cephalothorax dorsal to the cardiac stomach and ventral to the ophthalmic artery and the median dorsal sac. This communicates with the bladder by means of two lateral nephroperitoneal ducts which are also connected by a transverse commissure passing in front of the oesophagus. The renal sac is composed of a single layer of flattened excretory epithelium which both Weldon and Marchal found capable of absorbing indigocarmine and similar substances when they are injected into the blood of the living animal. From a study of the development of the excretory organs of *Palaemonetes varians*, Allen (1892*b*) demonstrated that the nephroperitoneal sac is formed by the fusion of the backward extensions of the two bladders. He also drew attention to the presence of a completely closed dorsal sac (the median dorsal blood sinus of Weldon) which lies upon the nephroperitoneal sac; it is probably coelomic in origin and persists in the adults of *Palaemon* (=*Leander*) and *Palaemonetes*. According to him it does not contain blood since blood corpuscles are never met with in the sacs of the larvae or of the adults; nor has any direct communication been observed between the dorsal sac and the blood sinuses of the body.

In Tables II and III are given the values of the osmotic pressure of the urine of *Palaemonetes* and *Leander*. In general the osmotic pressure of the urine is closely similar to that of the blood, though slight variations on either side are observed, as was also noted by Picken (1936) and Nagel (1934) in

Carcinus. The results also indicate that the general rule of isotonicity with blood is not affected by the differences in the nature of the medium, whether hypotonic or hypertonic. In *Palaemonetes* transferred to fresh water with a little NaCl, the urine was found to be slightly hypotonic to the blood, which also had a comparatively low value. But even here the extent of hypotonicity is so small that it may not be considered far outside the range of experimental variation, and further, the steady state had not been attained as evidenced by the low value for blood.

TABLE II. OSMOTIC PROPERTIES OF THE BLOOD AND URINE OF *PALAEMONETES VARIANS* IN DIFFERENT MEDIA. MARCH 1940

No.	Length mm.	Time	Osmotic pressure, % NaCl		
			Blood	Urine	Medium
1	28	1 week	2·606	—	4·780
2	29	1 week	2·583	—	4·780
3	27	1 week	2·692	—	4·780
4	27	10 days	2·745	2·615	4·225
5	27	10 days	2·546	2·500	4·225
6	27	15 days	2·315	2·318	3·410
7	28	1 month	2·224	2·290	3·246
8	29	Nat. habitat	—	2·158	0·468
9	28	Nat. habitat	—	1·952	0·468
10	28	Nat. habitat	2·013	—	0·468
11	27	Nat. habitat	2·188	—	0·468
12	33	15 days	1·907	1·918*	0·446
13	32	15 days	1·935	1·930*	0·446
14	32	15 days	1·945	1·969*	0·446
15	22	44 hr.	1·106	0·984	0·017
16	20	45 hr.	1·165	1·145*	0·017
17	28	Gradually acclimatized	1·892	—	0·010

* Sample from nephroperitoneal sac.
No. 15 showed signs of distress; no. 16 was a healthy specimen.

TABLE III. OSMOTIC PROPERTIES OF THE BLOOD AND URINE OF *LEANDER SERRATUS*. FEBRUARY 1940

Length mm.	Time	O.P. medium % NaCl	O.P. blood % NaCl	O.P. urine % NaCl
90 (ov. female)	2 months	3·246	2·960	2·991
94	2 months	3·246	3·046	2·955
90 (ov. female)	2 months	3·410	—	3·000
95 (ov. female)	2 months	3·400	2·934	2·958
55	1 month	3·210	2·608	2·598*
57	11 days	2·873	2·547	2·536*
57	11 days	1·646	2·180	2·190
62	11 days	2·873	2·500	—
75	½ hr.	Tap water	2·559	1·420 (?)
80	1 hr.	—	2·479	2·477

* Sample from nephroperitoneal sac. All others collected from the opening of the antennary glands.

The contents of the different parts of the excretory system of three specimens of *Leander* were removed separately and their osmotic pressures estimated to find if significant differences could be discovered (Table IV). The method adopted was to dissect the prawns under the binocular microscope, after blood had been collected from the heart and urine from the opening of the antennary gland. The contents of the nephroperitoneal sac and the dorsal

TABLE IV. ANALYSES OF SAMPLES FROM DIFFERENT PARTS OF THE EXCRETORY SYSTEM OF *LEANDER SERRATUS*. OSMOTIC PRESSURE IN % NaCl. MARCH 1940

No.	Medium	Blood	Urine from bladder	Nephro-peritoneal sac	Dorsal sac
1	3·400	2·781	2·792	2·691	2·701
2	3·280	2·760	2·698	2·720	2·770
3	3·280	2·810	2·779	2·791	2·828

sac were collected in separate micropipettes. While reasonably pure samples could be obtained from the nephroperitoneal sac, the possibility of contamination with blood could not be completely ruled out in collecting from the dorsal sac, though every possible care was taken to make sure that the sample was pure. The values obtained are nearly identical with the blood except for slight variations which are well within the limits of experimental error. Similar analysis could not be carried out with *Palaemonetes* owing to its smaller size. The fact that there is isotonicity of urine with blood in *Palaemonetes* acclimatized to water of extremely low salinity, and the almost similar values obtained for the contents of the different parts of the green gland of *Leander*, would point to the conclusion that there is no mechanism for the production of urine hypotonic to blood in either of these prawns.

It may be mentioned here that, in addition to the antennal glands, the gills may be expected to have a marked excretory function as shown by the work of Mollitor (1937) on the crab *Eriocheir*.

OSMOTIC CHANGES IN RELATION TO MOULTING

Interesting osmotic changes in accordance with the moult cycle were discovered by Baumberger & Olmsted (1928) in the crab *Pachygrapsus crassipes*. In sea water having a freezing-point depression of $-1{\cdot}975^{\circ}$ C. the tissue fluids of hard crabs had a Δ of $1{\cdot}327^{\circ}$, of pillans[1] $1{\cdot}893^{\circ}$, of hard crabs about to moult $2{\cdot}601^{\circ}$, and of newly moulted crabs $2{\cdot}193^{\circ}$. Thus the crabs are normally hypotonic to the extent of $-0{\cdot}648^{\circ}$ C. when they are far from moult, but in most other stages of the moult cycle the blood is hypertonic to the external medium. It has been concluded that there is a large increase in blood concentration of crabs just before moult and that a considerable amount of

[1] A stage intervening between 'hard' and 'about to moult' crabs, characterized by cracks on the carapace. Also called 'peelers'.

water is absorbed immediately after moult, which accounts for the reduction of osmotic pressure to normal. These changes have been confirmed in *Callinectes sapidus* (Baumberger & Dill, 1928) and in *Carcinus maenas* as regards water content (Robertson, 1937). There is no reference in the literature to osmotic changes in Macrura as influenced by the moult. In *Homarus* it has recently been found that osmotic equilibrium is established soon after moulting (Lowndes & Panikkar, 1941). The following observations are of interest, since in no stage of the moult cycle is *Leander* or *Palaemonetes* hypertonic or even isotonic to the environment, thus offering a striking contrast to the condition reported for *Pachygrapsus crassipes*.

In prawns it is by no means easy to judge the moult stages by appearance; nor is it possible to classify them into different moult groups as was done by Baumberger and Olmsted in *Pachygrapsus*. The prawns usually moult during the night and the moulting process itself is rapid; it was thus not possible to obtain a large number in which the time which had elapsed since the moult had been definitely ascertained. The procedure adopted was to keep isolated individuals under observation. The moulting has been described by Gurney (1923) in *Leander longirostris* and Nouvel (1933) in *L. serratus*, and I have little to add to these accounts. According to Nouvel the intermoult period varies from 10 to 20 days—the interval being longest in large prawns. The intermoult period was much longer in the batch of prawns observed by me; in young prawns it varied from 15 to 25 days, while the larger ones took about 30–35 days. In addition I have had under observation large prawns that had not moulted for 40–50 days.

TABLE V. OSMOTIC CHANGES OF *LEANDER SERRATUS* IN RELATION TO MOULT CYCLE. TEMPERATURE 6–12° C.

Date 1940	Length of prawn mm.	Time after moult	Osmotic pressure blood, % NaCl	Osmotic pressure medium, % NaCl
21. iii	60	½ hr.	3·030	3·400
22. iii	51	1 hr.	2·984	3·400
15. ii	41	12 hr.	2·870	3·400
12. iii	90	15 hr.	2·815	3·342
14. ii	51	24 hr.	2·820	3·332
14. ii	54	24 hr.	2·932	3·210
23. iii	60	25 hr.	2·730	3·400
23. iii	95	10 days	2·635	3·400
28. ii	45	24 days	2·688	3·341
28. ii	102	38 days (ov. female)	2·913	3·341
14. ii	100	Far from moult	2·990	3·332
18. i	94	Far from moult. New moult imminent as judged by appearance	3·046	3·246

The values given in Table V show that the prawns have a higher osmotic pressure than normal immediately after moult, but the concentration comes down to normal in about 10 days' time. Expressed in terms of difference

TABLE VI. WATER CONTENT OF *LEANDER SERRATUS*

Prawns from sea-water circulation except 1 and 2

No.	Moult stage	Length mm.	Gross weight g.	Dry wt. g.	% water	% water, cast included
1	15 min. after moult in Ca free sea water	55	1·416	0·307	—	78·32
2	½ hr. after moult in art. sea water with calcium	67	2·717	0·564	80·28	79·28
3	½ hr. after moult	60	1·180	0·242	79·5	78·17
		Moult alone	0·240	0·068		
4	2 hr. after moult	59	1·120	0·230	79·46	77·98
5	3 hr. after moult	60	1·348	0·278	79·38	—
6	1 day after moult	60	1·240	0·268	78·4	—
7	4 days after moult	55	1·166	0·287	75·39	74·57
		Moult alone	0·234	0·069		
8	9 days after moult	95	5·850	1·370	76·6	—
9	30 days after moult	63	1·630	0·448	72·75	—

%	Before moult	After moult
Water	72·7	79·23
Solid	27·3	20·77

Amount of water in a prawn of dry weight 2·73 g. after moult $= \frac{7{\cdot}923 \times 2{\cdot}73}{2{\cdot}077} = 10{\cdot}414$ g.

Therefore the amount of water absorbed $= 10{\cdot}414 - 7{\cdot}270 = 3{\cdot}144$ g.

Thus a prawn of fresh weight 1 g. absorbs 0·314 g. of water during moult.

between internal and external media the osmotic deficit is least in newly moulted examples and the deficit grows as days elapse after the moult. I have not been able to establish any remarkable rise in osmotic pressure immediately before moult, but it may be that I have not been able to pick prawns that were actually in the throes of moulting. In two prawns far from moult a slight rise in osmotic pressure was observed, and this is probably an indication of the activity prior to moulting. While a slight rise from 2·6 to about 3·0 % NaCl is indicated, there is no evidence that the blood attains hypertonicity with the external medium, nor even isotonicity, for the blood of a prawn only 10 min. after the moult gave a value of 3·00 % with an appreciable osmotic deficit of 0·37 % NaCl.[1] The general trend of osmotic changes in *L. serratus* during the moult cycle is similar to that of *Pachygrapsus*, with the difference that the range of variation is so limited that the hypotonicity of the blood is maintained in all stages of the moult cycle.

The exact mechanism whereby the increase in concentration is achieved prior to moult has not been satisfactorily explained in any crustacean. It would at least be partially explained by the conversion of accumulated glycogen into sugar as shown by Drilhon (1933) for *Maia*, though it should be admitted that the change in the sugar content of the blood observed in *Callinectes* (Baumberger & Dill, 1928) is insufficient to explain the whole osmotic rise. That the absorption of water is mainly responsible for bringing down the value to normal could be demonstrated by calculations based on

[1] This value is not included in Table V, only a single estimation having been made.

changes in water content. In prawns immediately after moult, the water content is about 79 %; a slightly lower value of 78 % is obtained if the cast skin is also included in the determination of gross and dry weights. On the other hand, prawns several days after moult have a water content of only about 72 % (Table VI).

It is noteworthy in this connexion that Robertson (1937), working on *Carcinus*, found that a crab having a fresh weight of 50 g. would absorb 35 g. of water during moult. The corresponding figures for *Maia squinado* (Drach, 1936), *Pachygrapsus crassipes* and *Hemigrapsus oregonensis* (Olmsted & Baumberger, 1923) are 58, 17 and 22 g. respectively, while the corresponding value for *Leander serratus* is only 15·7 g. Lowndes & Panikkar (1941) have found that *Homarus* of fresh weight 100 g. absorbs about 47 g. of water within less than 2 days after moulting. The difference between the amount of water absorbed by the stenohaline *Maia* and euryhaline *Carcinus*, and the much lower value for *Pachygrapsus crassipes* and *Leander serratus* would seem to be closely related to the differences in the permeability of the integument. We should expect a low permeability for animals that can maintain hypotonicity, and if the amount of water absorbed and the consequent osmotic changes taking place form an index of permeability, *L. serratus* would seem to be the least permeable of all the above-mentioned crustaceans.

TABLE VII. OSMOTIC CHANGES OF *PALAEMONETES VARIANS*

Date 1940	Length mm.	Time after moult	Osmotic pressure: Blood	Osmotic pressure: Medium
11. iii	40	6 hr. after moult	2·410	3·603
	40	Unmoulted for 32 days. Same lot as above	2·382	3·603
10. iii	27	1 hr. after moult	1·929	1·000
	30	Unmoulted for a very long time	2·034	1·000

I have not been able to make many observations on moulting in *Palaemonetes*, but Table VII gives values of osmotic pressure observed immediately after moult in two prawns that have been in experimental media. The figures, as compared with the normal, indicate that a slight rise may be expected, but distinctly smaller than in *Leander*; the amount of water absorbed after moult may be correspondingly low.

CHANGE IN WEIGHT OF PRAWNS IN DILUTED AND CONCENTRATED MEDIA

When a typical marine invertebrate is transferred to dilute sea water osmotic movements of water and salts take place resulting in a fall in the osmotic pressure of the internal medium. Direct determinations of osmotic pressure have shown that such a fall takes place in both *Palaemonetes* and *Leander*. If the dilution of blood is due to leakage of salts alone, there would be no appreciable difference in weight; but if it is caused by water entering the animal the

weight of the animal should show a transitory increase. The changes in weight of *L. serratus* after transferring from sea water to 50 % sea water are given in Figs. 6 and 7 which are self-explanatory. An increase in weight is observed in all the three instances, but the time taken to reach the maximum is different in different individuals owing probably to differences in size and in the moult cycle which has a considerable influence on permeability. An increase of 6–9 % of original weight is noticed, but an increase above 6·0 % would seem

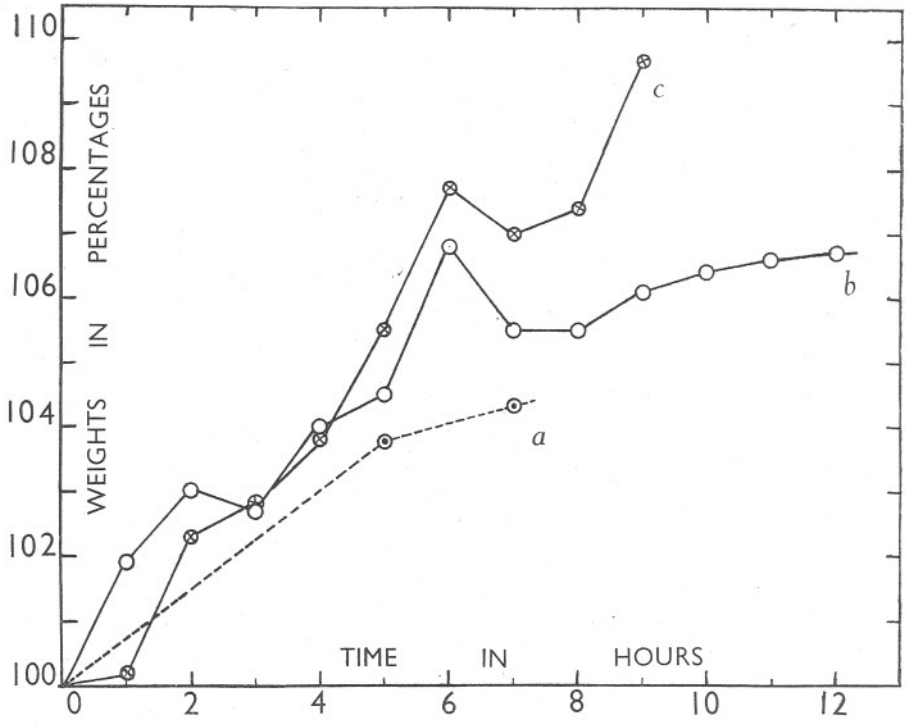

Fig. 6. Increase in weight of prawns in diluted media. Abscissae, time in hours; ordinates % of original weight. (*a*) *Palaemonetes* (dotted line), 10 specimens weighted collectively after transfer from normal sea water to 33 % sea water. (*b*) and (*c*) *Leander serratus* from sea water to 50 % sea water, single individuals.

to be fatal, as shown by the fact that the individual that survived for a long time after direct transfer showed only a maximum of 6·0 % and the values began to fall after about 25 hr. About 4 days were necessary for the prawn to show a constant weight, and this was about 2 % higher than the original weight. Experiments with individual specimens of *Palaemonetes* were not possible owing to their smaller size and the consequent tendency for the errors to be magnified, but a definite increase in weight has been observed in this species as well (Fig. 6*a*). Ten *Palaemonetes* having an initial weight of 1·86 g.

in sea water, where they had been living for months, showed an increase in weight of 0·07 g., i.e. nearly 3·8 %, after remaining for 5 hr. in 33 % sea water. The increase was well over 4 % after 7 hr.

Similar increase in weight has been noticed in many other marine crustaceans, such as *Cancer pagurus* (Schlieper, 1929), *Portunus puber* and *P. depurator* (Hukuda, 1932); but Bethe (1930) failed to notice any appreciable increase in *Carcinus maenas*. In the mangrove crab, *Heloecius cordiformis*,

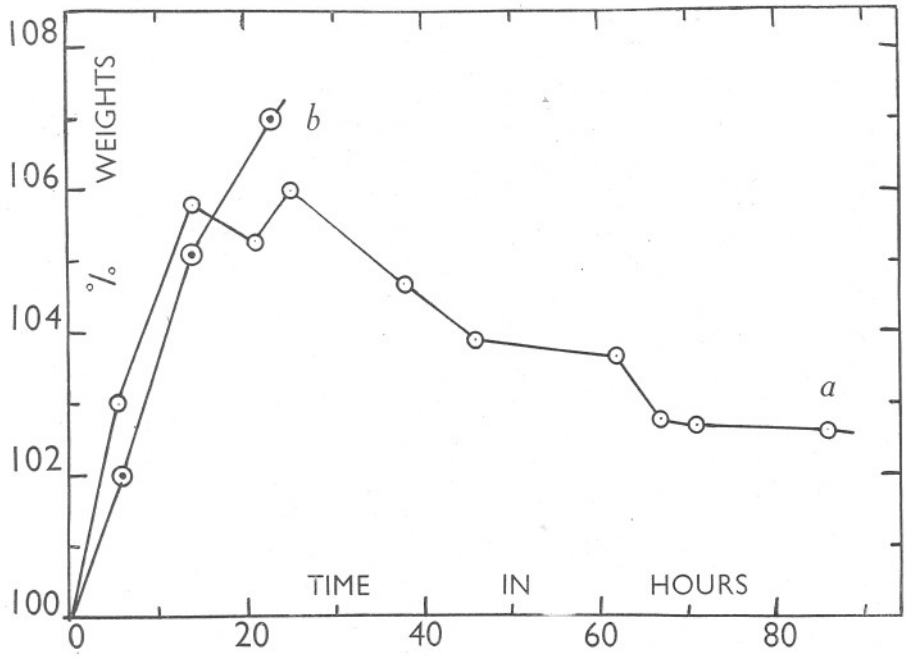

Fig. 7. Increase in weight of *Leander serratus* taken from sea water to 50 % sea water. (*a*) Specimen far from moult, which survived the experiment. (*b*) Prawn 2 days after moult which died in 24 hr.

Dakin & Edmonds (1931) failed to observe any change in weight corresponding to changes in the medium. In the brackish-water *Gammarus duebeni* and the freshwater *Gammarus pulex*, Beadle & Cragg (1940*a*) observed no appreciable change in weight; they conclude that in both these species changes in the osmotic pressure of the blood are due to salt and not to water movements. The changes in weight observed in *Leander* and *Palaemonetes* indicate definitely that the gills of both the species are permeable to water and that the dilution of the blood consequent on sudden transfer to dilute media is mainly brought about by the water that enters. A few hours after transfer a slight swelling and a high hydrostatic pressure of blood may be noticed in *Leander*. In the crabs studied by Hukuda a change of only about 4 % of weight was noticed and, by comparing with calculated figures assuming semipermeability,

the weight increase due to entry of water was shown to be insufficient to account for the fall in osmotic pressure of blood; he was therefore able to demonstrate the passage of an appreciable amount of salt to the exterior. Similar calculation with *Leander* would show that the amount of water that enters must be to a large extent responsible for the dilution of the blood, since the extent of dilution taking place is small owing to the low normal value for blood. The escape of salts to the exterior would seem to be inconsiderable at least in moderately dilute sea water, but it certainly takes place when the medium is very dilute.

When prawns are transferred from dilute to concentrated sea water there is a corresponding fall in weight, giving clear evidence of the escape of water to the exterior. The results obtained in experiments with *Palaemonetes varians* and *Leander serratus* are given in Fig. 8.

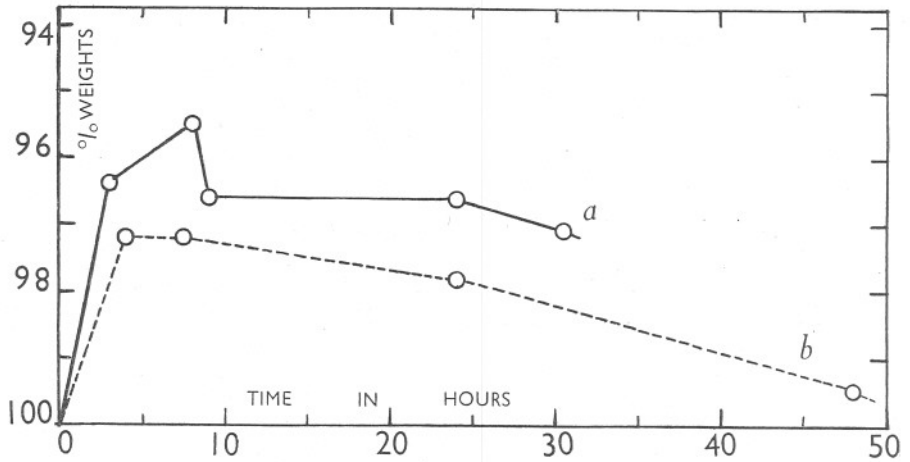

Fig. 8. Decrease in weight of *Palaemonetes* and *Leander* when taken to concentrated media. (*a*) *Leander serratus* acclimatized to 50 % sea water transferred to normal sea water. (*b*) *P. varians* acclimatized to 10 % sea water transferred to normal sea water; 10 specimens used collectively. (20. v. 40.)

Permeability of the Gills

In Crustacea the integument has a very low permeability, being heavily impregnated with chitin and protected by cuticle. The only possible surface through which water and salts may freely diffuse into the body of the animal is that afforded by the gills. The following experiments were performed to ascertain the extent to which the gills of *Leander* and *Palaemonetes* are permeable.

A strong solution of methylene blue was dialysed into sea water kept in a jar until the sea water was rich blue and opaque. Specimens of *Leander serratus*, *Crangon vulgaris* and *Carcinus maenas* were transferred to the jar

from the sea-water tanks. The *Leander* and *Crangon* chosen were approximately of the same size. The animals were removed at the end of half an hour, dissected, and the gills examined under the microscope. Of the preparations from the three species, the presence of methylene blue in the cells of the gill plates could easily be observed in *Crangon* and *Carcinus*, whereas no trace of the dye could be detected in the cells of *Leander serratus*. When the animals were left for about an hour, the gills of *Crangon* and *Carcinus* assumed a rich blue colour distinguishable even with the naked eye. In contrast to this, *Leander*, even if left for hours, failed to absorb the dye; the gills became faint blue after several hours, but this was owing to the presence of the dye between the gills and not in the gill cells proper.

The same experiment was performed with a methylene blue solution made in distilled water to which a small quantity of sea water was later added. This was necessary to prevent the early death of the experimental animals which would otherwise result. The osmotic pressure of this very dilute solution was 0·06 % NaCl. The results were similar to those of the previous experiment. The gills of *Crangon* and *Carcinus* took the dye in a very short time, the former being the first to show its presence. Even after 7 hr. of immersion the gills of *Leander* were colourless and the dye did not penetrate even 1 hr. after the death of the experimental animal.

Experiments with *Palaemonetes varians* gave the same results. The behaviour of the animal was also observed with methylene blue dissolved in concentrated sea water. The use of methylene blue is open to criticism owing to the fact that it may have been reduced after penetration (vide Gray, 1931). If an acidic dye like vital red is substituted, the dye has been observed to penetrate in *Leander* after prolonged immersion, while about half an hour is enough for *Crangon* and *Carcinus*. In regard to *Palaemonetes* the amount of vital red that penetrates (if any does) is extremely small, as judged by the examination of the gills of prawns appropriately treated. With a quick penetrating dye like neutral red, the gills of living *Crangon* are stained in a few minutes, while even after half an hour only a slight trace of the dye could be seen in *Leander*. These observations, though of a preliminary character, demonstrate that in *Leander* and *Palaemonetes* the permeability of the gills is very low as compared with the marine *Crangon* and the euryhaline *Carcinus*, or at any rate that the membrane properties of the palaemonid gills are different from that of the others.

Water-drinking Habits of Prawns

If a prawn is losing water by diffusion owing to the higher osmotic pressure of the external medium, one of the ways of making up for the water loss is for it to drink water. The technique employed to verify this water-drinking habit was essentially the same as that of Homer Smith (1930) who used phenol red as a suitable indicator. In addition to this dye, I have also employed vital red

and brom-cresol green. Concentrated solutions of these dyes were added in small quantities to jars containing prawns in experimental media, and the animals were examined after suitable intervals to find if the dye could be detected in the alimentary canal. Unlike the teleosts, the dyes seldom reach the hind-gut or even the posterior half of the mid-gut, since the absorption seems to take place in the hepatopancreas and part of the fore-gut. One difficulty encountered in this connexion was the presence of pigmented cells in the hepatopancreas which are themselves subject to colour variations. By careful examination of teased tissues under the microscope it was, however, possible to distinguish the foreign dye as distinct from the natural pigmentation.

Both *Palaemonetes varians* and *Leander serratus* have been observed to drink the external medium, especially when the latter happens to be sea water or concentrated sea water. It must be mentioned, however, that even when the external medium is hypotonic they have been observed to take in water as judged by the presence of dyes inside them. The results obtained have been on the whole erratic. While (after proper treatment for *p*H differences) the dye became detectable in the hepatopancreas of some specimens in a few hours, in others I have not been able to observe the dyes even after 14 hr. In one set of experiments when solutions of the same concentration were used as the external media (sea water) for both the species, it was found that the dye was seen in *Palaemonetes* in 1 day while it took 4 days in *Leander*. Experiments in sealing the mouth and preventing the entry of sea water were not successful, since the oral appendages are always at work and remove any foreign body near the mouth.

The fact that water drinking may occur in both hyper- and hypotonic media and the wide divergence in results obtained with different individuals in the same medium seem to indicate that it is not an essential part of the osmoregulatory mechanism. It is probable that water gets into the alimentary canal when the prawns feed or when they attempt to search for food. When directly transferred to very concentrated media there is some evidence from osmotic changes to show that salt water is taken in from the surroundings (see p. 326).

Discussion

Mechanism of Osmoregulation

If an animal is able to maintain a lower osmotic pressure of the blood than the sea water in which it lives, it must either be completely impermeable to water and salts or it must be doing continuous osmotic work. Complete impermeability is not practicable in marine animals that depend upon the external medium for their essential supply of oxygen which has to diffuse through the gills. If, on the other hand, salts from the external medium penetrate the integument and water escapes in accordance with the laws of osmosis, the animal has first of all to make up for the loss of water it sustains

and, secondly, it must get rid of salts to maintain the osmotic deficiency. The mechanisms possible are: (1) by producing a concentrated and markedly hypertonic urine, the animal can achieve elimination of excess of salts, and this may be further elaborated by the development of a special water reabsorbing mechanism; (2) it may drink sea water from time to time and thus make up for the loss of water and simultaneously excrete the excess of salt from the body, not necessarily through the agency of the kidney; or (3) the animal may absorb water from the surrounding sea water but not salt. The capacity to produce hypertonic urine has so far been observed only in vertebrates which have become terrestrial in habits, under conditions where the problem of conserving water is of paramount importance; none of the primarily aquatic organisms is known to possess it. In the marine teleosts, which have an osmotic pressure about one-third that of sea water, it has been demonstrated by Homer Smith (1930) and Keys (1931, 1933) that regulation is effected by the drinking of sea water and excretion of salts by the gills. We have seen that in *Leander serratus* and *Palaemonetes varians* the urine is isotonic with the blood whether the external medium is of extremely low or high salinity. The fact that the animals drink sea water and do this much more when in hypertonic media might suggest at once that the regulatory mechanism of palaemonid prawns is probably similar to that of marine teleosts. But this explanation alone would be insufficient to account for the osmotic independence of these prawns to wide variations in environment, and the ability, at least in *Palaemonetes*, to live in abnormally high concentrations of sea water which would be lethal to most marine animals—an ability comparable only with the classical instance of the brine shrimp *Artemia*. It seems likely that active water transport from outside to inside takes place in order to maintain hypotonicity in sea water in the same way as active salt absorption takes place in dilute sea water.

The total osmotic pressure curves of *Palaemonetes* and *Leander* indicate beyond doubt the homoiosmotic behaviour which they exhibit when their media are changed; this power is markedly developed in the former and partially lost in the latter. Within a range of nearly 5·0 % NaCl in its external medium the blood of *Palaemonetes* is affected only to the extent of about 0·8–1·0 % NaCl. The range of variation is much greater in *Leander*, but even here the blood is little affected by the dilution of the environment up to about 2·5 % NaCl; and it is probably reasonable to assume that under no circumstances is *L. serratus* subjected to changes in environment below this range under natural conditions. *Palaemonetes*, on the other hand, inhabits regions subject to wide fluctuations in salinity, not only seasonal but also tidal, the ditches in which it lives being liable to evaporation and to inundation by sea or river water. *Leander squilla* shows an intermediate condition which is what one would expect from its habits. But while the difference in homoiosmotic behaviour is consistent with the habits of these prawns, it does not enable us to judge how a new physiological ability has been acquired in one

genus that is anatomically so similar to another, or how the palaemonid prawns have acquired an osmotic behaviour so different from other marine prawns.

When *Palaemonetes* is in water of extremely low salinity it has an osmotic pressure equivalent to about 2·0 % NaCl under natural conditions and about 1·8 % NaCl under experimental conditions. If the curve be projected to the freshwater limit the value cannot be below 1·7 %, and under conditions in which the species has become naturally acclimatized to fresh water[1] it is likely that the value is even slightly higher. This is no doubt much higher than the values of the osmotic pressure of most other freshwater Crustacea. The crayfishes *Potamobius* and *Cambarus* have blood with freezing-point depressions of about −0·8° C. (about 1·37 % NaCl) and −0·65° C. (about 1·11 % NaCl) respectively (Duval, 1925; Schlieper, 1935; Lienemann, 1938), and freshwater insects such as the mosquito (*Aedes argenteus*, Wigglesworth, 1933) and chironomid larvae have lower values (Harnisch, 1934; Koch, 1938), while freshwater branchiopods (*Daphnia*, Fritzsche, 1917; *Chirocephalus*, Panikkar, 1941) have osmotic pressures lower than in any other crustacean. In these freshwater forms there is a regular osmotic stream of water entering the body through the gills and escaping through the excretory system. In *Potamobius*, Peters (1935) has conclusively shown that the danger of loss of salts through the discharge of large amounts of urine is overcome by the salt reabsorbing mechanism resident in the nephridial canals, which makes the final urine extremely hypotonic to the blood. In the same way as freshwater organisms depend upon an osmotic stream of water for copious urine production, the marine teleosts depend upon the continuous drinking of sea water to cope with the elimination of salts through the gills. If the question of osmotic regulation is entirely dependent upon the continuous entry of water or salts through a semi-permeable membrane and the consequent elimination of water or excretion of salts, it would hardly be possible for animals to survive sudden changes in environment, since equally sudden reversal of physiological processes could seldom happen. It becomes evident, therefore, that the ability to control the exit and entrance of substances in the body of aquatic animals is an integral part of the regulatory mechanism. It may be achieved by different methods, as by the development of a keratinous waterproof coat in the majority of teleosts, the secretion of mucus in the eels (Duval, 1925; Keys, 1933), or by the development of an almost impermeable cuticle in arthropods. The factor of salt retention, recently emphasized by Beadle & Cragg (1940*b*), would seem to be the result of the low permeability of the integument.

Permeability of the Integument: Water and Ion Transport

It is well known that the crustacean integument has a low degree of permeability except in the region of the gills, where exchange of substances can

[1] The value may be well below this in warmer latitudes (vide Panikkar, 1940*b*) as shown by Vialli's (1925) results.

take place in both directions. Different degrees of permeability have been noted by previous authors. In most marine Decapoda, Bethe (1929, 1930) has shown an almost indiscriminate permeability (of the gills) to water and ions; but these are forms in which the external and internal media are iso-osmotic. The ionic composition of the blood of these animals and that of sea water need not, however, be the same as shown by Bethe & Berger (1931) and by the recent analyses of Robertson (1939), but, in any case, a fairly constant ionic equilibrium is maintained. The extent of permeability is different even among stenohaline Crustacea; low permeability has been noticed in animals that show euryhaline tendencies, while true euryhaline animals are even much less permeable. *Carcinus*, for example, is less permeable to ions in both directions than marine crabs like *Portunus* and *Hyas* (Nagel, 1934); however, it is permeable to both water and salts as shown by the dilution curves of Margaria (1931), Bateman's (1933) conclusion that the gill membrane is almost impermeable to water being incorrect (Krogh, 1939; Webb, 1940). In the freshwater *Cambarus bartoni*, Maluf (1937) has shown that the gills are permeable to water but not to electrolytes, but in a later paper[1] (1939*b*) he admits that chlorides could pass through in both directions. The gills of *Eriocheir sinensis* are permeable to water and to substances like chloride and ammonia (Krogh, 1939). There is possibly no aquatic crustacean the gills of which are not permeable to water, for even the brine shrimp *Artemia* is permeable as indicated by experiments with heavy water (Krogh, 1939). The experiments made on the permeability of the gills of *Leander* and *Palaemonetes*, though only of a qualitative nature, show that the gills are much less permeable than in marine or even euryhaline animals like *Carcinus*. Changes in osmotic pressure and water content during the moult cycle also support this conclusion. The condition observed in these prawns is comparable to that of *Eriocheir* and possibly *Cambarus*; definite permeability in both directions exists for water and ions. However, the amount of water that enters the prawns when they are in osmotically inferior surroundings must be much less than that observed in freshwater Crustacea with salt-reabsorbing mechanism, since large-scale urine production will not be consistent with conservation of salts. Loss of water from the tissues when the prawns are in hypertonic media is inevitable, though the amount of water lost is not considerable owing to low permeability. If the loss of water is great, as for example in very concentrated media, it is probably made up by taking in sea water through the alimentary canal; but there remains the question of excretion of salts, the site of which has not been definitely proved, though circumstantial evidence seems to point to the gills.

The alternative possibility is an active transport of water (but not salts) into the body from the surrounding sea water. The osmotic work required to absorb water against an osmotic gradient would be of about the same order as

[1] Based on abstract in *Biological Abstracts*. The original paper was not available for consultation owing to the war.

that required for secreting salts to osmotically superior surroundings. It has to be mentioned, however, that active transport of water alone through a living membrane and against an osmotic gradient has not been demonstrated in any marine animal. Studying the ionic regulation of *Carcinus*, Webb (1940) brings circumstantial evidence in favour of water transport. That absorption of water takes place in Crustacea after moult is well known from the results of Baumberger & Olmsted (1928), Robertson (1937) and Drach (1939). In *Leander*, changes in water content and osmotic pressure during the moult cycle indicate the selective absorption of water immediately after moult so as to account for a rise of almost 5 % (cast included) in water content and a fall in osmotic pressure of about 0·2 % NaCl. If water and salts were absorbed without preference we should find a rise in the concentration of the blood after moult and not the gradual reduction actually observed, assuming, of course, that the excretory function remains normal. Since the highest osmotic pressure registered (3·030 % NaCl) is well below the value of sea water, this absorption takes place against an osmotic gradient and is, I think, sufficient proof of the ability of water transport in *Leander*, at least soon after moult. It is quite probable, therefore, that this capacity is not completely lost even during intermoult periods, and that active transport of water, probably across the gill membrane, plays a significant part in osmoregulation. Though technical difficulties in demonstrating this have not been completely overcome, it is hoped that analysis of ionic regulation will throw some light on this problem, for osmoregulation is mainly the result of the intensification of the processes at work in ionic regulation (Pantin, 1931; Webb, 1940).

The concept of salt transport, presumed by Schlieper (1929, 1935), conclusively shown in *Carcinus* by Nagel (1934) and explained on the basis of active ion absorption demonstrated by Krogh and his collaborators, accounts for the ability of many euryhaline and freshwater animals to maintain a high osmotic concentration. Intake of salts by *Palaemonetes* and *Leander* has been demonstrated. Though the possibility of at least some of the salts being absorbed through the gut cannot be ruled out, the quantity thus absorbed must necessarily be small owing to the fact that it would be unfavourable to the animal from the point of view of water economy. The only other place where salt assimilation can take place is the gills, and in view of the results obtained by Krogh (1939) and Koch (1934) the gills are presumably the site of ion transport in prawns.

Role of the Gills in Osmoregulation

From what has been said above, it will be clear that the gills of palaemonid prawns are the most vital organs in osmoregulation, especially in view of the relatively unimportant role played by the renal organs. They should have a low permeability to reduce the amount of osmotic work to be done when prawns are in hypotonic as well as in hypertonic media; they have to discharge salts to the exterior when the medium is hypertonic or alternatively transport

water into the body from the surroundings, and they have to perform the function of ion assimilation when the medium is hypotonic. Confirmation on these points is possible only after the direct histochemical observations on gills, which are being made, have been completed.

The following facts in regard to the structure of the gills of Palaemoninae are relevant in this connexion. Allen (1892*a*), in his account of the minute structure of the gills of *Palaemonetes varians*, describes the occurrence of numerous reticulous and clear glands on the axis of the gill. Cuénot (1895), who observed similar glands in certain other Crustacea, considered them to have the function of mucus secretion; but Yonge (1932), from a comparative study of similar structures, concluded that these tegumental glands are responsible for the secretion of the cuticle of arthropods. He finds the cuticle thickest where the glands are most numerous. Considering the fact that it is the cuticle that reduces the permeability of chitin, the presence of a large number of such glands on the gills is suggestive of the role they play in securing the low permeability of these prawns. In the freshwater *Palaemon*, Patwardhan (1937) mentions that the axis is protected by a thick layer of cuticle and that the gill plate itself is covered by a double layer of cuticle. From an examination of the gills of *Carcinus*, *Maia*, *Hyas* and *Eriocheir*, Webb (1940) concludes, however, that the cuticle is of little significance in controlling permeability and that the control is really exercised by the nature of the epithelium. That the gills have an excretory function is suggested by the fact that dyes injected into the animal are collected by certain cells lining the blood vessels of the gills[1] (Kowalevsky, 1889), and Cuénot (1895) has observed that their behaviour is almost identical to that of cells of the end-sac. According to Allen these cells surround the veins and possess vacuoles with excretory concrements. Whether or not they have a chloride-secreting function cannot be asserted at present, but they are definite evidence of active excretory processes taking place in the gills. In regard to ion absorption, Koch (1934) has demonstrated the curious affinity for silver salts of certain cells found in the branchiostegites of *Leander serratus* and *Palaemonetes varians*, and he suggests from a comparative study of similar cells in other arthropods that this property is evidence of active ion absorption taking place in the branchial epithelium.

Role of the Kidneys in Osmoregulation

The mechanisms employed to achieve osmotic independence of the surrounding medium are quantitatively and qualitatively different in the different groups of animals which have succeeded in establishing themselves in fresh water. Those which have developed the capacity to produce hypotonic urine have in general the lowest concentration of blood, in contrast to those producing blood isotonic urine which, though found in fresh water, have a high osmotic concentration of blood. Maluf (1938), reviewing the question of

[1] I have repeated these experiments and obtained similar results.

excretion in the Arthropoda, concludes that the nitrogenous wastes, which in Crustacea consist mostly of ammonia (Delaunay, 1931), are probably secreted into the lumen of the antennal kidneys by the cells of the labyrinth. That the actual filtration of water and crystalloids from the blood takes place first of all into the coelomic sac is evident from the work of Picken (1936) and Peters (1935). The morphological differences between the antennal glands of the marine *Homarus* and the freshwater *Potamobius* emphasized by Peters, and the significant changes in chloride content which he observed in the different parts of the green gland, have established conclusively that the nephridial canal of the freshwater crayfish is mainly concerned with the reabsorption of salts from the urine as it flows down from the labyrinth into the bladder. It has been found that the nephridial organs are usually provided with longer nephridial canals in the freshwater as compared with marine Crustacea, as shown by Schwabe (1933) in the freshwater *Gammarus pulex* and the marine *Gammarus locusta*. Exceptions to this rule have been found in crabs, such as *Eriocheir sinensis* and *Potamon*[1] *fluviatilis*, which though capable of living in fresh water have excretory organs similar to those of marine crabs (Schlieper, 1929; Schlieper & Herrmann, 1930). To these must now be added the palaemonid prawns which, though capable of penetrating into fresh water, do not have an excretory system specially modified for the purpose. The blood-isotonic urine in *Leander serratus* and *Palaemonetes varians* is not surprising when we consider the structure of the excretory organs, for neither has a urinary canal. Both the end-sac and the labyrinth are small and compact and very near the external orifice of the bladder. The structure of the bladder epithelium precludes the possibility of any active reabsorption taking place there. However, there is a distinctive feature in the excretory system—the enormous renal or nephroperitoneal sac which absorbs dyes injected into the animal and necessarily performs a certain amount of active excretion (Weldon, 1891; Marchal, 1892). From the fact that the walls of the sac are richly provided with blood vessels it would appear that a certain amount of filtration may possibly also take place there. Though the essential lay-out of the excretory systems in *Leander* and *Palaemonetes* is similar to that of the marine prawns *Pandalus*, *Hippolyte* and the shrimp *Crangon* (Weldon), they are enormously developed and fused to form a large storehouse of urinary fluid. This would seem to be advantageous in the production of large amounts of urine, since the rate of urine production is likely to be different when the prawns are in different media. Schwabe (1933), who examined sections of *Palaemonetes varians microgenitor* (brackish water) and *macrogenitor* (fresh water), could not discover any noticeable structural difference in the excretory organs of the two varieties. Similarly the description of the excretory organs of the freshwater *Palaemon* given by Patwardhan (1937) is essentially the same as that of *Leander*. On anatomical and osmotic grounds the evidence available is that adaptation to fresh water in the Palaemoninae has not been accompanied by

[1] Formerly known as *Telphusa*.

the development of a salt reabsorbing part of the nephridium and the ability to produce urine hypotonic to the blood. It is probably this very fact which has enabled the freshwater palaemonids to tolerate salt water.

Evolutionary Significance of the Osmotic Behaviour of the Palaemoninae

The osmotic behaviour of *Palaemonetes varians*, *Leander serratus* and *L. squilla* appears to be of great significance when viewed in relation to the habits and distribution of the group to which they belong. The family Palaemonidae includes four subfamilies, of which the Pontoniinae are marine, the Desmocaridinae and Typhlocaridinae are fluviatile, while the Palaemoninae include the following seven genera with habitats ranging from fresh to salt water:

Pseudopalaemon	Two species. Marine.
Brachycarpus	Two species. Marine.
Leander	About fifty species. Mostly marine; some estuarine; a few fresh water.
Palaemonetes	About fifteen species. Mostly estuarine or brackish water; a few fresh water.
Palaemon[1]	About seventy-five species. Almost entirely fresh water; a few estuarine.
Euryrhynchus	Two species. Fresh water.
Cryphiops	One species. Fresh water.

The genera of the subfamily have been discussed in some detail by Kemp (1925). Leaving out of consideration the exclusively marine and freshwater genera, we find that species of *Leander*, *Palaemonetes* and *Palaemon* occur both in salt and fresh water. *Leander* is mainly marine, but a good many species are estuarine; at least two occur only in fresh or brackish water (*L. gardineri* and *L. fluminicola*); not less than five species are known only from fresh water (*L. potitinga*, *L. cubensis*, *L. modestus*, *L. capensis* and *L. annandalei*); others, like *L. concinnus* and *L. paucidens*, seem to be indifferent to the salinity of the medium. Kemp observed *L. fluminicola* nearly 700 miles up the Ganges in India. Of the four species of *Leander* found on the English coasts, *L. longirostris* is known to ascend many miles up rivers (Gurney, 1923; Schnakenbeck, 1933). *L. serratus* is able to survive in brackish water for short periods and *L. squilla* is often observed in brackish water, though both are essentially marine species confined to the littoral zone. *L. adspersus* is a marine and brackish-water species.

Palaemonetes, though found only in fresh and brackish water under natural conditions, includes at least two species that can thrive in sea water. *P. vulgaris* is mainly an estuarine species but is also known from the sea on the American coast (Faxon, 1879). As mentioned before, there are few, if any, valid records of *P. varians* from the sea, but the prawn has been observed to live for many months and to breed in the sea-water tanks of the Plymouth Laboratory.

[1] Inclusive of the species referred by some carcinologists to the genera *Macrobrachium* and *Bithynis*.

Palaemon is an essentially freshwater genus, but a few species, such as *P. rudis*, *P. carcinus*, *P. malcolmsoni* and *P. lamarrei*, are known to migrate into brackish water during breeding periods (Kemp, 1915; Panikkar, 1937); the last-mentioned species is particularly tolerant to salt water and has been noticed in true brackish-water habitats (Panikkar & Aiyar, 1937). Menon (1938) has found the larvae of *P. rudis* and *P. carcinus* in brackish water.

It should be mentioned in this connexion that there are few reliable characters to distinguish *Palaemon* from *Leander*. While the main difference between the two is the presence of the hepatic spine in *Palaemon* and of the branchiostegal in *Leander*, there are two species (*L. potamiscus* and *L. fluminicola*) which, though obviously *Leander*, are without the branchiostegal spine, while in *Palaemon hildebrandti* the hepatic spine is sometimes absent; *P. mirabilis*, though technically a *Palaemon*, is remarkably like some species of *Leander* (Kemp, 1925). The specific characters of some of these species of *Palaemon* and *Leander* so overlap that their diagnosis is extremely difficult even for the specialist. Kemp, reviewing the species, considers that the genus *Palaemon* is probably polyphyletic in origin. The same view is held in regard to *Palaemonetes* (Kemp, 1925, p. 315), whose resemblance to certain groups of species of *Leander* in the adult as well as in the developmental stages is so marked that Gurney (1939) suggests there is nothing in the development of *Palaemonetes* to justify its separation from the genus *Leander*.

The discovery of osmotic independence in a purely marine species of *Leander* and of a highly developed homoiosmotic behaviour in *L. squilla* and *Palaemonetes varians* suggests that all the prawns known from varied habitats, included in the genera *Palaemon*, *Leander* and *Palaemonetes*, possess a well-developed mechanism of osmoregulation. Osmotic independence must naturally exist in all the freshwater genera of the Palaemoninae—*Palaemon*, *Eurythynchus* and *Cryphiops*. The fact that all species of *Palaemonetes* are found either in fresh water or in brackish water, considered in the light of the behaviour of *P. varians*, would indicate that relative osmotic independence is probably to be found in all the members of this genus. It is to be expected that a similar independence will be found in all the freshwater and at least all true brackish-water species of *Leander*. In regard to marine *Leander*, direct evidence has been obtained only in *L. serratus* and *L. squilla*, but I consider that osmotic independence is likely to occur in *L. longirostris*, as judged by its migratory movements, and in many Indian species, such as *L. styliferus*, which I have often found in brackish and sea water. The degree of hypotonicity when in sea water is bound to be varied; it may even be completely absent when the prawns are in normal sea water and may become apparent only in concentrated sea water as observed in *Eriocheir sinensis* (Conklin & Krogh, 1938). It would be of the utmost interest to know the osmotic behaviour of palaemonid prawns from different habitats in different parts of the world, for we might then secure data on their physiological adaptation which would throw light on their evolutionary history.

If an animal shows a high degree of osmotic independence relative to the external medium, especially if it involves ability to maintain hypotonicity, it is reasonable to consider that the animal must at some time in its evolutionary history have inhabited fresh water (Pantin, 1931; Baldwin, 1937), for such a mechanism would never have been needed for a purely marine life. The penetration of most marine animals into brackish water is closely associated with the development of osmoregulatory powers; but we find in them only an ability to maintain hypertonicity when the external medium is dilute sea water. This is brought about by decreased permeability to water and salts and active transport of ions from the outside to the inside. Thus, animals like *Carcinus maenas* can survive in brackish water of very low salinity, and maintain a high osmotic concentration of blood (Schlieper, 1930, and others), but isotonicity is established the moment they are taken back to sea water (Frédéricq, 1904; Duval, 1925). A further development of this ability to maintain hypertonicity enables a brackish-water animal to penetrate into fresh water. The adjustments required would either be increased ability to assimilate ions and a low permeability to water, or the development of renal salt-reabsorbing mechanism whereby loss of salts would be reduced to a minimum. We have found that in *Palaemonetes* acclimatization to fresh water has been brought about by the former method without any undue specialization of the excretory system, and it is by virtue of this that the prawn is able to live in external media of a wide range of concentration, a feature which it shares with many other Palaemoninae.

Two theories are possible to account for the osmotic independence shown by *Leander serratus* and *L. squilla*. One is that they have been derived from prawns that have been established in fresh water and developed a high degree of osmotic regulation, but that for some reason they began to penetrate back into brackish water and then into the sea. In the same way that a low permeability was advantageous in reducing the entry of water into the body of prawns when in fresh water, it was helpful in reducing the loss of water when the medium became hypertonic. The second possibility is that the present-day species of *Palaemonetes*, *Palaemon* and *Leander* are descendants of prawns which left the sea for life in inlets that eventually became land-locked and were thus subject to increase in salinity; in consequence they were obliged to develop a mechanism for maintaining hypotonicity and a low permeability. An argument against the latter theory is that these genera are world-wide in their distribution, and it is unlikely that these special conditions could have been so widespread. Both possibilities may have occurred and may account for the suggested polyphyletic nature of the genera in question. I consider the first explanation the more plausible in regard to *L. serratus* and *L. squilla*, since, if it were otherwise, the optimum value of total osmotic pressure would certainly have been higher than 2·8 and 2·6 % NaCl. Whether all *Leander* have had a freshwater ancestry or not cannot be asserted until the numerous other marine species have been subjected to close study. Investigations on

Brachycarpus and *Pseudopalaemon* would be of exceptional value, for if they are stenohaline they may represent forms which have not developed osmoregulatory powers in their history; on the other hand, if they have an osmoregulatory behaviour comparable to that of *Leander*, they would represent forms that have become secondarily marine in recent times.

The biochemical aspect of adaptation to fresh water emphasized by Needham (1930, 1937) throws some light on the question of the recolonization of salt water suggested in *L. serratus* and *L. squilla*. One important condition to be fulfilled if an animal is to establish itself in fresh water is that it should provide, within its egg, enough ash for complete development instead of having to depend upon the environment for inorganic substances essential for the organization of the embryo. The provision of ash in the embryo is therefore as necessary to enable a species to establish itself in fresh water as osmoregulation is to enable the individual to live in fresh water. It is well known that there are many animals which though they occur in fresh water have not overcome this embryonic obstacle, and are thus obliged to migrate into salt water in which alone their eggs can develop. This migration may also be necessitated by the absence or inefficiency of the osmoregulatory mechanism in the embryo, the mechanism, however, attaining its full functional significance in the adults which can therefore migrate into fresh water. Gurney (1923) found that in *L. longirostris* the eggs can develop only in salt water in spite of the adults being quite at home in fresh water.

It is well known that a large egg and a reduced or suppressed larval development is a characteristic of many freshwater animals, but the information we possess on this point in the Palaemoninae is widely scattered and not as precise as could be desired. In the crayfishes, the Astacidae and Parastacidae, the eggs are always large and the young are liberated in a form closely resembling the parents. In *Palaemon*, some species, such as *P. lamarrei*, have large eggs and a reduced development (Henderson & Matthai, 1910), while in others the eggs are small and there is no doubt a long series of larval stages. Some species with small eggs, such as *P. carcinus*, *P. malcolmsoni* and *P. rudis*, are known to migrate from fresh water to brackish water in order to liberate their young, and from this circumstance it is to be concluded that the osmoregulatory mechanism is absent or inefficient in the early stages but becomes functional after growth has proceeded and the young prawns are ready to ascend the rivers. In all probability, however, it would be incorrect to say that all species of *Palaemon* with small eggs must have access to brackish water during the breeding season; for it is likely that small-egged species are to be found in localities so far inland that regular access to the coast is an impossibility. In such species it is to be assumed that an osmoregulatory mechanism is functional from the earliest stages.

In the freshwater prawns belonging to the Atyidae, a family much more primitive than the Palaemonidae and therefore, as one may suppose, established in fresh water for a still longer period, the eggs vary greatly in their size, and

there are many small-egged forms which are certainly unable to migrate into brackish water.

What little we know of the reproductive habits and life history of *Palaemon* is sufficient to show that relative independence of the medium during developmental stages has not been attained at least in some species of an essentially freshwater genus, and it seems that although osmotic adaptation for freshwater life was achieved by the adults of *Leander*, a similar independence of the environment was not attained during larval life for embryonic or osmotic reasons. The habit of liberating the larvae in salt water, in an environment involving less osmotic work, may have brought about the slow suppression of the migratory habit, at least in some species of the Palaemoninae, until they became secondarily established in brackish water and finally in the sea.

Summary

1. The brackish-water prawn *Palaemonetes varians* and the marine prawns *Leander serratus* and *L. squilla* are hypotonic in normal sea water, the blood of these species showing osmotic pressures equivalent to 2·3, 2·8 and 2·6 % NaCl respectively, in an external medium of 3·5 % NaCl.

2. *Palaemonetes varians* is isotonic in water of about 2·0 % NaCl and the species is practically homoiosmotic, the difference in its osmotic pressure over a range of 5·0 % NaCl in the external medium being only 0·8–1·0 %. The species has a very wide range of tolerance from water that is nearly fresh to concentrated sea water equivalent to 5·2 % NaCl.

3. *Leander serratus* is much less homoiosmotic than *Palaemonetes*, and has a limited tolerance to dilution and concentration of the environment. Homoiosmoticity is maintained up to a dilution of 2·5 % in the external medium when isotonicity is reached; but in lower dilutions there is a steady decline in osmotic pressure and the regulatory mechanism evidently breaks down.

4. The osmotic behaviour of *Leander squilla* is very similar to that of *L. serratus*, but the homoiosmotic behaviour is more marked and it has greater tolerance to dilution of the environment.

5. When *Leander* and *Palaemonetes* are transferred to very dilute sea water, the internal osmotic pressure falls gradually for about 14–24 hr., varying according to the size of the individual. After the lowest value has been registered there is a slight rise, and a steady state is thereafter maintained.

6. Studies on the changes of weight of prawns when transferred to diluted media indicate that the integument (gills) is permeable to water and that, at least in *Leander serratus*, the amount of water entering is mainly responsible for the dilution of the blood. There is a similar fall in weight when prawns are transferred to concentrated media, due to loss of water.

7. The urine of *Palaemonetes* and *Leander* is nearly isotonic with the blood irrespective of the nature of the external medium in which the animal is placed. This suggests that the kidneys do not play a significant part in the

osmoregulation, not even in examples of *Palaemonetes* acclimatized to nearly fresh water.

8. The contents of (1) the dorsal sac, (2) the nephroperitoneal sac, and (3) the urinary bladder of *Leander serratus* have been separately removed and the osmotic pressures determined. They do not present significant differences; hence a salt reabsorbing or a water reabsorbing mechanism is absent in the excretory organs.

9. The osmotic changes during the moult cycle of *L. serratus* have been studied. There is a slight rise in the osmotic pressure of prawns about to moult and the value comes down to normal a few days after the moult. A prawn of fresh weight of 1 g. absorbs about 0·3 g. of water during moulting.

10. The gills of palaemonid prawns are definitely permeable to water in both directions and, to a slight extent, to salts. As compared with stenohaline animals, however, the permeability of the gills of *Leander* and *Palaemonetes* is shown to be very low, and especially so in *Palaemonetes*.

11. Both *Palaemonetes* and *Leander* drink the external medium as judged by experiments with dyes. This behaviour is, however, erratic and occurs even when in hypotonic media. The evidence is insufficient to assume that water drinking is essential for osmoregulation.

12. The prawns are able to assimilate ions from dilute solutions presumably through the gills. Part of the absorption of salts may also take place through the gut wall.

13. The possible occurrence of some salt-excreting mechanism is emphasized; probably in the gills. It has not been cytologically proved, but attention is drawn to the excretory cells occurring in the gills. The osmoregulatory role of the gill in the light of its histological structure is discussed.

14. Palaemonid prawns seem to achieve osmotic stability by active absorption of ions when in hypotonic media and there is strong circumstantial evidence for active transport of water against the osmotic gradient, probably very effective when in hypertonic media. The osmotic work required for adjustment is brought to a minimum by the low permeability of the integument (gills), which gives the prawns considerable powers of salt retention.

15. The significance of the osmotic behaviour of *Palaemonetes* and *Leander* in the evolutionary history of the Palaemoninae is discussed. It is suggested that *Leander serratus* and *L. squilla* are probably species that have taken secondarily to marine life.

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ADDENDUM

While this paper was in the press there has appeared an account of the osmotic pressure relations of nine species of crabs of the Pacific coast of N. America (Lowell L. Jones, 1941. *J. Cell. Comp. Physiol.*, Vol. XVIII, pp. 79–92). Of special interest is the fact that *Uca crenulata* shows remarkable powers of regulation and is even hypotonic in sea water, its behaviour being very much like that of the semi-terrestrial crabs examined by Pearse (*vide* Table I, p. 319). *Pachygrapsus crassipes* has been found to produce urine isotonic with blood. In *Hemigrapsus*, which has an osmotic pressure curve similar to that of *Carcinus*, a higher temperature seems to induce a slightly higher osmotic pressure. The author brings some evidence to indicate that crabs showing ability to maintain hypotonicity in sea water are better adapted for terrestrial life than others capable only of hyperosmotic regulation.

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THE ECOLOGY OF SOME PARASITIC COPEPODS OF GADOIDS AND OTHER FISHES

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From the Plymouth Laboratory

(Text-figs. 1–2)

CONTENTS

Introduction

During the study of the ecology of some fishes in the Tamar estuary in 1935–7 by one of us (Hartley, 1940), certain data concerning parasitic copepods of gadoids were collected, and as these were unpublished it was thought profitable to combine them with similar observations made during the period 1939–40 by the other author on gadoids caught in the sea nearby. The latter material was from two distinct habitats: (i) those fish caught in the trawl by the laboratory motor-boat *Gammarus* fishing within the limits of Plymouth Sound, together with those trawled by the *Salpa* in August 1939 from the Shagstone Grounds, which, although outside the Sound, are of similar depth (not more than 5 fathoms) and can be included with it under 'inshore waters'; (ii) other fish obtained from commercial trawlers fishing outside Tor Bay, Start Bay and over Rame Muds. The depth at which the latter were obtained is unknown,

but probably all were from water between 10 and 30 fathoms in depth; these grounds are considered as 'offshore waters'.

An analysis of these combined data throws some light on the distribution, and the factors controlling it, of some of the commoner copepod parasites of gadoids.

Lernaeocera branchialis (Linn.) (Fam. Lernaeoceridae)

The Relation between the Migratory Habits of Gadus merlangus *and* G. pollachius *and their Infection with* Lernaeocera branchialis

Length-frequency tables relating to *Gadus merlangus*, *G. pollachius* and *G. luscus* caught in the estuaries of the Tamar and the Lynher during 1936 and 1937 have already been published (Hartley, 1940, pp. 47–51). Though the numbers of fish were rather small, the results tend to show that these gadoids enter the estuaries when they are a few months old and remain there for some 10 months, growing rapidly in the spring and early summer and less so in the autumn and winter. Throughout the estuarine period there seems to be a gradual leakage of the stock out to sea, but the main exodus usually takes place in the following spring.

Lernaeocera branchialis is a large blood-feeding parasite found projecting from the gill chamber of our more important food species of gadoids, *Gadus morrhua*, *G. merlangus* and *G. pollachius*, but only the last two are common at Plymouth. The irregular distribution of this parasite and its effect on the young stock seemed worthy of investigation. We had at our disposal records of superficial examinations of over a thousand specimens of the two commonest gadoids from the Tamar estuary which were caught in the tuck-net during 1936 and 1937, and more detailed dissections of 245 of the same species from the sea nearby caught in the trawl during 1939 and 1940.

The chief points brought out by these data are: (i) that the young fish migrating inshore are free from parasites and become infected after entering inshore or estuarine waters, and (ii) that infected fish tend to behave abnormally and do not return to the open sea with the main stock.

Gadus merlangus

This species was twice as common as *Gadus pollachius* and *G. luscus* in the Tamar estuary; *G. pollachius* was also infected with *Lernaeocera branchialis* in the Tamar, but length data of infected fish are only available for *Gadus merlangus*, and these are set out in Table I A, and in histogram form in Fig. 1. The correlation between the migratory habits of this species and its infection with *Lernaeocera branchialis* in the Tamar estuary is indicated by the following summary of the facts illustrated in Fig. 1.

Recruitment of the small fish of year-class 0, with length range 4–10 cm., took place in May, but none of these young fish was infected. Though over 300 gadoids of less than 10 cm. long have been examined, no fish less than 11·0 cm.

TABLE I. INFECTION OF *GADUS MERLANGUS* WITH *LERNAEOCERA BRANCHIALIS* AND *CLAVELLA UNCINATA*

A. *From the Tamar Estuary*, 1936 *and* 1937 (*P.H.T.H.*)

Month	Total fish examined	No. over 10 cm.	A.M.L. for month	With *Lernaeocera*		With *Clavella*	
				No.	A.M.L.	No.	A.M.L.
1936							
i	41	41	20·14	9	20·16	1	(19·7)
ii	48	48	20·68	0	—	0	—
iii	16	16	19·13	8	20·63	0	—
iv	4	4	20·30	4	20·30	0	—
v	27	5	8·0	0	—	0	—
vi	5	0	(6·3)	0	—	0	—
vii	23	9	8·5	2	(19·0)	0	—
viii	68	64	12·05	9	12·94	20	12·35
ix	12	12	14·25	3	14·5	2	(14·5)
x	9	9	15·85	6	17·5	2	(13·5)
xi	7	7	16·5	5	17·0	1	(14·5)
xii	4	4	16·5	3	16·8	1	(15·5)
1937							
i	4	4	18·5	4	18·5	0	—
ii	1	1	18·5	1	(18·5)	0	—
iii	1	1	15·5	1	(15·5)	0	—
iv	0	0	—	0	—	0	—
v	0	0	—	0	—	0	—
vi	189	37	8·34	1	(11·5)	0	—
vii	65	58	12·0	3	14·5	0	—
viii	66	66	14·4	1	15·5	0	—
ix	49	49	15·8	5	16·1	1	(15·5)
x	10	10	18·6	1	(19·6)	1	(14·5)
xi	55	55	18·9	10	18·61	3	17·5
xii	11	11	16·2	1	(16·5)	1	(14·5)
Total	715	511		77		33	

B. *From Inshore and Offshore Waters near Plymouth*, 1939 *and* 1940 (*N.G.S.*)

Month	Origin	Total fish examined	No. over 10 cm.	A.M.L. for month	With *Lernaeocera*		With *Clavella*	
					No.	A.M.L.	No.	A.M.L.
1939								
viii	Sh.	24	24	29·5	6	30·0	5	29·8
x	R.	7	7	30·0	0	—	1	27·5
xi	R.	4	4	27·5	0	—	0	—
xi	P.	2	2	(15·0)	0	—	2	(15·0)
1940								
v	P.	4	4	15·0	3	14·7	2	15·6
vii	T.	31	31	23·8	2	24·0	2	22·9
vii	P.	4	2	8·6	0	—	1	(11·7)
viii	R.	14	14	24·0	0	—	4	24·0
ix	P.	1	1	(11·0)	1	(11·0)	1	(11·0)
x	R.	9	9	24·9	0	—	0	—
x	St.	4	4	24·2	0	—	0	—
xi	R.	4	4	24·8	0	—	1	(23·5)
Tota		108	106		12		19	

Note. A.M.L. = arithmetic mean length; P. = Plymouth Sound; R. = Rame Muds area; Sh. = Shagstone Grounds; St. = Start Bay; T. = Tor Bay.

was found infected (i.e. after 2–3 months of estuarine life). A length of 10 cm. has thus been taken as the arbitrary minimum, and only fish larger than this are included in the following analyses.

May to August was the period of rapid growth during which time the stock became infected. Infection did not necessarily take place at the same time for all fish, though information as to the state of maturity of the parasites relative to the length of the host is lacking for the Tamar material. In August 1936, 9 out of 64 of the rapidly growing new stock were infected, or 13·1 % (for the

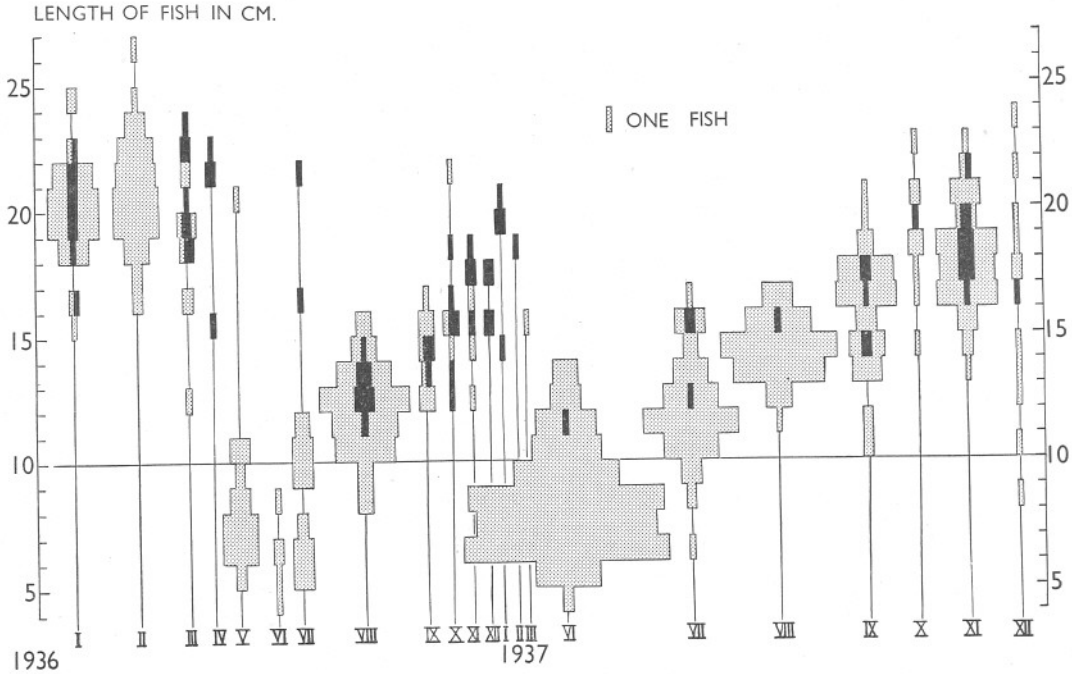

Fig. 1. Histogram representing the total fish examined from the Tamar estuary in 1936 and 1937. Infected fish shown black and uninfected fish stippled. Ordinates represent the length of the fish in cm. and the numerals along the abscissae indicate months.

whole period of rapid growth the infection was 9 out of 75 or 12·0 %). For the same period in 1937 only 5 out of 161 fish were infected, or 3·1 %. A period of less rapid growth followed, normally from September to the following spring, and ended in the run to sea; but in September 1936 though netting continued as before, there was a marked fall in the numbers of whiting caught. This may indicate a precocious run to sea of fish mostly less than 15 cm. in length. From September to December 1937 the slowly growing stock showed a four-fold increase in infection—17 out of 125, or 13·6 %. The 1935 stock persisted into the spring of 1936, and a similar persistence of the 1937 stock is hinted at, in spite of the irregularity in the numbers of fish caught towards

the end of the year. Apart from the anomalous absence of infection in February 1936, the rate of infection over the first 3 months of 1936 was fairly high, 17 out of the 105 fish, or 16·2 %, which is of the same order as that obtained for the period of slow growth at the end of 1937.

Factors governing the run to sea are unknown, but it does not seem to be dependent on the attainment of a certain minimum size or on the availability of food (cf. the precocious run to sea of small fish in midsummer of 1936); however, at some time after the period of rapid growth there is a sudden fall in the numbers of fish caught: the 1935 stock fell 76 % in March 1936, and the 1936 stock fell 82 % in September of the same year. It was immediately after this exodus that the remaining fish showed a sudden increase in the rate of infection which had a tendency to approach 100 %. These large infected fish lingered in the estuary for some months after the arrival of the new stock.

A comparatively few *Gadus merlangus* were examined from inshore waters (Table 1 B and Fig. 2*b*), but about half of the small fish (year classes 0 and i) were infected, and they are evidently a counterpart of the lingering post-exodus fish of the Tamar which had failed to reach the sea with the main stock. In spite of the small number of fish examined from offshore waters, their freedom from infection with *Lernaeocera* is remarkable: in Fig. 2*c* the two infected specimens came from a haul 'off Tor Bay' and may possibly have been taken in the shallower waters of the bay itself. It may be concluded, therefore, that the year class i whiting leaving the rivers and making out to sea in the normal way are very rarely parasitized by *Lernaeocera*, nor do they become infected after reaching the deeper waters.

Gadus pollachius

This species was only a third as common as *G. merlangus* in the estuary but about three times as frequently met with as the latter species in Plymouth Sound (Table II). A similar length distribution throughout the year was found in the Tamar for pollack as for whiting, but the growing fish showed less regularity in numbers (Hartley, 1940, Table VIII). An outstanding difference is the later date—in June—at which recruitment took place, which is explained by breeding starting some weeks later than that of the whiting. The length distribution of inshore pollack (Fig. 2*a*) shows what at first sight appears to be a bimodal curve, but this may be due to the irregular breeding of this species, which in some years breeds so late that young only appear inshore in the winter. This must have been so in 1939 to account for the double banking of the histograms for 1940. It is a remarkable fact that, at Plymouth, small bronze coloured pollack were taken at all times, except during midsummer, in the Sound; these fish appeared to be in good condition, and the examination of their stomach contents showed that their diet consisted mainly of crustaceans, but that in the autumn and winter small teleosts had been eaten, also Mollusca and vegetable matter such as *Enteromorpha* and *Zostera*. In the spring, 10–15 cm. fish usually contained polychaete remains;

TABLE II. INFECTION OF *GADUS POLLACHIUS*

A. *From the Tamar Estuary*, 1936 *and* 1937 (*P.H.T.H.*)

Month	Total fish examined	No. over 10 cm.	No. with *Lernaeocera*
i	13	13	2
ii	19	19	0
iii	8	8	1
iv	1	1	0
v	0	0	—
vi	130	0	0
vii	48	48	0
viii	18	18	0
ix	4	4	0
x	10	10	3
xi	19	19	4
xii	17	17	1
Total	287	157	11

B. *From Inshore and Offshore Waters near Plymouth*, 1939 *and* 1940 (*N.G.S.*)

Month	Origin	Total fish examined	No. over 10 cm.	A.M.L. for month	With *Lernaeocera*		With *Clavella*		With *Lepeophtheirus*	
					No.	A.M.L.	No.	A.M.L.	No.	A.M.L.
1939										
viii	St.	1	1	(38·5)	0	—	0	—	0	—
ix	P.	1	0	—	—	—	—	—	0	—
x	R.	4	4	28·25	0	—	2	(30·0)	2	(28·0)
xi	P.	2	2	16·0	0	—	1	(17·5)	0	—
xii	P.	3	3	12·66	1	(12·0)	0	—	0	—
1940										
i	P.	3	3	11·58	0	—	1	(11·5)	0	—
i	P.	1	1	25·5	0	—	1	(25·5)	1	(25·5)
ii	P.	4	4	12·14	0	—	2	(12·4)	0	—
iii	P.	2	2	13·7	0	—	0	—	0	—
iv	P.	13	13	13·6	4	14·3	2	(14·3)	1	(14·0)
iv	P.	1	1	22·5	1	(22·5)	1	(22·5)	1	(22·5)
v	P.	10	10	14·5	3	14·5	3	14·7	0	—
v	P.	1	1	21·0	0	—	0	—	0	—
vi	P.	4	3	16·6	2	17·0	2	17·0	0	—
vii	P.	14	3	15·4	2	16·9	1	17·7	1	(17·7)
viii	P.	43	29	11·4	4	16·1	3	13·5	0	—
ix	P.	6	6	12·55	0	—	4	13·3	0	—
x	P.	3	3	13·0	2	12·75	1	13·3	0	—
xi	P.	20	20	13·8	1	(15·8)	8	14·4	0	—
xi	P.	1	1	21·0	1	(21·0)	1	(21·0)	1	(21·0)
Total		137	110		21		33		7	

A.M.L. = arithmetic mean length; P. = Plymouth Sound; R. = Rame Muds area; St. = Start Bay.

C. *Fish Infected with* Lepeophtheirus pollachii. *Inshore Waters*, 1939 *and* 1940 (*N.G.S.*)

Length range	No. fish examined	With *Lepeophtheirus* No.	A.M.L.	%
10–15 cm.	87	1	(14·0)	1·15
Over 15 cm.	25	4	19·2	16·0
Offshore fishes, 1939 *and* 1940 (*N.G.S.*)				
Over 15 cm.	4	2	(28·0)	—
Total fish over 15 cm.	29	6	—	20·7
All pollack	116	7	—	6·05

it was extremely rare to find a stomach empty. The lingering of pollack infected with *Lernaeocera branchialis* is better demonstrated from our records of inshore fish than from those from the estuary. Estuarine fish showed a rate of infection for the two years of only 7 % (Tables II A and III A): 10 of these 11 infected fish were taken after the period of rapid growth (September–March 1936–7), when the whiting from the same area showed a remarkably high degree of infection (59 %), the infection rate for pollack for the latter period alone being 16 %.

Only four large fish (year class ii) were brought in from over the Rame Muds, but none of these was infected (Fig. 2 *a*, 'offshore'). Pollack, there-

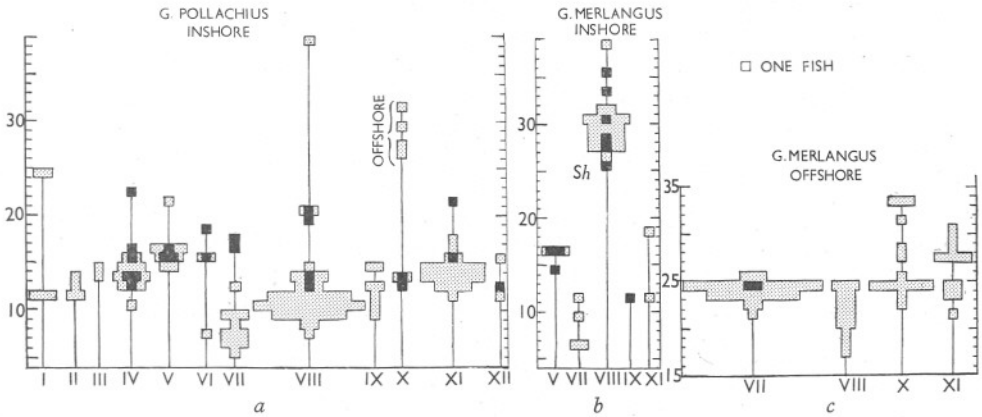

Fig. 2. Histograms representing the total numbers of fish examined from inshore and offshore waters near Plymouth during 1939 and 1940. Infected fish are shown black and uninfected fish stippled. Ordinates represent the length of the fish in centimetres (to the nearest half cm. above) and the numerals along the abscissae indicate months. *Sh*, fish caught from Shagstone Grounds, 1939.

fore, like whiting, become infected during their inshore or estuarine phase, more particularly in the former habitat, according to our observations.

The Relation between Parasitism and the Growth of the Fish

The causal trend of the correlation discussed above would seem to be that parasitism with *Lernaeocera branchialis* has some physiological effect on these gadoids which delays their seaward migration, rather than the fish remaining behind through some other set of circumstances and becoming parasitized owing to longer exposure to infection. The latter possibility can be tested by reference to Table III, in which the rates of infection for both species of fish are summarized according to their length groups for the three habitats. Owing to a significant number of *Gadus merlangus* being caught in the Tamar (A), the figures for this habitat are separated into the period of rapid growth (May–August) and the rest of the year when growth is slower (September–April). During the latter period the degree of infection is very nearly the same for both size groups, notwithstanding the small number of young fish,

whereas of the 226 larger fish only 53 were infected. If infection were a simple function of the size of the fish, i.e. depending mainly on the volume of potentially infective water passing over the gills, and on the length of time the fish remained exposed to infection, then the larger fish would be expected to show a much higher percentage infection than the smaller, whereas this was not so. That the larger fish show three times the infection of the smaller in the first period is, however, explained by referring to Fig. 1 which shows that they are represented by the lingering of the old stock which is very heavily infected; this same fact is reflected in the annual rates shown in the last column of Table III A.

In *G. merlangus* from inshore waters the 31 % total infection is very high, but it is made up as already suggested by small fish lingering behind the main stock on their way to sea from the rivers. The group of larger fish (probably in their third year), shown above *Sh* in Fig. 2*b*, were caught on Shagstone Grounds in the squid trawl in no more than 5 fathoms of water, so they may be included in the inshore habitat: incidentally, they show about the same rate of infection as the larger fish from estuarine waters.

G. pollachius from inshore waters show five times the infection in older fish than in younger fish, when figures for the whole period are taken (Table III B): no seasonal separation is justifiable owing to the irregularity in length distribution and the small samples available. That this marked difference is due to the 'lingering' phenomenon is evident from Fig. 2*a*.

Discussion of the possible Physiological Factors influencing Infected Fish

The nature of the physiological changes in these two species of gadoids effected by the presence of *Lernaeocera branchialis*, and having their main outcome in the postponement of the run to sea, is unknown. There are several lines of approach to the problem of the direct effect of this parasite on its host, and some of these may be considered here. The *condition* of parasitized fish as compared with non-parasitized has been examined during the 1939–40 investigations; the condition factor $K = \frac{w \times 100}{l^3}$ (where w is the weight of the fish in grams and l is the length in centimetres) has been used. These figures for each fish are not reproduced here, for it was found that they showed no correlation whatever with infection. The value of K is considered unreliable when individual differences are sought among fish at very different growth stages. The greatest error, perhaps, is introduced by the gross weight of the fish which includes the highly variable stomach contents. Though there are no records published of any measurement of the condition of fish infected with this parasite, Andrew Scott (1929, pp. 106–8) states that during the heavy infection year of 1928 the young whiting from the shrimping grounds between Mersey and Dee showed so marked an emaciation that infected ones could be picked out at a glance. Thomas Scott (1909, p. 90) makes the statement that 'whitings and other gadoids have been captured reduced almost to

skin and bone, having one or more large worm-like *Lernaea* [=*Lernaeocera*] hanging at their gills full of the red blood they had extracted therefrom'. Such emaciation has never been observed by us.

TABLE III. INFECTION OF *GADUS MERLANGUS* AND *G. POLLACHIUS* WITH *LERNAEOCERA BRANCHIALIS* SUMMARIZED ACCORDING TO THE GROWTH OF THE FISH

A. *From the Tamar Estuary*, 1937 *and* 1937 (*P.H.T.H.*)

Gadus merlangus

Length range	May to August: No. fish examined	May to August: Infected fish No.	May to August: Infected fish %	September to April: No. fish examined	September to April: Infected fish No.	September to April: Infected fish %	All months: No. fish examined	All months: Infected fish No.	All months: Infected fish %
10–15 cm.	216	11	5·1	37	8	21·6	253	19	7·5
15–25 cm.	32	5	15·6	226	53	23·2	258	58	22·3
Total catches	248	16	6·45	263	61	23·5	511	77	15·0

Gadus pollachius

No length data of infected fish from Tamar available.
No infection recorded during the period May–August; October–April: of the 82 fish examined above 10 cm., 11 were infected (13·4 %).
Infection over the two-year period = 7 %.

B. *From Inshore Waters*, 1939 *and* 1940 (*N.G.S.*)

Gadus merlangus

Length range	No. fish examined	No. infected fish with *Lernaeocera*
10–20 cm.	8	4
Over 20 cm.	24	6
Total	32	10 (=31·2 %)

Gadus pollachius

Length range	No. fish examined	With *Lernaeocera* No.	With *Lernaeocera* %
10–15 cm.	84	9	10·7
Over 15 cm.	22	12	54·5
Total	106	21	19·8

C. *From Offshore Waters*, 1939 *and* 1940 (*N.G.S.*)

Gadus merlangus

Two infected fish from 73 examined (2·7 %)

Gadus pollachius

No parasitic copepods were found in the four fish (A.M.L. 28·25 cm.) examined in October

Had there been a retarding effect on the growth of the fish by the presence of the parasite it would have been evident in our records. In Tables I and II the arithmetic mean lengths of the monthly catch are given for comparison with the A.M.L. of the infected fish, and the unexpected correlation is found that, on the whole, the infected fish are larger than the uninfected, even when

fish of the same size group are considered together, as they are for inshore and offshore fish. It is difficult to account for this anomaly, though two possibilities suggest themselves: first, that mere size is a favourable factor, a larger fish presenting a greater infective surface. Though this possibility was rejected above as an explanation of the higher rate of infection of large fish as a whole, it may possibly be operative here when a group of fish of a restricted length range, whether large or small, is being considered. The second possibility is that the smaller infected fish of each length group have been eliminated from the samples. Owing to the loss of blood (see below) and consequent reduction in respiratory efficiency, the weaker fish (i.e. those under the A.M.L. of the length group) may perhaps enter the surface water where the oxygen tension is higher; if so, they would not be caught in the trawl or in the tuck-net which operate over the bottom. Indeed they may, in these higher levels, become an easy prey for gulls and other predators.

Since infection has no measurable effect on these two species of gadoids at Plymouth, it would appear that they have, to a large extent, become tolerant of the parasite.

We have calculated that the capacity of the intestine of a fully grown *Lernaeocera* is at least 100 c. mm., which is considerable in relation to the blood volume of a young gadoid. It is not known at what rate the parasite feeds, i.e. how often this volume is extracted from the host, though it is shown elsewhere that feeding is not a continuous process (Sproston & Hartley, 1941). Granting, then, that the blood loss to *Lernaeocera* is probably far greater than that to any other parasite (that is, relatively and absolutely great), it would be of great interest to know the blood-cell counts of infected and uninfected fish; for in this way it might be possible to measure the effects of parasitism. Some degree of 'secondary anaemia'[1] is to be expected, for the loss of blood to the parasite would be made good by absorption of tissue fluids, via the capillary walls in the first instance, and finally equilibrated by absorption of water from the external medium (see below); the net result being a dilution of the tissue elements of the blood, and, perhaps, a greater or less strain on the haemapoietic organs. The various well-known symptoms of anaemia would contribute to a chronic cachexia which may well account for the parasitized fish failing to respond to the normal urge to run to sea.

A second possibility is tentatively suggested to account for the lingering of parasitized gadoids in estuarine and inshore waters: this concerns the osmoregulatory mechanism of the fish. The absorption of fluid from the external medium to make good the loss to the parasite is complicated by the fact that the gadoid is living in hypertonic media, whether it is in the sea or in estuarine waters. The salinities at various points in the estuaries of the Tamar and Lynher were determined by Milne (1938) who worked concurrently with one of us (P.H.T.H.) during the work on the Saltash tuck-net fishery there. It is

[1] 'Secondary anaemia' is used here in the clinical sense, to indicate the result of simple blood loss, in contrast to 'primary anaemia' which is defective blood-cell production.

notoriously difficult to determine the exact salinity level at which fish are living in an estuary, but in all probability these gadoids normally remain near the bottom where changes in salinity are not so great as elsewhere. Though these fish were taken in the upper reaches of both estuaries in summer, in winter they were only caught in the neighbourhood of Saltash Bridge where the salinity did not fall below 25–24 $^{0}/_{00}$; this represents the minimum bottom salinity recorded, and probably a similarly low figure would be obtained from certain places in Plymouth Sound (Barn Pool, for instance, where a large proportion of our gadoids were obtained during the latter series of observations). The large 'lingering' infected gadoids found in the seaward end of the estuaries in autumn and winter when the rivers are in flood are therefore inhabiting media of a much lower salinity than the normal fish of the same size which have migrated to offshore habitats.

It is well known that the blood of marine teleosts has an osmotic concentration of only about a third of that of sea water. From the work of Smith (1930) and Keys (1933) it is now clear that the mechanism of regulation consists of drinking sea water and excreting the excess of salt through the gills. Excretion of salt against an osmotic gradient necessarily involves the expenditure of considerable energy. To quote from Smith's review of the subject (1932, p. 11): 'a large fraction of the ingested and absorbed sea water is excreted extra-renally...nearly all the water excreted as urine must be procured from the sea water at the expense of considerable osmotic work.... Consequently the normal fish tends to keep the urine flow and the ingestion of sea water down to a minimum for the sake of physiological economy.' The choice of habitat may therefore be a physiological response to the necessity of absorbing additional sea water to maintain the normal blood volume (i.e. pressure equilibrium), and it is possible that to reduce the concomitant osmotic work the parasitized fish would tend to remain in media having the minimum salinity compatible with life.

It was hoped that these two hypotheses concerning the exact nature of the physiological upset of parasitized fish could have been tested experimentally; but so far it has been found to be impossible to keep the fish infected with *Lernaeocera* alive in the laboratory. This, in itself, is a pertinent fact. When batches of gadoids were brought into the aquarium only a few of the uninfected fish died in transit, but nearly always the infected fish would be found dead, floating venter upwards, with the operculum widely distended and the mouth open to the fullest extent; any surviving infected fish always died within an hour or so of being placed in the tanks. This, in fact, is the only direct effect of physiological upset observed by us in infected fish.

The Susceptibility of the different Gadoids to Infection with Lernaeocera *spp.*

From Table III it would appear that *Gadus merlangus* was twice as susceptible to infection with this parasite as *G. pollachius* in the estuaries, and that the susceptibility was 3:2 in inshore waters. When, however, our total

figures for all habitats are taken together there is little difference between them:

Species	Total no. of fish examined	Total no. of fish infected	% infection
G. merlangus	616	89	14·5
G. pollachius	267	32	12·0
Both species	883	121	13·7

G. minutus migrates inshore during its first year, though from the data (Hartley, 1940, p. 50) it does not appear to ascend the estuaries with any regularity. Of the 27 specimens examined from the Tamar and the Lynher during 1936 and 1937, none was infected with copepods. During 1939 and 1940, 120 specimens were examined from the sea and were free from copepods with the exception of two fish, both well above the average size of our samples; one of them had a mature specimen of *Lernaeocera branchialis* attached to the anterior angle of the fourth gill arch. This is the first record of an adult of this species being found on *Gadus minutus*. Hesse (1870, and 1891, p. 191, as quoted by Wilson, 1917, p. 40) records what is considered to be a larval form on the gills of the same host. The occurrence of the parasite on this host seems to be rare, for *G. minutus* is quite as common as *G. pollachius* in inshore waters, and Ford (1931) considers it to be the commonest gadoid on the local trawling grounds outside Plymouth Sound; yet the previous investigators of parasitic copepods at Plymouth who must have examined large numbers of this fish have never recorded the presence of *Lernaeocera branchialis* on it.

Gadus luscus is infrequent inshore, but of the eleven specimens from Rame Muds, three were infected with *Lernaeocera lusci*. During the previous investigations (1936, 1937) 289 fish of this species were examined from the Tamar, of which 246 were over 10 cm. in length; but only two of these were infected with *Lernaeocera*, which, in all probability was *L. lusci*, though they were not identified at the time. This parasite is specifically distinct from *L. branchialis* (a parasite rarely found on *Gadus luscus*): apart from certain morphological characters it differs from the latter species in its position of attachment to the host, being found attached to the gill arches at about the middle of their length and penetrating only into the branchial vessels. The host reaction is also different to this species: at the 'neck' of the parasite the branchial mucosa of the host is hypertrophied: a traumatic overgrowth in the form of a swollen collar which obscures much of the body of the parasite. This has also been observed by Vanden Berghe (1933), but is not mentioned by any other observer. It is common to find multiple infections of this species; one of the fish from Rame Muds had six *Lernaeocera lusci*. Since so low an infection rate (0·81 %) was found for *Gadus luscus* in the Tamar, and a comparatively high one (25 %) for the offshore fish it would seem that for this species infection takes place after the run to sea. It is possible, therefore, that the intermediate host carrying the copepodid stages and the males is different from that of *Lernaeocera branchialis* (*Pleuronectes flesus*), which is an active vector mainly in estuarine and inshore waters; so that the whole life cycle of

L. lusci would seem to be passed in deeper offshore waters, a marked contrast to that of *L. branchialis* (see below). Scott & Scott (1913, p. 145) record *L. lusci* from *Gadus luscus* caught 10 miles off Aberdeen in January 1901.

Gadus morrhua is infrequent in the Tamar estuary, and only two out of the 20 fish examined from this habitat were infected with *Lernaeocera branchialis*; no fish of this species have been examined by us from the sea at Plymouth. A. Scott (1929, p. 108) says that the cod caught with the highly infected whiting were rarely infected off the Lancashire coast.

No *Lernaeocera* spp. were found by us on other gadoids: a few each of *Gadus virens* and *Urophycis blennoides* were examined from offshore, also 23 *Onos mustela* and a few each of *O. tricirratus* and *O. maculata* from inshore waters. Numbers of *Callionymus lyra* were examined from the estuary in 1936 and 1937, and some from inshore in 1939 and 1940, but they were free from copepod parasites.

The Duration and Distribution of the Developmental Phases of Lernaeocera branchialis

The life history of this parasite is well known, though the records of the developmental forms are not numerous in the literature. There is a free-swimming copepodid stage which becomes attached to the gill filaments of the flounder; here a series of modified copepodid stages are passed, finally giving rise to pelagic forms (redescribed by Sproston, 1941). The female is still cyclopoid in appearance but has an elongating genital segment; the male is similar to the infective copepodid. These pelagic forms remain within the gill cavity of the flounder for a short time, attachment being effected by the chelate second antennae; copulation takes place here and the male soon dies. The fertilized female leads a pelagic existence until it finds itself within the gill chamber of a gadoid; it then becomes attached near the anterior angle of the fourth gill arch and undergoes a peculiar metamorphosis. The long genital segment first twists and then dilates, and the cuticle becomes thicker. Meanwhile anchoring processes grow out from the sides and top of the cephalothorax, these becoming the bifurcating 'antlers' of the adult. Penetration continues, at first through the branchial vessels and then into the ventral aorta and often into the auricles. Occasionally fibrous and fatty tumours develop, but usually the only histological response is a fibrotic thickening of the vascular system around the anchoring structures. The suctorial form of mouth is situated on the end of a conical proboscis embedded in the blood vessel. The swollen genital segment becomes a darker red as the blood-feeding habit continues. Two very long thin egg strings are extruded just above the small cylindrical abdomen; they are tightly coiled in a boss-like tangle and can often be seen projecting with the abdomen and part of the genital segment from the operculum of an infected gadoid. Owing to the permanent mode of attachment, the 'antlers' remain in situ after the death and fragmentation of the rest of the parasite.

During the investigations by the first author (1939–40) data were collected with a view to determining the longevity of *Lernaeocera* and the age of the host when infection occurs. The development of the adult female on the gadoid has been separated, somewhat arbitrarily, into seven substages as

TABLE IV. SUBSTAGES OF *LERNAEOCERA BRANCHIALIS* ON *GADUS MERLANGUS* AND *G. POLLACHIUS*

From Inshore and Offshore Waters near Plymouth, 1939 and 1940 (N.G.S.)

A. *Distribution of Substages Throughout the Year*

Month	i	ii	iii	iv	v	vi	vii	viii	ix	x	xi	xii		Totals
P	—	—	—	1	—	—	—	—	—	—	—	—	1 P	
U	—	—	—	—	1	—	—	—	—	—	—	—	1 U	19 (U+V+W)
V	—	—	—	—	1	—	1	—	1	—	—	—	3 V	
W	—	—	—	2	2	1	2	5	—	1	1	1	15 W	
X	—	—	—	2	2	1	1	7	—	1	—	—	14 X	16 (X+Y)
Y	—	—	—	—	—	—	—	1	—	—	1	—	2 Y	
Z	—	—	—	—	4	—	1	1	—	—	1	—	7 Z	

Symbols used to denote the substages found

P = 'Pennella' stage.
U = Very young form: torsion of body just complete, but genital segment not yet swollen. Pale red in colour.
V = Very young form: antlers showing first bifurcation and penetrating into the branchial vessels. Medium red in colour.
W = Young form: antlers penetrating as far as ventral aorta, but no external egg strings. Deeper red, especially in the abdomen.
X = Full grown form: external egg strings emerging but not ripe.
Y = Fully mature form: egg strings hatching or spent, and body very dark red in colour; abdomen almost black.
Z = Remains of dead antlers embedded in sublingual and vascular tissue.

B. *Distribution of Substages on Fish of different Size Groups*

Length range	No. of infected fish	No. of parasites	Sub-stages	Intensity of infection per fish: Single	Double	Triple
10–15	11	11	P 2V 5W 2X Z	11	0	0
16–22	14	21	U 7W 6X Y 6Z	9	3	2
Over 22	8	11	V 3W 6X Y	5	3	0

Notes. (1) The minimum length *G. merlangus* infected was 11 cm.; *G. pollachius* 12 cm.
(2) Minimum length fish bearing dead parasite: *G. merlangus* 13·5 cm. and *G. pollachius* 15·5 cm.
(3) Of the 8 multiple infections found, 7 were composed of parasites in two distinct substages. Proportion of multiple infections: 8/33 (24·3 %).
(4) In all respects the parasites from the two host species were similar in their distribution, and for this reason the data are considered together.

shown in Table IV. The 'Pennella' stage was so called by Scott (1901) to denote that stage of the fertilized female before the antlers grow out and just prior to torsion.

The occurrence of these various substages throughout the year is shown (Table IV A), and the last column shows the relative abundance of these stages

over the period of observation. It is seen that the immature and mature stages are scattered in time, so that no definite infection or breeding period can be assigned to *Lernaeocera*.

Stekhoven (1936) and Stekhoven & Punt (1937) have studied similar material from the cod from continental inshore waters at Helder, and they conclude that there is a simple annual cycle, that maximal infection of the intermediate host, the flounder, takes place in April and May, and that the mass infection of the cod is in midsummer. In their Table III (1937, p. 653) they give a summary of the details concerning *L. branchialis* from some dozen cod, all about $1\frac{1}{2}$ years old. These data, however, do not seem to lend very convincing support to their conclusions, for the parasites were by no means of a similar age: about half of the number examined belonged to various immature stages (P to V in our nomenclature), and of the rest, about a half were mature and the remainder consisted of dead remains. Apparently the collections were made only in the months of May and September, but the results show what is probably a typical cross-section of any population of *Lernaeocera*: for, in general, they agree with our findings on the distribution of the substages throughout the year. Our conclusions are, however, very different from these authors', for the data we have show conclusively that generations are being produced continuously all the year round.

Table V shows that the few flounders examined between December 1939 and December 1940 were all infected with the copepod developmental stages except those two fish below 10 cm.

TABLE V. THE INFECTION OF *PLEURONECTES FLESUS* WITH THE DEVELOPMENTAL STAGES AND MALES OF *LERNAEOCERA*

(December 1939 to December 1940, N. G. S.)

Month	ii	ii	ii	iii	iv	iv	ix	ix	xi	xii	xii
Length of fish	12·2	13·4	13·5	9·0	10·8	11·5	12·1	17·3	9·8	22·5	12·3
No. of *Lernaeocera*	3	8	7	0	16	7	1	2	0	20	10

On other fish: *Cyclopterus lumpus*, caught inshore on 3. iv. 40, had three mature males of a lernaeoceran on the gills, and *Solea solea* (also a fully grown fish), caught off Roscoff (France) on 3. x. 38, had a single half-grown copepodid on the gills, which also belonged to this family of parasites (see Sproston, 1941).

All the flounders were caught in strictly inshore waters at Plymouth, and most of them from the seaward side of Laira Bridge in the Cattewater: Mr Spooner confirms our view that salinity and other conditions here are comparable with those near Saltash Bridge in the Tamar estuary. Records of hosts for the copepodid stages are sparse in the literature and they usually refer to the occurrence 'on flat fish such as the flounder' and similar vague terms. Claus and Metzger's original material came from the flounder as did Stekhoven's; it is curious that there are no records by the Plymouth workers at any time for these distinctive young stages. During the survey of the

flounders in connexion with the Saltash Tuck-Net Fishery (P.H.T.H.) no special search was made, unfortunately, for gill copepods; indeed, there are no records extant for them from flounders taken in rivers or offshore, and the only localities mentioned are the inshore waters of Europe. It is also curious that so far no larval lernaeoceran has been recorded from the Western Hemisphere, though a species described by Wilson (1917), closely allied to ours, is common on gadoids of the Atlantic seaboard. We think that it is safe to state that the normal intermediate host at Plymouth is *Pleuronectes flesus*, for though a thorough microscopical examination has been made of the gills of 100 other flat fish caught at Plymouth between August 1939 and December 1940, no larval forms were found. The numbers of flat fish were distributed between nine species as follows: *P. platessa* 55, *P. cynoglossus* 1, *P. microcephalus* 2, *P. limanda* 21, *Rhombus laevis* 4, *Arnoglossus laterna* 3, *Solea solea* 9, *S. variegata* 1, and *S. lutea* 4.

It is regrettable that no flounders were obtainable during the summer months at Plymouth; but that breeding of the parasite was taking place during this time is evidenced by the occurrence of mature adults shedding nauplii (see Table IV A, substage x)—so that our evidence is complete for the continuous breeding of *Lernaeocera*.

By a careful examination of the facts at our disposal we can form some estimate of the length of the life cycle of this parasite. Eggs have been hatched in the laboratory in plunger jars containing sea water, and the pelagic copepodids were swimming actively on the second day. Some of these had attached themselves to the spent part of the egg strings, for want of other substratum, and had moulted, once at least, into the pupoid form which is described elsewhere (Sproston, 1941); this took place overnight and they lived for only a few hours afterwards. It is possible that these copepodid stages on the flounder are run through rapidly, for whenever seven or more were found together at least one was in the process of moulting. Stekhoven & Punt (1937, p. 653) consider that as the pelagic fertilized female is seldom encountered, it must usually pass directly from the flounder to the cod when the latter migrates inshore. This, in general terms, is borne out by our observations on other gadoids, but the term 'directly' is misleading. It is improbable that so small and ill-equipped a crustacean could survive planktonic conditions in the open sea, and finally sink to the bottom and seek out a suitable gadoid; it is much more likely that the cycle is completed in the shallow inshore waters and in estuaries; but any direct transmission by actual contact is most unlikely. That these pelagic fertilized females can exist as free animals for short periods has been shown by Andrew Scott (1901, p. 44), who collected them from filters placed over the outflow from the Peel flounder hatcheries: he says that he also caught males in this way, but their presence was accidental, since they normally die soon after copulation on the gills of the flounder. The ratio of males to females was 1:25. Up to the beginning of the century these forms had only been taken by I. C. Thompson (1893, p. 212, pl. 26, fig. 7) in

tow-nettings off Puffin Island off the coast of Anglesey; the succeeding stages—the 'Pennella' stages of Scott—have since been found on the gills of gadoids, and recently Stekhoven & Punt (1937) have found an unbroken series (described in their Tables I and II). This series lacks, however, the extremely elongated form, showing the incipient antlers as small bulges on the cephalothorax, which was discovered by Thomas Scott in 1900 on the gills of a whiting in the Bay of Nigg, Aberdeen, and has never been found again (A. Scott, 1901, pl. IV, fig. 6). Regular tow-nettings have been taken outside Plymouth Sound throughout the year for a number of years, and Dr Lebour and Mr F. S. Russell, who have examined the plankton, inform us that they have never found a larval stage of *Lernaeocera*; this bears out our suggestion that a gadoid once out at sea does not become infected with *L. branchialis*.

For arriving at an approximation to the minimum length of the gadoid phase of the life cycle, the following rationale was employed. Since the youngest gadoid found infected was 11·0 cm. long and the youngest bearing a dead parasite was 13·5 cm., then the whole phase may be completed while the fish is growing some 2·5 cm. From the histograms this time may be estimated, very roughly, at 8 weeks.

Additional data in support of this are obtained from the age of epizootic organisms. In August 1939 a third-year whiting was caught on the Shagstone Grounds bearing two *Lernaeocera*: one was immature (stage V) and the other, with half-spent egg strings, was covered with an epizootic hydroid. We are indebted to Mr W. J. Rees for examining this at the time and for his report on the specimen, which is as follows:

> On the exposed gravid genital segment (the so-called body) of the *L. branchialis* there was a well developed colony of *Clytia hemisphaericum* (Gronovius) = *Clytia johnstoni* (Alder, 1857). The hydrocauli were branched and numerous gonothecae were present containing medusa buds. The colony was normal in every respect and there was nothing to suggest that it was parasitic on the copepod. It was probably at least 6 weeks old and may have been two or three months old.
>
> Many examples of a triple association between a fish, a parasitic copepod and a hydroid are known. Jungersen (1911) cites several instances and also describes a hydroid, *Ichthycocodium sarcotretes* occurring on *Sarcotretes scopeli* Jungersen, 1911 (Lernaeoceridae). This hydroid he regards as semi-parasitic because the polyps possess no tentacles and no nematocysts.

A. Scott (1929, p. 108) gives the following information regarding epizootics: 'The *Lernaeocera* apparently live quite a long time. I have had specimens overgrown with zoophytes and young mussels 1·3 mm. long.' The length of mussel spat at the time of settlement appears from the records to be rather variable as it depends on local conditions; Matthews (1913, pp. 557–9) indicates that laboratory-bred spat settled when lengths were between 1 and 2 mm., but that one young larva collected from the plankton had not settled though it was 4 mm. long. Dr Lebour has kindly given us her opinion on the

probable length of time during which Scott's specimens had been attached; this, she considers, may have taken place shortly before they were collected, and in any event they could not have settled more than a month previously.

Returning to Table IV, the relative frequency of the occurrence of the adult substages throughout the year may be taken as an approximate measure of the relative duration of those substages. The suggestion, in general terms, is that the frequency with which any phase is encountered in a regular and continuous sequence is proportional to the time occupied by that phase in the sequence. For instance, of the 36 living *Lernaeocera* found on the two species of gadoids, 20 were immature and 16 were mature, so that approximately five-ninths of the time is spent in the immature condition and four-ninths of the time in the mature condition. Similarly, one-seventh of their time is taken up in penetrating from the outer to the inner vascular tissues, growing the antlers and completing the torsion of the body (substages P to V), the rest of their time being divided about equally between continuing the growth of the genital segment, both in length and girth, and the final stage during which the egg strings are extruded and the ripe eggs shed. After this the animal remains alive for a short time (only two Y were found, so that it is fairly rare, i.e. probably a brief stage); death is followed by rupture and fragmentation of the projecting parts, leaving the antlers and some part of the 'neck' embedded in the tissues of the host.

Two direct observations on the living *Lernaeocera* are noted here as they have some bearing on the time factors of these substages. There was usually no difficulty in keeping the adult parasites alive in circulating sea water in the laboratory provided they were in no way injured during the dissection from the host. One W stage had been under observation for some hours when it started to extrude egg strings; after about 3 hr. approximately half the normal bulk of egg strings were extruded, but the eggs were pale and there was no sign of nauplii in the distal ones. At this time, unfortunately, these observations had to be concluded owing to an air-raid. Other egg-bearing females have been kept under observation for some days; in one the nauplii were hatching from the distal eggs as these became detached, over a period of 4 days' observation, and though at the end of this time the animal was sacrificed for another experiment, there was no marked diminution in the mass of the remaining egg strings. Though no measurements have been made to determine the rate of shedding of the eggs it seems that this is protracted over, perhaps, 2 or 3 weeks. Under natural conditions the eggs may be shed more rapidly owing to the projection of the end of the genital segment of the ripe female into the surrounding water, a little beyond the operculum of the fish.

Owing to this protracted shedding of ripe eggs the metanauplii from the first eggs liberated may well have passed through several, or even all, their stages on the flounder by the time the last sister egg has just hatched out. Nevertheless, it is unlikely that the same flounder is the recipient of the whole

gamut of larvae from one female *L. branchialis*, though superficially the facts suggest this: for in all the infected flounders examined bearing seven or more larvae, some of the latter were males and females in the fertilization stage and some were newly arrived copepodids which had not yet become attached by the rostral filament, but were merely hanging on to the gills by their chelate second antennae. This variety of stages probably represents infection from several parents; this is an acceptable view considering the extremely high infection rate of the flounders from the seaward end of estuaries.

From the above account it seems probable that *L. branchialis*, at least at Plymouth, has about four or five consecutive generations a year, and that these generations show an infinite degree of overlap.

Multiple Infections of Gadus merlangus *and* G. pollachius *with* L. branchialis

The records of multiple infections of fish caught in the sea near Plymouth are shown in Table IV B: and since seven out of eight of those recorded within the limits of inshore waters consisted of parasites of distinctly different ages, any possibility of immunity is precluded. This has already been demonstrated by the similar findings of Stekhoven & Punt (1937, Table III). That the chances of reinfection increase with the age of the fish (provided that the latter remains inshore or in the estuary) is demonstrated by the absence of reinfection in fish less than 15 cm. long, and its presence to the extent of 24·3 %

TABLE VI. DISTRIBUTION OF *LERNAEOCERA BRANCHIALIS* ON *GADUS MERLANGUS* AND *G. POLLACHIUS*

Intensity of Infection per Fish from the Tamar Estuary, 1936 *and* 1937 (*P.H.T.H.*)

Length range	Infected fish	Multiple infections		No. of parasites per fish			
		No.	%	1	2	3	4
G. merlangus:							
Under 15 cm.	19	5	26	14	5	0	0
Over 15 cm.	58	14	24	44	7	6	1
Total	77	19	24·7	58	12	6	1
G. pollachius:							
Total fish	11	3	27·3	8	1	2	0
Both species	88	22	25	66	13	8	1

in fish over 15 cm. long from inshore waters. In Table VI similar results from the estuary are given as far as our records permit. The multiple infections are shown here also for both host species together for comparison with those from the sea. One fact which is difficult to explain is that in whiting from the Tamar there is no increase of multiple infection with age of the fish as shown above. The other outstanding result is the remarkable agreement in each fish and in each habitat of the proportion of multiple infections to the

total number of infections (about a quarter). Considering the number of fish examined, this figure is perhaps rather high; and it is regrettable that larger numbers of fish were not examined from both habitats so that the results could have been treated statistically, to test the possibility of infection being a predisposing factor for reinfection.

Clavella uncinata (O. F. Müller) (Fam. Lernaeopodidae)

Some Factors Influencing its Distribution on Gadus merlangus *and* G. pollachius

The distribution of *Clavella* shows, in some respects, a marked contrast to that of *Lernaeocera*, particularly in relation to the inshore and offshore migrations of the hosts. Its occurrence month by month in the three habitats on *Gadus merlangus* and *G. pollachius* is shown in Tables I and II respectively. The incidence on fish of known length has been examined for *G. merlangus* in the estuary (P.H.T.H.) and for both species from the sea (N.G.S.), but as there is no very important correlation these details are not reproduced here. The seasonal occurrence is at first sight significant in the estuary, for it was found on *G. merlangus* only during the last third of the year; this, however, is more likely to be a length correlation, since a similar seasonal variation was not found inshore.

Table VII. Infection of *Gadus merlangus* and *G. pollachius* with *Clavella uncinata* summarized according to the Growth of the Fish

A. *From the Tamar Estuary*, 1936 *and* 1937 (*P.H.T.H.*)

Gadus merlangus

Length range	No. fish examined	With *Clavella* No.	With *Clavella* %
9·5–15 cm.	253	25	9·9
Over 15 cm.	258	8	3·1
Total	511	33	6·45

Gadus pollachius

Of the 157 fish examined only one was infected with *Clavella* (in November 1936): 0·64 %

B. *From Inshore Waters*, 1939 *and* 1940 (*N.G.S.*)

Gadus merlangus

Length range	No. fish examined	With *Clavella* No.	With *Clavella* %
9·5–15 cm.	4	3	(75)
Over 15 cm.	29	8	27·5
Total	33	11	33·3

Gadus pollachius

Length range	No. fish examined	With *Clavella* No.	With *Clavella* %
9·5–15 cm.	87	20	23
Over 15 cm.	25	11	44
Total	112	31	27·7

C. *From Offshore Waters*, 1939 *and* 1940 (*N.G.S.*)

Gadus merlangus

Of the 47 fish examined, all of which were over 20 cm., eight were infected: 17 %

Gadus pollachius

Of the four fish examined two were infected (A.M.L. = 30·0 cm.)

When the results are summarized according to the growth of the fish (Table VII), some interesting factors suggest themselves: first, the marked difference in the infection rates of estuarine and inshore fishes. The total infection rate

of *Lernaeocera branchialis* on the two species of host in the estuary and inshore was 13·2 and 22·5 % respectively, whereas for *Clavella uncinata* it was 5 and 31 %. That the increase in infection with *Lernaeocera branchialis* inshore is due to the 'lingering' effect of parasitized fish being intensified, has already been suggested. Parasitism with *Clavella*, however, does not seem to have any such repercussions on the migratory habits of the host, and the six-fold increase in infection of inshore fish as compared with those from the estuaries seems to be brought about by a different set of factors. The most probable is its lower tolerance to dilution of the external medium. Experiments described elsewhere (Panikkar & Sproston, 1941) have shown that *Lernaeocera branchialis*, by virtue of a normal osmotic concentration below that of sea water, can tolerate dilution of the external medium at least down to 2·24 % NaCl, which is probably lower than that normally tolerated by the host. Unfortunately, no experiments on the osmotic behaviour of *Clavella* have been made, but casual observations have repeatedly shown that it dies quickly when very little tap water is added to the sea water in which it will live for some hours in the laboratory. *Lernaeocera*, on the other hand, has been kept alive under laboratory conditions (isolated from the host) for over a week on several occasions.

An important paper published recently (1939) by Poulsen, on the biology of *Clavella uncinata*, contains a great deal of interesting information; the distribution of this parasite on the cod in Danish waters was extensively investigated and it was found to be fairly common, especially in the Cattegat and in the Belt Sea, but infection decreased with salinity and Poulsen considers that of 16 ‰ to be lethal. He also remarks on the difficulty of determining the exact salinity level at which fish are found; from his maps (Figs. 6, 7) it seems that the relatively highly infected fish of the Belt Sea area were caught in 10–20 m. of water, or even beyond the 20 m. line. Even if fish were confined to the bottom layers where salinity is highest, this is appreciably lower than the mean bottom salinity in the Tamar estuary. Poulsen quotes Jacobson's figures for the Belt Sea at 20 m. as 20–26 ‰, but the bottom salinity of the Tamar estuary where other species of gadoids were found does not fall below 24 ‰ even in winter. It may be that a local race of *C. uncinata* has become adapted to the more brackish conditions of the Danish waters. It would be interesting to know the distribution of *Lernaeocera* spp. in the same region.

Poulsen found that no year-class 0 fish were infected, but that the highest infection occurred in the year-class i fish (38 %), thence falling steadily as the age advanced. The smallest cod found with *Clavella* was 11·0 cm. long, and this Poulsen includes in year-class i. There is a possibility that the sudden increase in infection in the yearling cod may be correlated with their migratory habits: a relatively high minimum salinity being the limiting factor. Graham (1934) discusses the state of our knowledge of the migration of young codling and he considers that, while it is true that there is a winter visitation of these

young fish into inshore waters, Meek's theory (1916) of a periodic (annual) in- and offshore migration is by no means proven. He thinks it more likely that there is a general scatter of the post-larval phase and, since they seem to like a rough bottom where crustacean food is abundant, any fish entering inshore waters may remain there provided that the bottom is suitable, in spite of other inclement factors; though Graham was careful to point out that the data in his possession were insufficient to make more than a working hypothesis. If the young codling are in inshore Danish waters, where the salinity is lower than elsewhere, then it is possible that the absence of *Clavella* on the year-class 0 fish may thus be accounted for, and that they become infected after their first offshore migration.

Among the infected *Gadus merlangus* in the Tamar estuary there was one small fish (8·8 cm.) which was certainly in its first year; the smallest inshore infected fish measured 9·6 cm. and as this was the only one infected below 10 cm., 9·5 cm. was taken as the arbitrary minimum, and only fish above this length are considered in the following analyses. From Table VII it is seen that in the estuary the infection of *G. merlangus* dropped to a third of its intensity after the period of maximum growth, but on the other hand, inshore *G. pollachius* showed nearly double the infection after the period of maximum growth. We can offer only one explanation for this result; perhaps the decreasing salinity conditions met with by the growing fish in the river as the season advanced were such as would be unfavourable to the developing nauplii, so that few succeeding generations were forthcoming to reinfect the stock. However this may be, it seems that conditions in the Sound were altogether more favourable to the parasite, for here the degree of infection is of a similar order to that found by Poulsen in Danish waters for the allied species of host of the same age.

The susceptibility of the various species of gadoids to infection with *Clavella* is similar to that with *Lernaeocera*, except that we have not found the former on *Gadus luscus*; one specimen of each parasite was taken from *G. minutus* from the sea but none from the river. In spite of the unequal distribution of *Clavella* on the gadoids of the estuary (Table VII A), the total infections from our combined records are not very dissimilar:

Species	Total no. of fish examined	Total no. of fish infected	% infection
G. merlangus	591	52	8·8
G. pollachius	273	34	12·5
Both species	864	86	10·0

The Duration and Distribution of the Later Developmental Phases of Clavella uncinata

Unlike *Lernaeocera*, *Clavella* has only a single host in its life cycle, so that the hazards of the free living stages are enormously reduced as compared with those of the former type of parasite. Perhaps as a direct consequence of this

Clavella produces fewer eggs. Gurney (1934) has given the first and only account of the complete developmental cycle of this copepod. He had considerable difficulty in rearing copepodids from eggs under laboratory conditions at Plymouth, but later he was able to collect a series of intermediate forms from gills of local pollack.

Some 150 gadoids have been examined throughout the year by one of us (N. G. S.), but the pupa-like developmental stages described by Gurney have not yet been found. The various adult substages have, however, been found in several months of the year. As Gurney did not suggest any grouping of these substages, we have tentatively divided them into three as indicated in Table VIII; in section A of this table their distribution is shown throughout the year.

TABLE VIII. SUBSTAGES OF ADULT *CLAVELLA UNCINATA* ON *GADUS MERLANGUS* AND *G. POLLACHIUS*

From Inshore and Offshore Waters near Plymouth, 1939 and 1940 (N. G. S.)

A. *Distribution of Substages Throughout the Year*

Month	i	ii	iii	iv	v	vi	vii	viii	ix	x	xi	xii	Total
X	1	—	—	—	—	—	2	13	3	—	8	—	27 X
Y	—	3	—	1	1	—	2	8	2	1	11	—	29 Y
Z	2	—	—	2	4	2	2	18	3	13	13	1	60 Z

Symbols used to denote the substages of the 'adult' parasite

X = all very young forms in which the genital segment has not swollen and is about one-third of the body in size.

Y = young immature forms in which the genital segment has swollen but in which the egg strings have not emerged.

Z = mature forms with external egg strings, including those with spent egg strings.

B. *Distribution of Substages on Fish of Different Size Groups*

Length range	Infected fish	No. of parasites	Substages	Multiple infections: No.	%	No. fish (No. parasites)
9·5–15	22	29	10 X 11 Y 8 Z	4	18	18 (1), 3 (2), 1 (3)
16–20	11	26	6 X 7 Y 13 Z	4	36	7 (1), 1 (2), 1 (3), 1 (5), 1 (9)
Over 20	15	62	11 X 11 Y 40 Z	8	53	8 (1), 2 (2), 1 (3), 1 (7), 1 (9), 1 (10), 2 (11)

Notes. (1) Minimum length of *G. merlangus* infected 11·0 cm.; *G. pollachius* 9·6 cm.

(2) Minimum length of *G. merlangus* with Z stage 11·5 cm.; *G. pollachius* 14·0 cm. No dead and disintegrating parasites were found.

(3) Among the multiple infections in the three groups the following proportions of fish had dissimilar stages occurring together: smallest $\frac{1}{4}$, medium $\frac{3}{4}$ and largest $\frac{4}{5}$.

(4) As with *Lernaeocera* the two hosts showed similar distribution of parasites in all respects.

There is a slight indication of two main breeding seasons, though the number of specimens is insufficient to warrant a definite conclusion. The youngest adult substage occurs throughout the summer and autumn but is absent in the spring, but the maturing forms (Y) were found all the year round, though the mature forms (Z) were always more abundant, with a concentration in summer and autumn of about four times the average monthly distribution. It is not likely that the few Y group individuals recorded in the spring were remnants of the previous year's brood, for we have every reason

(see below) to think that these substages are passed through fairly quickly; it is more probable that they represent an early brood of the same year: the X substages corresponding to these were not found by us. Using the same approximation as was employed above for *Lernaeocera* in order to form a rough estimate of the incidence in time of these substages, it will be seen that in the last column of Table VIII A the numbers of immature forms (X and Y) together are about equal to the number of mature (Z) forms, also that the numbers of X and Y are nearly equal. Thus, it is probable that the time spent in completing the X and Y phases is nearly the same, and that each is about half that of the mature phase Z. The smallest whiting and pollack infected in inshore waters, from which these data were mostly collected, were respectively 11 and 9·6 cm. in length, and the smallest infected with a mature egg-bearing *Clavella* were 11·5 and 14·0 cm. Accordingly, at least in the whiting, the X to Z substages are passed through within a few weeks, perhaps about a month at a rough approximation. From Table VIII A it thus seems that reproduction takes place throughout the year as it apparently does also in *Lernaeocera*.

In Table VIII B it is seen that the number of mature egg-bearing females increases with the length group of the fish, i.e. it is in accordance with the probability of infection increasing with the time of exposure of the host under favourable conditions for infection. (It was assumed that estuarine conditions were not so favourable, hence the decreasing infection rate with age of fish—vide Table VII A.) Further, the rapid increase in the numbers of Z forms may be due to the longer persistence of this stage, causing, in effect, a 'piling up' of this final stage on the older fish.

Multiple Infections

The increasing percentage of multiple infections with the length group of the fish from the sea (Table VIII B) clearly demonstrates that there is no immunity to superinfection developed by the fish. In half of the multiple infections the parasites were of markedly dissimilar ages (see note 3, Table VIII). In the third-year whiting caught offshore it was common to find a large number of parasites of graded ages, and in some of them there were no

TABLE IX. DISTRIBUTION OF *CLAVELLA UNCINATA* ON *GADUS MERLANGUS* AND *G. POLLACHIUS*

Intensity of Infection per Fish from the Tamar Estuary, 1936 and 1937 (P.H.T.H.)

All of the 33 fish infected were less than 15 cm. long, the smallest infected fish was 8·8 cm.

No. of parasites per fish:	1	2	3	4	
No. of fish infected:	26	4	2	1	Multiple infections: 7/33 or 21·2 %.

mature Z forms; so that these successive stages must represent superinfection from other fish. It is quite probable that autoinfection occurs on one host, for spent females have also been found in company with very young X forms.

The state of maturity of the parasites found on the gadoids from the Tamar

estuary was not determined, but data were collected regarding multiple infection and are given in Table IX. Since all the infected fish from this habitat were of the first length group dealt with from the sea, they are comparable and, indeed, they do show a similar rate of superinfection (21 % as compared with 18 %).

Direct Effects on the Host resulting from Infection by Clavella

As with *Lernaeocera*, the values for the condition factor, *K*, of the hosts have been determined for fish infected with *Clavella*, but as they show no correlation whatever with infection, they are not reproduced here. Similarly, there is no consistent variation in the arithmetic mean lengths of monthly groups of fish infected and not infected with *Clavella* (see Tables I, II); so as far as our data go, it may be said that *Clavella* has no appreciable effect on the host.

Clavella is much smaller than *Lernaeocera* and it seldom occurs in very large numbers; moreover, it is probably mainly a mucus feeder, though it may also feed on detritus and on gill tissue. In the pollack its habitat is distinctive, and in all specimens except one the parasites were found on the tips of the gill filaments, and usually on the first gill of each side (though sometimes also on the second gill), and situated towards one or the other end of the gill rather than in the middle region of the gill arch. When full-grown parasites were found on the pollack the adjacent four or five gill filaments, both on the gill to which it was attached and on the next gill, were shorter and appeared to have been browsed down to the extent of one-fifth or one-sixth of the length of the normal filaments. These browsed gill filaments did not, however, show a marked anaemia, though they were usually adherent, suggesting a hypersecretion of mucus resulting from the irritation and feeding of the parasites. Dawes (1940) has made a much-needed contribution to our knowledge regarding the histological effect of trematode gill parasites on their hosts, and though no such detailed investigations have been made in the present study, it seems quite possible that the effects are comparable. Poulsen (1939, p. 241, Fig. viii) gives a figure of an eroded fin of a cod caused by the browsing of *Clavella*: this shows a whitish edge which is thickened on the eroded margin. He considers that the effects on the gills would cause a more vital injury, and that in both instances the mechanical presence of the parasite would affect the movements or reduce the efficiency of the respiratory current in the branchial cavity. Hence, he suggests that *Clavella* may have a distinct detrimental influence on the growing stock, since at least in Danish waters the incidence of infection is fairly high. He has also determined the condition factors for infected and uninfected fish, but his results do not show any significant correlation. His explanation is that those fish taken to be uninfected have probably been infected in the recent past, and their lowered condition factor would produce a spuriously low mean value among the 'uninfected' fish.

The *Clavella* found on the whiting were never attached to the gill filaments:

on the smaller fish they were invariably found attached to the gill rakers on the first gill and more rarely on those of the second: they are actually more common on the small spinose bosses on the inner margin of the gill arch, though they are also found quite frequently on the long rakers on the outer margin of the first gill. In the older whiting, however, it was not unusual to find *Clavella* also attached to the roof and sides of the buccal cavity and sometimes on the tongue, but they were rarely found on the inner face of the operculum and never (as they are on the cod) on the outer surface of the body. Though there is no specific difference between the *Clavella* infecting the two common gadoids at Plymouth (Gurney, 1934) there is a distinct influence exerted in some way by each kind of host which determines the characteristic habitat taken up by the infective copepodid of the *Clavella*.

It may be added that the whiting may be expected to suffer much less from the presence of this parasite than the other gadoids, for the situation of the parasite is usually well removed from soft tissues and, on this host, it must be largely a detritus feeder.

The Genus *Lepeophtheirus* (Fam. Caligidae)

Like all other caligids, the species of *Lepeophtheirus* have a single host in their life cycle; in this they resemble *Clavella*, but unlike the latter they are not permanently attached to the host. As a group they are among some of the least modified of parasitic copepods. They are primarily marine, and owing to their thin cuticle, which may be subservient alike to excretion and gaseous exchange, they are unable to withstand marked or rapid changes in salinity of the external medium. Their sensitivity to reduced salinity when the host enters more or less brackish waters is illustrated by the distribution of *L. pollachii* and *L. pectoralis* at Plymouth.

Gurney (1933, p. 334) quotes Hutton's observations on *L. salmonis*. Hutton has found the parasite 85 miles from the sea and has shown that it can withstand fluviatile conditions for two weeks or more; but this is exceptional, and the fact that the egg strings very soon break away from the females denotes that conditions are certainly not suitable for breeding. The females are said to be less viable than the males. Gurney endorses the popular belief that their presence on a fish is strong evidence that it is freshly run from the sea. The present records do not include this species.

L. pollachii may have been overlooked in the samples taken from the Tamar estuary as no special search was made for it; but it was occasionally taken during 1939 and 1940 from the seaward end of the estuaries and from Plymouth Sound, as well as from offshore habitats, so that it is capable of withstanding a slight dilution of the external medium. Details of its occurrence are given in Table II C.

Perhaps the chief factor in limiting its distribution to the larger fish is its rigorous preference for a peculiar habitat: it is invariably found attached to

the under-side of the tongue of pollack, though sometimes it occurs in other positions in the sublingual space after the death of the host. It has never been found on the operculum or outer surface of the body, and never on any other gadoid. Chalimus stages have not been found. Its absence from the smaller fish is therefore explained by the habitat being too small to accommodate it. There is no obvious seasonal variation in numbers, but our collection was too small to study this species from the point of view of breeding phases.

L. pectoralis is usually considered a very abundant parasite on pleuronectids, and unlike the foregoing species shows no marked preference for a particular species of host. Its habitat is not quite so constant as that species, though it is usually found in the neighbourhood of either of the pectoral fins. That it is even more sensitive to dilution of the external medium is shown by its absence at all times from *Pleuronectes flesus* and *P. platessa* caught at the seaward end of the estuaries at Plymouth, although it was found occasionally on these fish caught in the Sound. Over 2500 flounders and a considerable number of plaice from the Tamar estuary were examined by one of us (P.H.T.H.) during 1936 and 1937, but not a single specimen of *Lepeophtheirus pectoralis* was found, though a careful search was made for them. A single (20 cm. long) specimen of *Pleuronectes flesus* caught in the Sound harboured this species. The incidence of infection of *P. platessa* in the Sound, as estimated from casual observations, seems to be lower than that usually met with in offshore waters. The low percentage of infection in our records is probably due to the majority of our fish being caught at the seaward end of the estuaries; these fish were young (0–i year class).

TABLE X. INCIDENCE OF *LEPEOPHTHEIRUS PECTORALIS* ON *PLEURONECTES PLATESSA*: 'INSHORE'

Year class	0	i	ii	iii	iv	Total
Length range (cm.)	Up to 9	9–18	18–24	24–30	Over 30	
No. of fish examined	2	26	11	6	9	54
Length of infected fish	—	12·5	—	24·0	34·0, 38·0	4 fish
Month of capture	—	Jan.	—	Aug.	Oct. and Feb.	7·4 %

NOTES ON THE DISTRIBUTION OF *LERNAEENICUS* SPP. ON THE SPRAT

This genus of parasites belongs to the same family as *Lernaeocera* and may have similar powers of toleration to brackish water. No sprats have been examined from the sea during the present investigations; but the following records, made during the study of the Saltash tuck-net fishery of the Tamar estuary (Hartley, 1940, p. 42), are included here for comparison with the related *Lernaeocera* on gadoids from the same habitat. As was found with *Lernaeocera*, the several species of *Lernaeenicus* have characteristic habitats which are adhered to rigorously: *Lernaeenicus sprattae* is always found on the eye and *L. encrasicola* is embedded into the body musculature, the head

sometimes reaching through to the coelom. The distribution of infected specimens of *Clupea sprattus* with the two species of *Lernaeenicus* is summarized in Table XI. The incidence of infection is rather low, and about a third of the infected sprats probably belonged to the stock of the previous year. This may be another instance of the lingering inshore of fish infected with parasites of this family. It is also interesting to note that the 8·0 cm. fish caught in September 1936 had a triple infection of *Lernaeenicus sprattae* on one eye. In this host, therefore, as in gadoids, there is no immunity developed.

TABLE XI. INFECTION OF *CLUPEA SPRATTUS* WITH *LERNAEENICUS* IN THE TAMAR ESTUARY, 1936 and 1937 (P. H. T. H.)

Year	No. of fish examined	With *L. sprattae*		With *L. encrasicola*	
		No.	%	No.	%
1936	477	5	1·12	1	0·21
1937	899	4	0·445	2	0·22
Total	1376	9	0·655	3	0·22

Lengths of fish infected with Lernaeenicus

Date	A.M.L. for sample	Lengths of infected fish: With *L. sprattae*	With *L. encrasicola*
1936			
23 May	8·78	10·1	8·4
19 Aug.	4·88	5·4	—
18 Sept.	4·82	6·8; 8·9; 8·0	—
2 Oct.	7·25	7·5	—
1937			
21 Jan.	5·21	—	6·6
1 Feb.	4·92	5·1	—
April	5·33	5·8	5·5
8 May	6·02	5·7	—
27 July	3·63	3·6	—

Neither *L. sprattae* nor *L. encrasicola* is very common round our coasts.[1] Scott & Scott (1913, pp. 156–9) mention a catch of 600 sprats from the shrimping grounds off Blackpool which yielded a 2·3 % infection with *L. sprattae*. Leigh-Sharpe (1935, pp. 270–5, Figs. 1–7) redescribes and figures these two parasites from material obtained from the Tamar estuary in the spring of 1934; he obtained 29 from 960 fish (2·9 %), and he states that Dr Gurney examined a catch of over a thousand fish from the same locality at about the same time and obtained a similar percentage of infection. He does not record any multiple infections, though among Scott's material one fish had three on one eye.

[1] Mr Ford recalls examining large catches of sprats at Plymouth among which some specimens had black holes in the eye, which he took to indicate the sites of previous infection with this parasite. Unfortunately these specimens were destroyed by enemy action, but the possibility remains that the real rate of infection of sprats is higher than the apparent rate given above.

L. encrasicola is even less common. A. Scott (1907, p. 94) examined some hundreds of sprats caught off Blackpool in February 1906 and only found a single infected fish, and Leigh-Sharpe found only one among the 960 fish from the Tamar; the 1936 and 1937 catches from the same locality yielded a slightly higher percentage (0·2 %). We have no data as to the condition of the infected sprats, but observers state that *L. sprattae* causes blindness, which would indirectly lead to malnutrition and possible death; both species would cause a certain amount of debility through loss of tissue fluids on which they feed. These combined effects may explain the rarity of these two parasites.

Summary and conclusions

The migratory habits of *Gadus merlangus* and *G. pollachius* are examined in relation to the variations in the rate of infection of these fish with *Lernaeocera branchialis*. A sudden increase in infection in estuarine and inshore waters is found to coincide with the offshore migration of the main stock. The infected fish apparently linger behind and may live for some time in the old habitats after the arrival of the next recruitment. The offshore migrants are seldom infected, and it is probable that infection does not take place in water deeper than about 5 fathoms.

The possible immediate causes of this 'lingering' phenomenon are examined, and two suggestions are made as to the nature of the physiological upset brought about by the presence of *L. branchialis* on these gadoids. Other factors influencing the distribution of the parasite are discussed.

The relative susceptibility of the different species of gadoids to infection with species of *Lernaeocera* is examined as far as the data allow.

The duration and distribution of the developmental phases on the intermediate pleuronectid and final gadoid hosts are estimated: the former being probably not more than two weeks, and the latter about six to eight weeks. Corroborative evidence for the length of the gadoid phase is obtained from certain epizootic organisms. The production of a vast number of eggs and their protracted shedding result in a scattered series of larval forms at all times of the year on the gills of flounders caught at the seaward end of the estuaries. There is likewise a scattered series of adult substages on gadoids. The numerical abundance of each phase is taken as being proportional to the length of time spent in each phase. There may be as many as five or six consecutive generations a year, but these generations show an infinite degree of overlap. The infection of the 0 year-class fish after their few weeks' sojourn in inshore and estuarine waters is correlated with the large numbers of larval forms found on flounders in this neighbourhood.

The lingering of parasitized gadoids, in effect, ensures the reinfection of the flounders: the ripe eggs from the female *Lernaeocera* on the gadoid are thus shed in shallow water where the conditions are optimum for the early development and for the minute free-swimming copepodids and their sub-

sequent infection of the flounders on the gills of which they begin their metamorphosis. The coherence of these facts, and the rarity of infected gadoids in offshore habitats, suggest that *L. branchialis* is, *par excellence*, an inshore parasite. On the other hand, the relatively frequent occurrence of *Gadus luscus* infected with *Lernaeocera lusci* in offshore waters suggests that infection takes place there and that the intermediate host is a pleuronectid more adapted to deeper waters than *Pleuronectes flesus*. That this intermediate host can also live inshore is evidenced by the occurrence of occasional infected *Gadus luscus* among the young stock in the estuary during 1936–7.

Multiple infection with *Lernaeocera* of different ages, both on the flounder and on the gadoids, indicates that these fish do not develop an immunity to reinfection, and it may even be possible that infection and the consequent prolonging of inshore and estuarine life may favour reinfection. The distribution of the adult substages on *Gadus merlangus* and on *G. pollachius* is very similar in all respects.

The distribution of *Clavella uncinata* has been similarly studied on the two common gadoids from estuarine, inshore and offshore waters near Plymouth. *Clavella*, unlike *Lernaeocera*, is confined to a single host throughout its life cycle; it is therefore not subject to the same restrictions regarding breeding habits as the latter parasite, and successful egg production can take place at any time during the life of the host: it is independent of the migratory habits in this respect, though other limiting factors appear to be operative. As with *Lernaeocera*, infection is first recorded in the 0 year-class fish a few weeks after their inshore migration. In strictly inshore waters (Plymouth Sound) the rate of infection continues to increase with the age of the whiting and pollack, but in estuarine waters nearby the infection is at first moderate but later (after the period of maximum growth of the fish) tends to fall off: this may be an expression of inclement conditions for the infective stages, such as the reduced salinity of this habitat during the autumn and winter. The effect of reduced salinity on *Clavella* parasitizing the cod in Danish waters is also discussed. *Clavella* appears to be about equally common on the cod, whiting and pollack, but it is rare on *Gadus minutus* and it has not been found by us on *G. luscus*. The duration and distribution of the later developmental substages are estimated in the same way as for *Lernaeocera*, and it is concluded that all the adult substages are completed in a few weeks, and that the complete cycle is perhaps not longer than two months. Reproduction takes place throughout the year, though since conditions are more favourable at certain times the main recruitment to the *Clavella* stock takes place in the autumn in the sea near Plymouth. Multiple infections were also common with this parasite so that it induces no immunity in its host.

The direct effect on the host is discussed; as with *Lernaeocera*, there is no discernible difference in the condition factors of infected and uninfected fish. The difference in the habitat and food of *Clavella* on three species of gadoids is indicated.

The species of *Lepeophtheirus*, like other caligids, are similar in body form and organization to the first stages of *Clavella* and *Lernaeocera*, and owing to their minute size and probable lack of osmoregulatory powers are unable to withstand salinity changes of any magnitude in the external medium as, for instance, when the host migrates into brackish waters. *L. salmonis* is more resistent than most caligids. *L. pollachii* has not been found in fish from estuarine waters and only occasionally in those caught inshore, but it is fairly common in fish caught out at sea. Its occurrence is primarily limited, however, by mechanical factors, i.e. the size of the sublingual space of the pollack, which is its invariable habitat. *L. pectoralis* is apparently most highly sensitive to reduced salinity, for it is strictly marine in occurrence: though quite adequate samples of *Pleuronectes flesus* were examined from estuaries no infected fish were found.

Data on the distribution of *Lernaeenicus sprattae* and *L. encrasicola* from *Clupea sprattus* in the estuary are included for comparison with *Lernaeocera*; though this is a consistently rare parasite, the records show that older infected fish tend to linger with the newly recruited estuarine stock of the following season. The effect on the host is discussed.

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OBSERVATIONS ON THE BIONOMICS AND PHYSIOLOGY OF *TREBIUS CAUDATUS* AND *LERNAEOCERA BRANCHIALIS* (COPEPODA)

By N. G. Sproston[1] and P. H. T. Hartley

From the Plymouth Laboratory

(Text-figs. 1–5)

CONTENTS

The Habitat and Distribution of *Trebius caudatus*

Trebius caudatus Krøyer is a parasitic copepod belonging to the family Caligidae: a remarkably uniform group which is among the least modified by the parasitic habit. The body form is similar to that of *Caligus* and *Lepeophtheirus*, but unlike the former it lacks the pair of frontal suckers, and unlike both of these common genera the fourth legs are biramous in both sexes. The most obvious adaptation to the parasitic habit in this family is the roundish flattened carapace, which has a sucker-like action and prevents the animal from being washed off the surface of the fish on which it lives. This action is easily seen when caligids are transferred to a glass vessel: if an attempt is made to remove them, the previously swimming animal comes to rest and applies its carapace to the smooth surface with surprising tenacity. Another adaptation is the presence of strongly chitinized hooks on various cephalothoracic appendages.

All those who have worked with caligids remark on their peculiar habit of swimming to the edge of the sea water when placed in vessels in the laboratory. If left for a time they will swim out of the water and perish by becoming dry. We have seen this in several species and in no instance did they attempt to return to the water. This peculiar taxis is difficult to explain in relation to their normal habits.

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The life cycle of all caligids is a simple one, involving only one host. For the most part the species are host-specific; but some are found on hosts of closely related genera, and these parasites are notably those which are not confined to a particular habitat on the host, being found in various locations and on both surfaces in flat fish. The strictly host-specific ones, such as *Lepeophtheirus pollachii*, found under the tongue of the pollack, are usually confined to a special habitat. Even those showing host specificity to a less degree, such as *L. pectoralis* on various pleuronectids, very rarely migrate to fish of other species in a crowded aquarium. Fish heavily infected with caligids have been brought to the laboratory from the quay packed together in baskets, and in no instance have we found parasites characteristic of one species transferred to the adjacent fish of other species, though the parasites were alive and active and conditions were moist enough for them to move from fish to fish had there been any tendency to do so. An example of an exceptional degree of freedom is *Caligus rapax*. This very common parasite is found most frequently on gadoids, but it is also found on a large variety of other teleosts and even on elasmobranchs; moreover, it is not uncommonly met with in the plankton (Lebour, 1931, p. 173). *Trebius caudatus* has an intermediate degree of freedom: it is confined to elasmobranchs and is most common on Rajidae. Scott & Scott (1913, p. 82) mention that it is found '...on skates, rays, dog-fishes, etc.', but they do not give any specific names. It appears from the records that *T. caudatus* is confined to European waters, from which it is the only species of the genus recorded. Three other species have been found in the western hemisphere, also on elasmobranchs (Wilson, 1907, 1921). We have been able to add four new hosts to the records for this parasite, and three new hosts for the Plymouth district; the complete host list is given below.

Summary of Distribution of Trebius caudatus *according to Hosts*

Raja clavata Linn.
Plymouth: Hartley, December 1934 (vide infra).
Roscoff: Sproston, June 1937, on the upper and lower surfaces of the head, and rather more numerous (young, paler forms) in the gill cavities, spiracles and a few in the mouth.

R. maculata Mont.
Plymouth: Oakley (in Leigh-Sharpe, 1933), 'on the dorsal surface'. Hartley, December 1934 (vide infra).
North Sea area: cited by Lint & Stekhoven (1936).

R. microcellata Mont.
Plymouth: Hartley, December 1934 (vide infra).

R. blanda Holt & Calderwood.
Plymouth: Hartley, December 1934 (vide infra).
Irish Sea: Great Orme to New Quay Head (Scott, 1929); Red Wharf Bay, N. Wales. Scott (1929) found seventeen females and three males on one fish.
North Sea area: cited by Lint & Stekhoven (1936).

R. fullonica Linn.
North Sea area: cited by Lint & Stekhoven (1936).

R. batis Linn.
Polperro, Cornwall: Norman (1859) (in Norman & T. Scott, 1906).
Plymouth: Bassett-Smith (1896), on the dorsal surface of the head and in the nasal cavities.
Irish Sea: Belfast Bay, W. Thompson, September 1838 (in Baird, 1850). Great Orme to New Quay Head (Scott, 1929).
North Sea area: cited by Lint & Stekhoven (1936), 'head and nasal cavities'.
R. marginata Lacepède.
Roscoff: Sproston, June 1937, three medium-sized fish all had a few of these parasites, mostly on the underside of the head. One large fish had 18 adults on the upper and lower surfaces of the head, 10 young (pale) forms in the spiracles and 5 in the nasal cavities. None was found on medium and small fish examined in September–October 1938 at the same place.
Galeus vulgaris Fleming.
Cited by Gerstaecker (in Bronn, 1866).
Acanthias vulgaris Risso.
Roscoff: Sproston, September 1938, on the gills.

Note. The nomenclature of these fish is that used in the *Plymouth Marine Fauna*, 1931.

It is unfortunate that those studying parasitic copepods have seldom investigated their ecology, so that we have few records of the exact location of these animals on their hosts or other data concerning the host-parasite relationship. MacCallum (1916, p. 23) was one of the first to stress the importance of such observations when he drew attention to the nasal cavities of elasmobranchs as favourite habitats for ectoparasites—both for copepods and monogenetic trematodes—which habitat, he says, seems to have been overlooked. For instance, it was not until 1918 that Leigh-Sharpe first discovered *Lernaeopoda globosa* in the nasal cavities of *Scylliorhinus caniculus* (L.) Blainville, 1816; this parasite has since been found very frequently at Plymouth and at Roscoff, invariably in the same situation. Considering the large numbers of dog-fish examined annually in laboratories it is remarkable that it escaped notice until so recently.

Of the species of *Raja* examined by one of us (N.G.S.) at Roscoff in 1937 and 1938, it was noticed that most of the large fish were infested with *Trebius caudatus*, while only a few of the medium-sized fish and none of the small fish had them. They occurred on both surfaces of the head near the anterior end, and when immature forms were found they were invariably in the mucus of the nostrils, gill cavities and spiracles, and on two occasions a few in the mouth near the internal aperture of the spiracle. These immature forms were always pale in colour in contrast to the rather darkly pigmented adults. They were often associated in the mucus with the filamented eggs of *Rajonchocotyle* sp., a monogenetic trematode not uncommon on the gills. Of the twenty fish examined in detail at Roscoff, four, or 20 % (large to medium fish), were infected; this is about the same degree of infection as that found at Plymouth (see below).

During December 1934 one of us (P.H.T.H.) had the opportunity of examining several trawled species of *Raja* from the Mewstone Grounds and from Cawsand Bay, Plymouth. Special attention was given to the exact location on the host of the *Trebius caudatus* found, and other ecological data were noted. The distribution of these copepods on the different hosts from the two habitats is set out in Table I, and their distribution on the host is shown in Fig. 1.

TABLE I. THE DISTRIBUTION OF *TREBIUS CAUDATUS* ON *RAJA* FROM TWO LOCALITIES NEAR PLYMOUTH IN DECEMBER 1934

	R. blanda	*R. clavata*	*R. maculata*	*R. microcellata*	All species
Cawsand Bay					
No. of fish examined	28	13	20	9	70
No. of infected fish	2	1	5	2	10
Percentage infected	7·2	7·7	25	(22)	14·3
Mewstone Grounds					
No. of fish examined	0	14	7	1	22
No. of infected fish	0	5	3	0	8
Percentage infected	—	35·7	(43)	—	36·36
Both localities					
Total fish examined:					
Number	28	27	27	10	92
Length range: cm.	25–35	5–70	10–55	20–60	5–70
Mean length: cm.	23·0	30·3	29·1	37·5	28·5
Infected fish:					
Number	2	6	8	2	18
Percentage	7·2	22·2	29·6	20·0	19·6
Mean length: cm.	20·0	52·5	42·2	52·5	44·4

In spite of the small size of the samples, it may be permissible to point out certain items of interest in these observations at Plymouth in 1934.

The *Raja* caught on the Mewstone Grounds are between two and three times more heavily infected than those caught at the same time in Cawsand Bay. The depths of water in these two localities are respectively 10–15 and 3–5 fathoms, so that other things being equal, deep water appears to be more favourable to *Trebius caudatus* than shallow. One possible factor is that the parasite is less disturbed by currents in deeper waters. The fish themselves move but little, so that we feel justified in regarding the two habitats separately (see Steven, 1936).

On the whole, the larger the fish the more likely is it to be infected. No fish below 10 cm. long was found infected. The length distribution in three arbitrary groups in relation to the infection rates is given below (Table II). The arithmetic mean length of all the fish examined in 1934 was 28·5 cm. and that of the infected fish was 44·4 cm.

TABLE II. LENGTH DISTRIBUTION OF *RAJA* SPP.

Length group cm.	No. of fish examined	Infected fish	
		No.	%
5–25	48	2	4·15
25–50	34	11	29·5
50–70	9	6	66·6

The mean rate of infection of the species of *Raja* both at Roscoff and at Plymouth was about 20 %. The mean intensity of infection per fish at Plymouth was only 1·45 parasites per fish, but at Roscoff it was 6·0. The latter figure includes only the adult forms on the skin; the immature forms from the head cavities were not included. Unfortunately, these cavities and their contained mucus were not examined in the Plymouth samples, and no immature forms were found.

At Plymouth both sexes of fish were about equally parasitized: ten females and eight males.

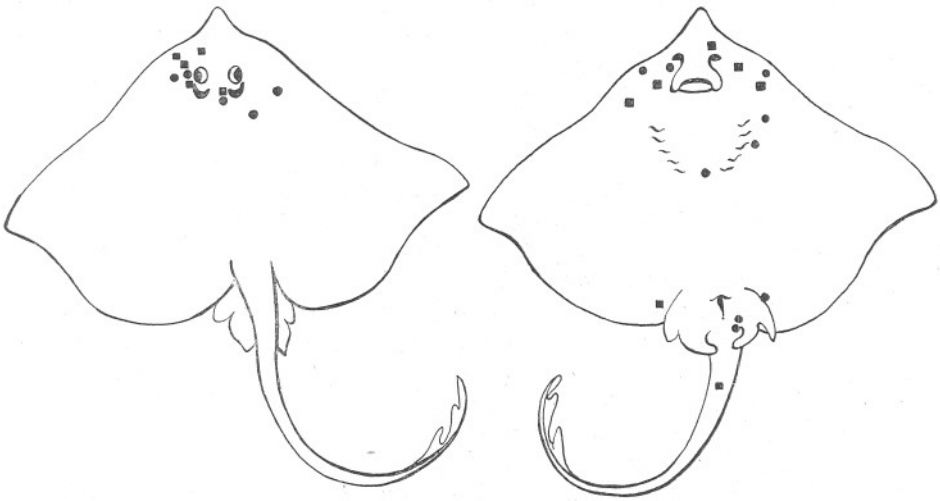

Fig. 1. Generalized diagram of *Raja* showing the location of each individual *Trebius caudatus* found in Dec. 1934. The black disks indicate males and the black squares females.

From Fig. 1 it is seen that the parasites tend to collect near the orifices on both surfaces of the body—ten on the upper side and sixteen on the under side—and that this distribution is also that of greatest concentration of mucus glands on the skin. It is understandable that this secretion would attract the parasites, for it is a necessary lubricant on so rough a surface and also is believed to form a large part of their food.

Of the *Trebius caudatus* collected at Plymouth, twelve were females and fourteen were males; the Roscoff records are not quite complete, but the proportion of the sexes was nearly equal, with a slight preponderance of females among the adults. This nearly equal distribution of the sexes is most unusual in caligids, in which the males are as a rule far less common than the females.

Intestinal Respiration in *Trebius caudatus*

The cuticle of free-living copepods such as *Calanus* and *Cyclops* is very thin, and it is generally assumed to permit gaseous exchange, not only through the thin appendages but also over the whole surface of the body. In the

larger copepods, however, the specific surface (i.e. surface per unit volume) decreases significantly and the cuticle becomes thicker to withstand the stress of stronger musculature, so that superficial respiration alone is evidently inadequate.

The term 'anal respiration' is generally applied to that phenomenon in various invertebrates whereby a respiratory current is taken in by the anus. In Copepoda it includes peristalsis of the gut (which is only partly concerned with respiration), various movements of the hind body as a whole and the actual gaseous exchange through the gut wall; at the same time these movements are transmitted to adjacent organs and the body fluid between them, so that they serve as the motive power for a two-way circulation. We prefer to use the term 'intestinal respiration', because it does not exclude the homologous phenomena in those parasitic copepods in which there is no anal current.

Various authors have described rhythmic gut movements (peristalsis and 'anti-peristalsis') in copepods, and in those lacking a heart such movements have been considered as supplying the motive power for general circulation of the body fluids. Claus was strongly opposed to Weismann's interpretation of these movements as respiratory in function, in spite of numerous authors' observations of a current of water entering and leaving by way of the anus, in copepods, and in other crustacea of the same order of size. According to Hartog (1888, p. 26), Claus's chief difficulty in accepting anal respiration as a normal function lay in his conception of the function of the rectal dilator muscles as solely concerned in defaecation. Hartog, in his monographic account of the morphology of *Cyclops* (1888), shows that it is these muscles which are primarily concerned in admitting and ejecting the respiratory current through the anus. He shows that their action, in series with those of the higher reaches of the gut on the one hand, and that of the circulatory disposed muscle fibres in the walls on the other, have the combined action necessary to effect anal respiration. The events are thus described by Hartog (p. 25): the anus is dilated when the gut is stretched to its maximum extent, which allows free ingress of water, the anal valves then close and the rectum is pulled forward: the stomach meanwhile begins to expand and becomes strongly arched so that the anterior region of the gut assumes a marked sigmoid curve in the antero-dorsal direction, which causes a forward movement of the water recently taken in. With the immediate flattening of the sigmoid curve the stream of water is reversed and flows again into the hind gut as this, in turn, straightens out, and makes its exit as soon as the anal valves open. A fresh stream of water enters at the recommencement of the cycle, just as the rectum is being drawn forward and immediately before the anal valves close.

Probably the fine teeth or coarse setae described by Hartog (1888, p. 24, Pl. 3, fig. 2) as occurring on each anal valve, serve to filter the respiratory current, though he does not offer this as an explanation. Apparently the valves

in the intestine (also figured and described by Hartog) are too fine to allow the passage of food with the current, but he states (p. 26) that the rhythmic cycle is interrupted when a bolus of faeces collects in the rectum, but immediately on its expulsion movements are resumed with an extraordinary vigour.

This author claims to have observed a similar regular rhythmic anal respiratory current in three groups of free-living copepods and also in *Caligus* and in *Argulus* (as well as in cladocerans, gammarids, *Asellus*, larvae of *Apus* and *Zoea*). Wilson (1905, pp. 516–17) states that he has observed movements associated with anal respiration in *Lepeophtheirus edwardsi*, *Caligus rapax* and *C. bonito*, and that it was '. . . exactly like that described by Hartog for *Cyclops*, save that it was not as regular'. He does not, however, give any details of his observations. Scott (1901, p. 20) describes an intermittent two-way peristalsis in *Lepeophtheirus pectoralis* and a concomitant jerky two-way circulation of the blood; but though he describes anal dilator muscles in this species he remains sceptical of anal respiration. This scepticism is maintained by Scott & Scott (1913, p. 14).

We have both observed this phenomenon in various species of caligids from time to time, and we find that the underlying mechanism seems to be substantially the same as that described by Hartog for *Cyclops*, the action of the longitudinal muscles attached to the gut working in harmonic sequence, superimposed on the peristaltic movements of the gut itself. Here, however, the agreement ceases. Some years ago, one of us (P.H.T.H.), working at Plymouth, made an attempt to investigate the rhythmic nature of respiration in *Trebius caudatus*. It appeared far more complicated than that described by Hartog, and at first sight it seemed that the welter of pulsations was entirely arhythmic. The irregularity was apparent not only in numerous individuals on different occasions but in the same individual when examined over periods of 6–9 minutes. The main results of these observations on *Trebius* are summarized below:

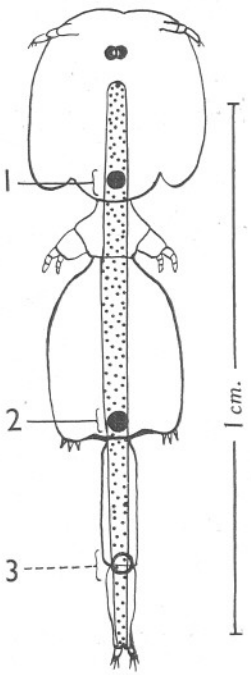

Fig. 2. *Trebius caudatus* (female) (diagrammatic), showing gut and the three centres controlling peristalsis.

(1) Peristaltic waves proceed along the entire length of the gut in both the forward and backward direction.

(2) In regard to these movements the three sections of the gut, the cephalothoracic, that in the genital segment and that in the abdomen, appear more or less isolated, and each has its own set of peristalses. These may, or may not, be transmitted to the adjacent segments.

(3) The peristaltic waves appear to be initiated from three centres which are situated towards the posterior end of each gut section: these are shown dia-

grammatically in Fig. 2. The exact situation of the abdominal centre was difficult to determine and its approximate position is indicated by an open circle.

(4) Each section of the gut is able to initiate contractions in either direction, but at times a wave of contraction started in one section may be continued into another. Forward peristalses in the abdominal gut, the only type of continuation observed with any frequency, were often continued into the genital gut. Backward peristalses of the genital gut were twice seen to be continued into the abdominal section. In one set of observations only, backward peristalses in the cephalothorax were seen to be continued into the genital section on three occasions during 6 minutes.

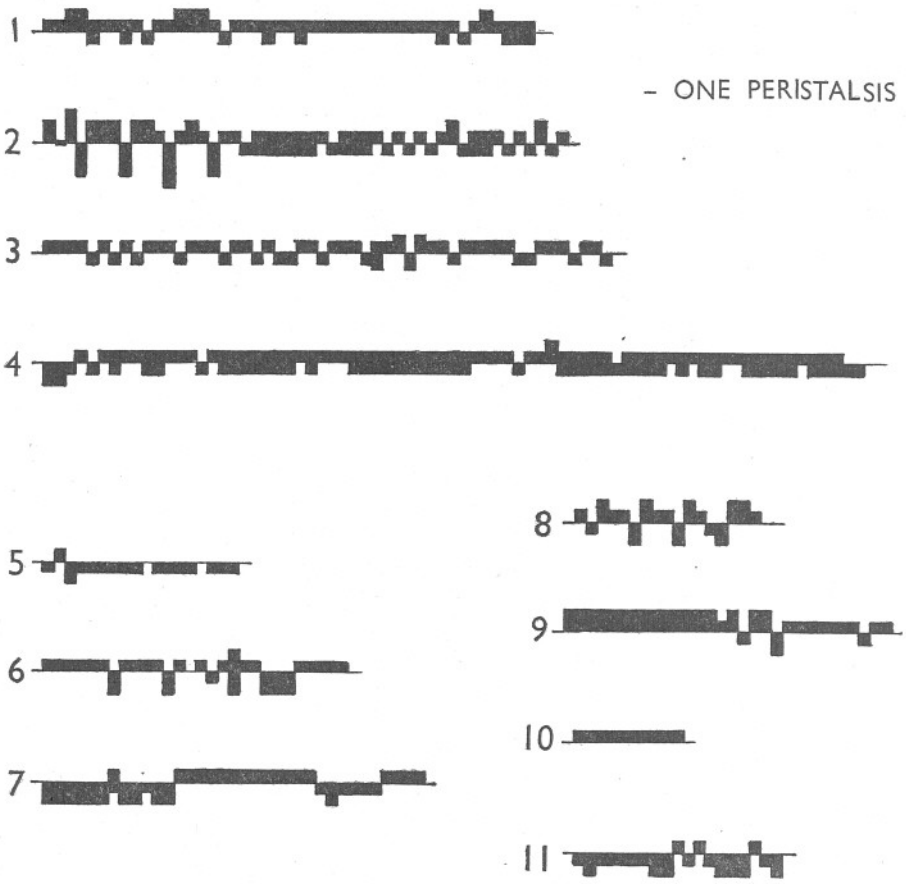

Fig. 3. Graphical representation of the direction and relative intensity of the peristalses in *Trebius*: 1–4 from the cephalothoracic section of the gut, 5–7 from the genital segment, and 8–11 from the abdominal gut. The records are of selected typical examples, and the blocks above and below the central line represent forward and backward peristalses respectively.

(5) Peristaltic contractions were more numerous per unit time in the cephalothoracic than in the other two sections.

(6) Simultaneous peristalses in both directions were characteristic of the cephalothoracic section. They were very infrequent in the genital section (1·5 % of the total observations only), and unknown in the abdominal section.

(7) The duration of peristalsis in either direction was extremely variable, as was the intensity (strength of beat) and the frequency of the waves of contraction.

(8) It is beyond doubt, however, that forward peristalses were the more numerous.

Fig. 3 illustrates the change of direction and the relative intensity of some selected typical series of peristalses in each section of the gut. The value of the time axis is not given. These particular examples show extreme irregularity in the maintenance of peristalsis in a given direction; the relative intensity of the beats is also apparently capricious. Table III gives the means of the frequencies of peristalses in the complete series of observations.

TABLE III. THE FREQUENCY OF PERISTALSES IN *TREBIUS CAUDATUS* (PER MIN.)

	Total peristalses			Backward peristalses only		
	Cephalo-thorax	Genital segment	Abdomen	Cephalo-thorax	Genital segment	Abdomen
	6·85	1·40	5·00	1·57	0·80	0·5
	4·36	0·60	1·28	0·535	0·20	0
	4·84	1·00	1·66	0·526	1·00	0
	11·00	3·86	9·5	3·67	1·17	0
	12·00	1·50	0·50	2·33	3·00	0
	9·00	5·83	3·16	7·33	0	2·67
	12·16	2·33	—	4·00	—	—
	5·00	7·00	—	8·83	—	—
Arithmetic means	8·15	2·94	3·52	3·60	1·03	0·53
Means of forward peristalses				4·55	1·91	2·99
Ratio of forward/total peristalses (%)				55·8	65·0	85·0

These observations were made under approximately similar laboratory conditions, and though the temperature factor was not controlled (albeit highly important), the high degree of variation observed during the short periods of observation cannot be due to this variable: it is probably a normal attribute of the process. Owing to this high degree of scatter, the number of observations is insufficient for the results to be treated statistically, so the arithmetic means alone have been reproduced in the table.

It is instructive to note that the frequencies of the forward peristalses expressed as percentages of the frequencies of the total peristalses (when the means are employed) increase rapidly from anterior to posterior ends of the gut. This—the increasing residuum of forward over backward peristalses, from head to tail—is one of the most significant features of that type of intestinal respiration which involves an anal current, for it ensures that the

contents of the gut are retained. It would seem that there is a necessary fall in the pressure gradient along the gut towards the anterior end, and that this prevents too great a movement in the middle region of the intestine where digestion is believed to take place; it would also reduce the chances of regurgitation through the mouth to a minimum. Much of the irregularity of the gut movements may be due to the impedance caused by the presence of more or less bulky food and faecal matter.

It was not possible at the time to make simultaneous observations on the relation of the peristaltic movements as a whole to the actual intake of water by the anus. A gaping of the anal lips followed by a closure strongly suggestive of the intake of water was seen repeatedly in *Trebius*; and in *Paracalanus* sp. the uptake of beads of water was seen distinctly on several occasions. In *Trebius* there was also a definite rubbing together of the anal lips: they showed, immediately after the intake of water, a differential up and down movement—along the antero-posterior axis—as if one side contracted and then the other. Lastly, it may be mentioned that the intake of water was always accompanied by a brisk wagging of the abdomen as a whole, a movement so violent that often it affected the whole body. This up and down beating of the hind body is especially characteristic of the chalimus stages of parasitic copepods, and is always very obvious in the 'cyclopoid' and 'pupal' stages of *Lernaeocera branchialis* on the gills of the flounder: this species is dealt with below.

The highest frequency of total peristaltic beats occurs in the cephalothorax (Table III): this may well have a digestive rather than a respiratory significance, for simultaneous amphidirectional peristalsis was the general rule in this region, and would help the trituration process in the stomach. The amphidirectional peristalsis took place by a wave of contraction starting forwards from centre 1 (Fig. 2) which was balanced by a posterior wave starting, apparently at the same time, from the anterior limit of the gut: it had, in fact, the appearance of being reflected from the anterior wall. The observed effect was a regular wave, the wave-length of which was equal to the length of this section of the gut. If another initiation centre is present at the anterior limit of the gut, it is necessarily only concerned with backward peristalses.

The subdivision of the gut into three sections is a further aid to the retention of food in the respective regions for a sufficient time for digestion to be completed. Scott (1901, p. 16) has shown that there are no valves separating the sections, but Wilson (1905, p. 510) points out that there is a constriction at the junctions of these regions '. . .like the beginning of a sphincter muscle, but the opening cannot be closed'. Hartog has, however, demonstrated valves in *Cyclops*. Another safeguard against the premature passage of food from the stomach is the normal discontinuance of the cephalothoracic peristaltic waves into the genital segment. The rare instances in which this was seen may mark the passage of food through the sphincter-like constriction, and so be part of normal alimentation.

The continuation forwards of peristaltic waves from the abdominal into the genital gut, on the other hand, is part of the respiratory movements and is necessarily the rule in this section of the gut: handing on, as it were, the respiratory current from the rectum to the intestine.

The vigorous action of the longitudinal muscles moving the water current from stage to stage along the gut is doubtless the cause of the movements of the hind body as a whole. This is naturally more conspicuous in so light an animal as a chalimus, suspended as it is by its frontal filament: the muscular contractions cause it to swing through a considerable arc. The peristaltic movements serve to stir the water (oxygen vector) in each section of the gut and so effect maximum efficiency in the gaseous exchange through the walls. The generalized peristaltic movements are transmitted to the blood in the surrounding haemocoele and account for the discontinuity in blood circulation already referred to, and observed by Scott (1901, p. 20) and Wilson (1905, p. 515) as well as by many earlier authors.

The respiratory movements in caligid copepods show, therefore, an irregular regularity.

The Relation of the Internal and External Environment to the Feeding of *Lernaeocera branchialis*

Lernaeocera branchialis (Linn.) presents a complete contrast to the caligids in almost every respect: it is one of the most highly modified of the parasitic copepods. The adult female is permanently fixed to a gadoid with its head in the vascular tissue in the region of the heart; its food is almost entirely the blood of the host; its cuticle is relatively highly chitinized, so that it is more independent of its environment; and its life history is complicated by the interpolation of an intermediate host which the male never leaves. The first copepodid stage is free swimming as in *Trebius* and other caligids, and in general body form it is not unlike them. This stage finally settles on the gills of a flounder (*Pleuronectes flesus*) where it undergoes part of its metamorphosis (see Sproston, 1941), resulting in a free swimming cyclopoid male, and a female which is similar except for an elongated genital segment. Temporary attachment of these latter forms is effected by the chelate second antennae. After pairing, the male may return to the gill filaments, though it does not live for long; but the female becomes pelagic and seeks certain gadoid fish on which the metamorphosis is completed.

The following observations on *Lernaeocera branchialis* were undertaken by one of us (N. G. S.) at Plymouth during the period August 1939 to November 1940. It was mentioned above that respiratory movements were observed in the developmental forms on the flounder, and was accompanied by vigorous motion of the body as a whole which was visible to the naked eye. Detailed observations were only made on the adult female; although peristalsis in the thinner part of the hypertrophied genital segment could be seen while the

parasite was in situ, no details could be made out until it was excised and put in a strong transmitted light under a low power microscope. The parasites were accordingly carefully dissected away from the tissues of the host and placed for a time in circulating sea water, in which they remained apparently healthy for periods up to 9 days. The rate and vigour of the peristalsis did not appear to have diminished and it was assumed that the animal had not suffered any marked derangement in its functions by removal from the host. Important consequences of this, however, would include the gradual starvation and changes in osmotic equilibrium consequent on the change in internal environment, viz. the gradual substitution of sea water for gadoid blood in the intestine. These suppositions are supported by direct measurements of the osmotic pressure of the body fluid of the adult female *Lernaeocera* (Panikkar & Sproston, 1941).

It was found that the adult female *Lernaeocera*, unlike most marine invertebrates, is able to maintain a body fluid hypotonic to its external medium so long as it is attached to the host. It presents a complex osmotic system by virtue of its feeding on hypotonic blood, on the one hand (that of *Gadus pollachius* having an osmotic pressure equivalent to 1·443 % NaCl), and by being bathed externally by sea water on the other, the body fluid of *Lernaeocera* having an osmotic pressure equivalent to 2·0–2·8 % NaCl. When the parasite is removed from the host, however, isotonicity is established with the external medium owing to water being taken in through the mouth in place of the blood of the host. *Lernaeocera* was nevertheless still able to tolerate dilution of the external medium up to 2·243 % NaCl, which represents a salinity probably lower than that normally tolerated by the host.

Since the above paper was written other experiments have been conducted (by N. G. S.) which show that isolated *Lernaeocera* is unable to survive 50 % sea water for much longer than an hour. On three occasions perfectly healthy *Lernaeocera* were transferred to normal sea water after removal from the host, and when normal peristalsis had been verified they were subjected to a gradual dilution of the external medium and left in approximately 50 % sea water. They all became pale and peristalsis was increasingly disturbed and weakened, finally ceasing altogether; by this time the blood in the gut had been lost through the mouth (the actual stream of blood seen in one specimen only). Death, as judged by cessation of peristalsis, and complete pallor occurred in about an hour in two examples and in a little longer period in a third. The physiological effect was probably that of a violent endosmosis of the now hypotonic medium into the tissues of the parasite most probably via the alimentary canal—some vacuolation was seen in the body tissues of one specimen.

Since in the natural condition on the host a marked hypotonicity is maintained by the body fluid, any intake of water by the anus is precluded; indeed, the anus was carefully observed continuously for periods up to 20 min. (the period including several changes in direction of peristalsis), but no ejection

of fluid was seen or any movement in this region which could suggest that the anus was functional. To verify this, fine particles in suspension were watched in the neighbourhood of the anus but no movements occurred suggestive of an anal current.

The abdomen of this species has been figured in section by Scott (1901, Pl. V, fig. 4), in which the anus is shown as an aperture, but neither he nor subsequent authors actually mention that it is functional.[1] From the physiological considerations elaborated above we consider it non-functional, and examinations on the living animal do not disprove this. On microscopical examination of the whole parasite, a flattened funnel-like depression is seen at the posterior extremity, but it does not perforate the cuticle, and the rectum apparently ends blindly. It is not surprising that *Lernaeocera* has become aproctous in its final adult stage, for like many blood-feeding parasites the digestion is slow but complete.

No movement of fine particles in suspension in the neighbourhood of the mouth was observed in living (isolated) *Lernaeocera*, and it was assumed that this aperture was also closed during the period of observations. Though the mouth is morphologically of the suctorial type, we do not consider that it is functional, as such, in the adult female; for suction would certainly be superfluous against the pressure of the blood stream of the fish. Furthermore, the histological changes taking place as the result of traumatic injury by the parasite, as recently shown by Stekhoven (1936), consisting of a fibrotic thickening of the ventral aorta and bulbus arteriosus, produce a stenosis of the lumen in this region and a consequent increase in the blood pressure. The difficulty for the parasite, would, it seems, be the exclusion of an inrush of blood through its mouth. In view of these considerations it is not surprising to find that the mouth is normally closed, probably by valves, and that it opens only at well-spaced intervals to admit a meal of blood which is retained in the gut for a long period. In support of this it may be mentioned that the isolated specimen which survived 9 days in circulating sea water showed only a slight fading of the original red colour of the gut at the end of this time.

The actual feeding depends, in our opinion, on the maintenance of a dynamic equilibrium of the osmotic system referred to above, and involves the following set of factors. A slow osmosis occurs through the body wall, i.e. water will pass out owing to the tendency for isotonicity to be established, and if salts enter they must do so far more slowly than water escapes. The gradual loss of water in this way would finally reduce the hydrostatic pressure in the gut of *Lernaeocera* to a limiting value which would determine the intake of a

[1] Brady (1880) quotes Krøyer's observation that on touching the body of a mature female *Lernaeocera* a jet of liquid was forcibly ejected from the hind end of the abdomen. No one has been able to confirm this observation. We suggest that the animal was inadvertently punctured and that the contents, being under pressure, would, and do, escape in the form of a jet.

fresh supply of blood through the mouth: that is, the internal pressure in the gut gives way to the blood pressure of the host. This discontinuous feeding is supported by the findings of Panikkar & Sproston (1941, Table II) on the osmotic pressure of *Lernaeocera* in situ, where a considerable variation in values was observed for the body fluid: the low values would represent a comparatively recent meal of the hypotonic blood of the host, and the higher a corresponding degree of starvation—the body fluids having been concentrated by gradual loss of water through the cuticle.

Microscopical examinations made on the contents of the gut (by withdrawing it through fine glass cannulae as described in the paper cited) while the parasite was still attached to the host, and some 2 hr. after the death of the latter, showed a few fish erythrocytes which were quite normal in appearance and had no signs of crenation or other effects of digestion. The body fluid was examined in a similar way: it was reddish yellow in colour and contained even fewer cells; these were minute stellate cells which rapidly disintegrated, and were considered to be thigmocytes (proper to the blood of Crustacea). Stekhoven has made similar investigations (1936) and he found no fish erythrocytes in the body fluid; he considers that the red colour of the latter is due to a specific respiratory pigment which may, he thinks, be a 'reconstructed' pigment from the blood of the host, dissolved in the body fluid of the copepod, and acting as an oxygen carrier. He maintains that it is distinct from haemoglobin on the grounds of different colour; but he admits that he has not submitted it to micro-spectrographic tests. It is regrettable that our material was insufficient for such tests. In our opinion the different colour (reddish yellow) of the body fluid is due to its own intrinsic yellowish colour partly masking the haemoglobin, and that this yellowish tinge is partly the result of digestion, for fat droplets were frequently seen in suspension which were deep yellow in colour. We should expect to find that the reddish tinge was due to haemoglobin in solution, this having passed through the gut wall after the breakdown of the erythrocytes and accumulated in the body fluid as the parasite grew older. The more advanced the *Lernaeocera* the deeper we found its coloration to be: those females with partly spent egg strings were deep red, and those with spent egg strings were purplish red and the abdomen almost black. When the excised parasite was placed in circulating sea water the colour faded but little, except on two occasions when the specimens died prematurely. In these specimens the gut lost the red colour on the third day and on the succeeding day the body became increasingly pale until it died: the final colour on death was a brownish yellow.

Vanden Berghe (1933) made some investigations on the alimentary canal of *Lernaeocera*. In his discussion of the subject he rightly dismisses the remarks of Hesse (1863) on the subject of feeding in *Lernaeocera* as being confused and fantastic. Hesse also 'describes' (without figures) a heart and blood vessels which Vanden Berghe very justly denies, though his further remarks to the effect that both heart and blood vessels are unknown in

Copepoda are not accurate, for a saccular heart is present in Calanidae and Pontellidae and Ed. Van Beneden demonstrated blood vessels in *Lernanthropus* (also in *Congericola* and *Hatschekia*), and proved that they contained haemoglobin in solution (see his claim to the priority for these discoveries, 1880); the complex vascular system in *Lernanthropus* was later described by Heider (1879) in his monograph on the subject, and, in American species, by Wilson (1922).

Vanden Berghe examined a large number of *Lernaeocera* from *Gadus morrhua* and *G. luscus* at Wimereux during the summers of 1931 and 1932, but his findings differ curiously from those of Scott (1901), Stekhoven, Punt (1936, 1937), and our own. He says that the red colour resides entirely in the haemocoele and that the gut of all his specimens was devoid of red colour. He examined the fluids by making an incision across one of the antlers and states that the haemocoele fluid which issued was of a bright red colour and labile; but on making a deeper incision into the body the liquid from the gut was liberated, which was viscous and yellow in colour. We can only assume that during the difficult process of dissecting out the parasite from the host tissues, some unnoticed injury occurred, perhaps to the proboscis, that the original contents of the gut escaped before the above examinations were made, and that the liquid he observed coming from the gut was merely the residuum of the original contents mixed with the tissue fluids. From the absence of all but traces of fish blood in the gut, and because he was unable to extract an anticoagulant from any part of the alimentary system, he concluded that meals are taken at well-spaced intervals and rapidly digested. We, on the other hand, while agreeing that meals are rare, consider digestion to be a very slow process for, as explained above, by the micro-dissection methods employed we had no difficulty in extracting red liquid from the gut which was certainly no more viscous than the haemocoele liquid. It is difficult to see how Vanden Berghe reconciled these conclusions with his other findings on the rapid propagation of peristaltic waves in the intestine. In our experience the peristalses were conspicuous; for as the waves of compression and rarefaction travelled along the gut its red colour showed up as a band of colour of varying intensity against a background of a less intense red, which was tinged with yellow in the younger specimens.

Intestinal Respiration in *Lernaeocera branchialis*

Observations were made on the peristaltic movements of *Lernaeocera branchialis* both from the whiting (*Gadus merlangus*) and from the pollack (*G. pollachius*); the length of continuous observations was between 20 and 40 min., but on two occasions it exceeded an hour. These two long records are reproduced in graphical form in Fig. 4 together with two others. Fig. 4 is in no way comparable with Fig. 3, for in it the abscissae represent the time in minutes during which the animal was observed and the ordinates, the number

of peristalses per minute passing through the mid-point of the genital segment; both forward and backward peristalses are recorded on either side of the abscissa passing through the origin. *a* is a recording from a freshly isolated parasite from the pollack: A–B was taken after the parasite had been in circulating sea water for only 2 hr., and C–D after the same specimen had remained in circulation for 48 hr., and it is seen that there is little difference in the type of recording. In both the backward wave was accompanied by a forward one

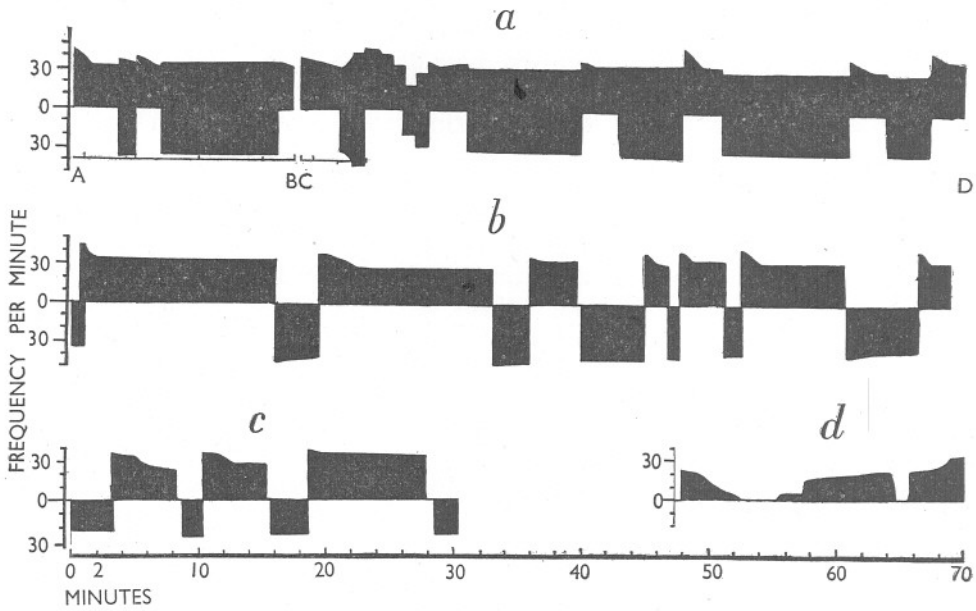

Fig. 4. Graphical records showing the frequency and direction of peristalsis in the genital segment of adult female *Lernaeocera branchialis*. Anterior and posterior peristalses shown respectively above and below the central line; frequency of waves per minute represented along ordinates and time in minutes on the abscissae. *a*, specimen from *Gadus pollachius*—A–B, 2 hr. after isolation of parasite from host; C–D, same specimen 48 hr. after isolation. *b*, specimen from *G. merlangus* 24 hr. after isolation. *c*, another specimen from same host 24 hr. after isolation. *d*, specimen from *G. pollachius* which had remained in situ on dead host for 7 days at 2° C.; recorded after specimen had been isolated and had remained in sea water for 3½ hr. at room temperature.

and these latter waves continued throughout the period of observation. This simultaneous, amphidirectional, peristalsis was rather unusual in our specimens. *b* is a record from a parasite removed from *Gadus merlangus* after it had remained in sea water for 24 hr., and *c* is one from another specimen from the same species of host on another occasion; in neither is there an amphidirectional peristalsis for more than a few beats at a time and this only occurred at the change-over from the backward to forward direction. In the change from forward to backward there was sometimes a slight pause (never more than half a minute, and usually less) at the actual change-over.

In each of these records (as in others not reproduced here) the initial forward contractions were stronger and more frequent in unit time than the subsequent ones: they started off at high speed which rapidly diminished, reaching a normal value within 1·5–2 min., and this frequency was then maintained steadily till the end of the phase. Only on two occasions did the backward peristalses start with a higher rate of contraction than the normal, and on a few they started at a lower rate which was soon brought up to normal.

As in *Trebius*, there appeared three initiation centres for the peristaltic waves in *Lernaeocera* (these were mapped out by Sproston before Hartley's observations were made known to her) and it is of great interest to note that their positions are homologous in two such widely differing copepods. Fig. 5 is a diagrammatic optical section of *L. branchialis* in which the approximate positions of these centres are indicated. Some attempt has been made to show the relative thickness of the cuticle in different regions of the body wall by the thickness of the outline: that of the antler-like outgrowths of the cephalothorax is very thick indeed, so that there is little space within them; elsewhere, particularly in the posterior two-thirds of the genital segment, there appeared a wide area of haemocoele (notably in advanced females) and at times some movement could be seen therein as a direct consequence of the peristalsis; but no movement, i.e. circulation, could be made out in the cephalothoracic region. The abdomen is also strongly chitinized and this made observations of peristalsis very difficult except in young females.

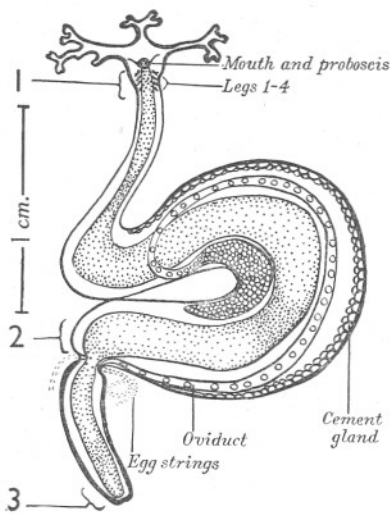

Fig. 5. *Lernaeocera branchialis* (adult female) (diagrammatic), showing the regions from which peristalses arise. These three 'centres' are homologous with those of Fig. 2.

The comparatively few observations we have on the abdominal peristalses agree in that they seldom coincide with those in the genital segment. Forward peristalses were less frequent in unit time than those in the genital segment so that they appeared out of phase, and they were never continued forwards past centre 2 into the genital segment; their frequency was fairly constant—from 16 to 20 per minute—but their intensity was feeble. Backward peristalses were also less frequent than the corresponding waves in the genital segment; they were fitful and sometimes the impulses failed to be transmitted; they appeared to originate from centre 2 and not be merely 'handed on' from those of the genital segment which originated from centre 1. Other instances of impedance were met with in the genital gut immediately on the cessation of a

set of forward peristalses, when there was a pause before the impulses from centre 1, in the hind region of the cephalothorax, were manifested as backward waves (Fig. 4 *b*, *c*). On the other hand, immediately on the cessation of the impulses from centre 1, centre 2 appeared to be relieved of some depressor action and the forward impulses were abnormally strong and frequent (Fig. 4 *a*, *b*, *c*). On rare occasions the depressor action was insufficient to quell the normal impulses from centre 1, and they continued concurrently with those from centre 2, resulting in the amphidirectional peristalsis observed throughout this specimen (Fig. 4*a*).

A further demonstration of impedance in centre 1 was found when a specimen of *Lernaeocera branchialis* was kept in situ on a pollack in the refrigerator at 2° C. for 7 days. On removal at the end of this period the parasite was opaque and no movement was visible within it; it was dissected out and placed in circulating sea water at laboratory temperature, in which it became translucent and peristalsis was resumed—very fitfully at first, but after 3 hr. it became stronger and more or less continuous but in the forward direction only. The recording after $3\frac{1}{2}$ hr. is shown in Fig. 4 *d*. No backward waves were seen. After 24 hr. the forward peristalses were still in progress, with pauses of 1–5 minutes; the animal was then sacrificed for osmotic pressure determinations (Panikkar & Sproston, 1941, Table II (5)).

Stekhoven & Punt (1937) investigated the temperature effects on the peristalsis in *Lernaeocera branchialis* from the cod; they found that lowering the temperature reduced the frequency of the peristalsis, and also that the backward peristalses were almost completely inhibited by reducing the temperature to 5·5° C. for short periods (about 15 min. only in their experiments), but resumed their normal frequency when the temperature was raised again. They also determined the duration and frequency, under normal laboratory conditions, of the peristalses of seven specimens of *L. branchialis* and of two specimens of *L. lusci* (which gave substantially the same results). We have summarized their data and calculated the arithmetic means for comparison with our own (Table IV).

TABLE IV. THE DURATION AND FREQUENCY OF THE ANTERIOR AND POSTERIOR PERISTALSES IN THE GENITAL SEGMENT OF *LERNAEOCERA BRANCHIALIS*

Summary of Results obtained by Stekhoven (1936), *Stekhoven & Punt* (1937) *and Sproston* (*present paper*)

	Forward		Backward	
	Duration min.	Frequency per min.	Duration min.	Frequency per min.
	Stekhoven: Stekhoven and Punt			
Arithmetic means	2·75	44·0	2·12	37·5
Range	0·6–7·8	25·0–72·6	0·58–3·75	22·5–60·5
	Sproston			
Arithmetic means	5·7	33·2	3·3	30·6
Range	2·0–15·4	19·0–48·0	1·0–15·0	19·0–48·0

From the preceding summary in Table IV and from Fig. 4 it is seen, without going into a statistical analysis, that our values vary over a wide range, but an approximate idea of the duration and frequency of peristalsis in both directions is given by the arithmetic means of our observations. The mean duration of backward peristalsis is just over half (58 %) that in the forward direction in our specimens, but the mean frequency per minute is only slightly less. The corresponding results obtained on the same species of parasite from the cod by Stekhoven (1936) and by Stekhoven & Punt (1937) show similar relative differences; but the durations of peristalses in a given direction were always much shorter than those obtained by us, i.e. about half, when the means of each set of observations are calculated. The frequencies of the peristalses in their specimens are, however, slightly higher than in ours. Considering the wide variations in both sets of results, we do not feel justified in analysing them further.

The conclusions arrived at by Stekhoven (1936) and Stekhoven & Punt (1937) are that there are two automatic centres controlling the gut movements of *Lernaeocera branchialis*: one associated with the circumoesophageal commissure and the other near the end of the mid-gut (also referred to as the caudal centre). The exact location of these centres is, of course, hypothetical; but it is curious that these authors do not refer to a centre in the abdomen, nor do they record the, admittedly weaker, series of peristalses in this region. In their analysis of the peristalses in the genital segment they state that the impulses from the hinder centre (centre 2 in our figure) have a greater frequency, are stronger and are also maintained for a longer time than those originating from the cephalic centre. Our observations also support this conclusion. Now in the change-over in peristalsis from one direction to another, Stekhoven mentions an overlap of waves; he implies that this normally takes place at each change-over, but that it is of a shorter duration in the backward-forward change-over. He did not record any pauses in peristalsis. On the other hand, we observed pauses occasionally, but they only occurred at the forward-backward change-over. Overlap of waves from both directions, i.e. amphidirectional peristalsis, was also occasionally seen by us, but only during the backward-forward change-over (Fig. 4). Though these details are slightly at variance with the results obtained by Stekhoven & Punt, their implication is the same: namely, that the forward impulses (those from centre 2) in the genital segment are in every way more effective, and that there is a lag or impedance imposed upon the backward impulses (coming from centre 1).

As Stekhoven & Punt overlooked Vanden Berghe's paper it is necessary to review some of the interesting results obtained by this author on the peristaltic movements of *Lernaeocera*. Vanden Berghe finds that the initial contractions in each set of waves are stronger and more frequent than the succeeding ones (a point not mentioned by Stekhoven & Punt); they begin at about 44 per min. and gradually diminish, and the average duration for the backward phase is about 3 min., but that in the reverse direction it is about

twice as long. This agrees very well with our findings (Fig. 4). He also mentions amphidirectional waves—disordered surgings to and fro—lasting for 2 or 3 sec. occurring at the backward-forward change-over, though he does not state exactly what happens at the other change-over. Like Stekhoven, he does not record pauses between the phases. He publishes very few actual records, and those, only of the number of waves of an entire phase of peristalsis. The means of these ten observations are: backward phases 65 and forward phases 107; he emphasizes this marked dissimilarity but he has found that it is far less marked in the younger specimens which had periods of the order of 50 and 70 respectively. We have not yet been able to confirm this.

Like Stekhoven & Punt, Vanden Berghe only recognizes two initiation centres: one near the mouth and one at the other extremity of the body, but unfortunately his statements are not very clear in regard to them, and not once does he mention that he noticed waves originating from the hind end of the genital segment. This is to be regretted for he goes on to describe some very interesting experiments in which he ligatured the body of a young specimen, first in one place and then in two places along the body. In the absence of diagrams the value of these experiments is diminished, for we do not know the position of the ligature in relation to the centre 2 in our figure. When one ligature was used an alternating peristalsis was seen as before, but the waves 'originating' from the ligature in both directions were weak and markedly less numerous than those starting from either of the ends. When two ligatures were used the central region between the ligatures showed no sensible movement. After consultation with V. Willem, Vanden Berghe concludes that, on a myogenic theory, these movements need not involve a special nervous intervention; for as peristaltic contraction is an intrinsic property of the gut wall, the reversal is explicable on the basis of fatigue and that the return wave at the single ligature is a reflected one.

We cannot wholly subscribe to this explanation, for it does not account for the difference in character of the peristalses in the genital segment and in the abdomen, nor does it cover the initial 'kick' in the forward waves, nor again the reduced facility of propagation of the backward series and their final suppression when submitted to low temperatures. We therefore maintain that it is probable that the intrinsic contractility of the muscle fibres in the gut wall is controlled by at least three autonomic nerve centres in copepods, and that the depressor action of centre 1 is significant in retaining the contents of the gut in those forms which utilize an anal current in respiration.

The Evolution of the Respiratory Mechanism in Parasitic Copepods

It is of interest, in passing, to review briefly the evolution of the respiratory mechanism in parasitic Copepoda. In *Trebius* and other caligids as well as in the developmental stages of the Lernaeoceridae an anal current introduces the water of the external environment which acts as the oxygen vector.

Hartog (1879) considered respiration in *Cyclops* to be 'entirely anal'. The gut movements are subservient to the circulation of a colourless blood in the lacunar system of the haemocoele. In the Dichelesthiidae, in such forms as *Congericola*, *Hatschekia* and *Lernanthropus*, there is a peculiar development of vessels containing blood with haemoglobin in solution, and this oxygen carrier supplements the respiration which takes place by means of an anal current and a lacunar system in the haemocoele. In *Lernanthropus* there are foliose outgrowths of the cuticle along the margins of which the large vessels run, and these are connected by a network in the substance of these appendages. The haemoglobin is probably obtained by diffusion through the gut wall in these blood-feeding forms. In the Lernaeoceridae an evolutionary series can be made out which shows a decreasing dependence on the anal current, or in other words, a tendency to become independent of the external environment. In *Lernaeenicus* the long thin body of the adult female hangs quite freely in the water and it is possible that cuticular respiration is of importance in this genus; it is probably not a blood feeder, but is nourished largely by the coelomic and tissue fluids of the clupeoid host. *Pennella*, though deeply imbedded in the skin of Cetacea, is not red in colour, and probably feeds on tissue fluids only. That it is still largely dependent on its external medium is shown by the presence of delicate feathery caudal appendages which are thought to aid cuticular respiration; also it is well known that *Pennella* is killed when the whales migrate into the colder polar waters. In *Peroderma* the short body is largely imbedded in the muscles of the back of the sardine and the head bears a tuft of thin-walled digitiform 'rhizoids'. This parasite has been the subject of numerous papers by Monterosso (1921–30), who shows that though the alimentary canal is still functional (he does not state whether the genus is aproctous), the cephalic rhizoids serve as absorptive organs both of nutriment in solution and of oxygen from the blood of the host. The rhizoids, he maintains, are syncytial in structure as are all the other organs of the body (Caullery (1926, p. 19) doubts this, and thinks that the syncytial appearance may be due to defects in fixation) and their growth in the renal tissue of the fish causes vascular hypertrophy, so that the rhizoids are bathed in host blood in the lacunae in which they come to lie. The haemoglobin which either passes into the haemocoele via the rhizoids, or via the gut wall, or both, acts as an accessory vector of oxygen; there may or may not be an anal current, but active peristalsis is noted in the gut, especially in the median section, and waves are said to be initiated in the posterior region. He emphasizes the kinetic function of the gut in relation to the body fluids.

In *Lernaeocera* the cuticle has become particularly heavily chitinized and the cephalothoracic outgrowths are purely mechanical anchors; cuticular respiration is reduced to a minimum and both oxygen and food enter the animal through the mouth in the form of the blood of the gadoid. A vigorous two-way peristalsis keeps the ingested blood in circulation, so that absorption of solutes is facilitated, and at the same time a feeble circulation is transmitted

to the haemocoele fluid. Independence of the external environment has reached a high level as shown by the osmotic gradient and by the toleration of brackish water by this genus of parasites.

A comparison of the details of respiration in *Lernaeocera* and in *Trebius*, which represent the two extremes in the above series, is particularly instructive. The two-way respiratory current in the gut of *Trebius* is complicated by the muscular action of the relatively powerful mouth parts, the trituration of food in the stomach and its periodic expulsion as faeces by the rectum. In *Lernaeocera* digestion is apparently slow, and during this process the gut is closed at both ends, so that the two-way peristalsis is unimpeded by the exigencies imposed upon it in *Trebius*, and a higher frequency is observed. The freedom from extraneous movements enabled the characteristics of the peristalses of *Lernaeocera* to be studied in detail: whereas in *Trebius* the movements had to be analysed to show the preponderance of forward waves (which enables the food to be retained in the gut for a sufficient time for digestion to be effected), in *Lernaeocera* this emphasis on the forward peristalses is very obvious. In the latter type of parasite this inequality is clearly a relict from the earlier stages when peristalsis had to be controlled with a view to the retention of food in the gut in the face of a continuous current via the anus.

Acknowledgements

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Summary

A study of the distribution of *Trebius caudatus* from four species of *Raja* trawled from two localities of different depths at Plymouth shows that there is a higher infection rate in the deeper water.

Both at Roscoff (France) and at Plymouth the mean total rate of infection is about 20 %, and the larger fish are more often infected than the smaller. Both sexes are equally parasitized and the copepods tend to collect near orifices on both surfaces of the fish (regions where the mucus secretion is maximum). Immature forms were found at Roscoff in the mucus of the orifices themselves. The collections from both places contained about equal numbers of male and female parasites.

Intestinal respiration, a feature common to free living and parasitic copepods, includes the gaseous exchange through the gut wall, the associated movements stirring the oxygen vector (which is either the external medium taken in by the anus, or the food current when this is the blood of the host), and the circulation of the body fluid as a direct result of these movements. The significance of the gut movements, which include the peculiar two-way peristalsis, is examined in detail in *Trebius caudatus* and *Lernaeocera branchialis*:

the former has a functional anus which admits the external medium and the latter has a closed anus and employs the blood of the host as the oxygen vector.

Peristalses in the three sections of the gut in *Trebius* are not synchronous and wide variations are found in the frequency and strength of the waves and also in the duration of the backward and forward phases of peristalsis. Much of the irregularity is due to the presence of food and faeces in the gut and to ancillary muscular activity.

In *Trebius* the maximum number of total peristalses per minute is in the cephalothoracic section of the gut, but the waves are never transmitted to and fro between this and the genital segment, though they are regularly passed forwards from the abdominal gut. The three sections of the gut are virtually separate in regard to peristalsis, which is controlled by three centres situated towards their distal ends. On analysis an increasing residuum of forward over backward waves is found from the fore to the hind end of the body; this is effective in retaining the food in the gut.

In *Lernaeocera* the cephalothoracic gut is practically absent and the peristalses in the abdomen are fitful and not synchronous with those in the large genital segment, where a two-way peristalsis is clearly seen. The peristalses in this species are unimpeded by the alimentary exigencies imposed upon them in *Trebius*, so that the superiority of the forward waves is obvious: they have greater strength, a higher frequency and a longer persistence than those in the backward direction, i.e. those originating from centre 1. This anterior centre has a depressor effect on the backward waves and is the most sensitive to temperature changes: complete inhibition is brought about by previously subjecting the animal to low temperatures.

Peristalsis in both species is controlled by three homologous autonomic nerve centres which have varying powers of stimulation and inhibition. The significance of the emphasis on the forward peristalses appears to be lost in *Lernaeocera*, for it is clearly a relict of the earlier stages when food had to be retained in the gut in the face of a continuous to and fro current through the anus.

The feeding of *Lernaeocera* takes place at rare intervals and digestion is slow. The intake of blood from the host is dependent on the same mechanism which maintains the dynamic osmotic equilibrium between the contents of the gut and the external medium.

The evolution of the modes of respiration in parasitic copepods is summarized.

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A WIRE-ANGLE INDICATOR FOR USE WHEN TOWING PLANKTON NETS

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(Text-figs. 1–4)

The old type of wire-angle indicator, which consisted of a pendulum swinging across an arc of a circle graduated in degrees, and which had to be applied by hand to the wire, was clumsy to use, especially in bad weather, and when the wire led out over the stern of the ship. It needed someone to attend to it, which often resulted in the wire angle not being checked more than once every five or ten minutes when the crew were busy. This instrument was therefore designed to remain in place throughout the tow, and to give a large

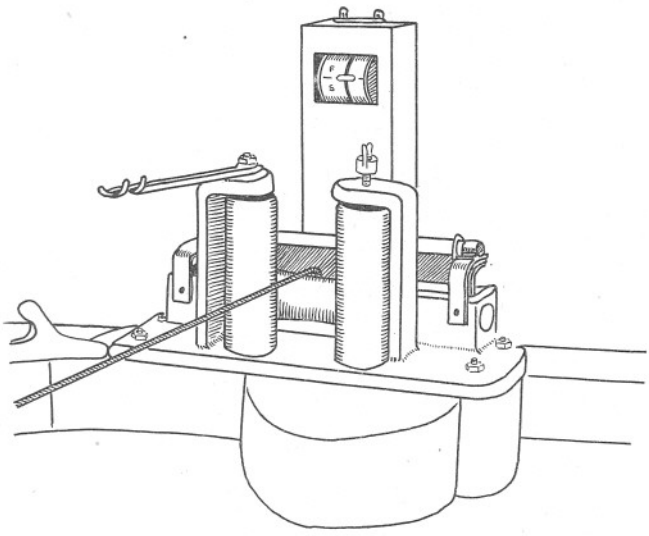

Fig. 1. Angle metre in situ on the stern of the *Culver*.

dial reading which could be easily seen from a distance. On the research ketch *Culver* there is an engine throttle control mounted close to the wheel, and all the man there has to do is to adjust the engine speed so that the large indicator in the instrument remains on the red line. In practice it was found that the wire angle rarely varied more than about $\pm 2^{\circ}$, except in very bad weather, and so the depth of the nets could be regulated very closely. The instrument is oil damped, so that the pitching of the ship and the vibration of

the wire during hauling do not affect it. It has been used for about two years on the *Culver* at Bermuda; it has needed no attention in that time, and, which is a good point, the crew like using it.

The framework of the instrument consists of two plates ([1]2*c*, 3*c*, 4*c*), spaced apart by three rods (2*i*, 3*h*, 4*e*), bolted on the outside (4*d*). Inside this frame, a pendulum weight (3*f*, 4*f*) is suspended by two arms (3*b*, 4*b*)

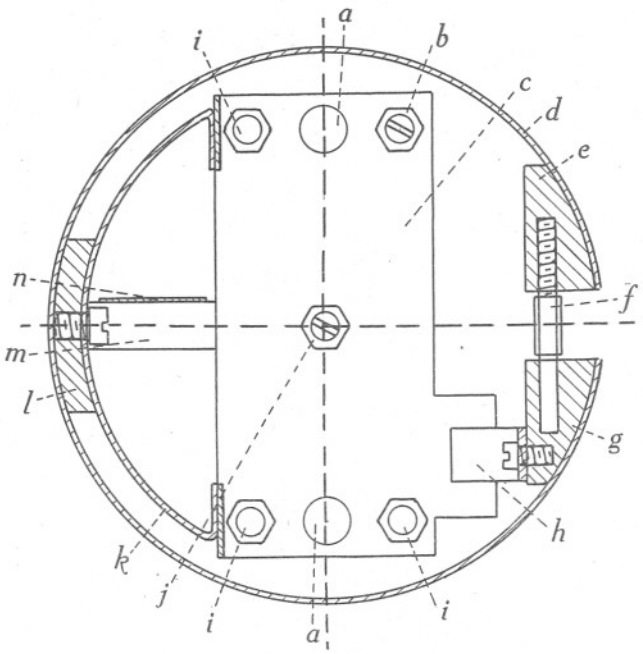

Fig. 2. Section of angle metre from side, taken through outer locking strip (Fig. 4*i*).

from an axle (3*a*, 4*m*) which turns in a conical seating in the end of adjustable screws (4*k*) in projections (4*l*) of the main frame. The pendulum is prevented from swinging too far by two stop screws (3*i* and 4*h*) attached by brackets to the main frame. In front of the pendulum is a small block (3*d*), which also turns on an axle (3*e*) in conical seatings in projections (2*j*) of the main frames. This block is coupled to the pendulum by a strip (3*g*, 4*g*), in such a way that a small movement of the pendulum imparts a much larger rotatory movement to the block. The block carries in front a strip (2*m*, 3*l*), on the end of which is the indicator (3*m*) seen on the face of the dial in Fig. 1. On the top of the strip is a flat plate (2*n*, 3*n*), mounted at right angles, and acting as a damper to the swing of the strip.

The indicator (3*m*) moves over a semicircular dial (2*k*, 3*k*), which is

[1] Numbers in brackets refer to text figures.

mounted in two halves on brackets on the main frame, and the strip (3*l*) moves up and down in the slit between the two halves of this scale.

The whole instrument is mounted in a glass jar, and is held inside this by two locking strips (2*d*, 4*a*, *i*), bent into circles, one at each end of the jar. Each strip is broken at one point, where it has two blocks (2*e*, *g*) attached, and these can be pushed apart by set screws (2*f*, 4*j*), thus tightening the strips

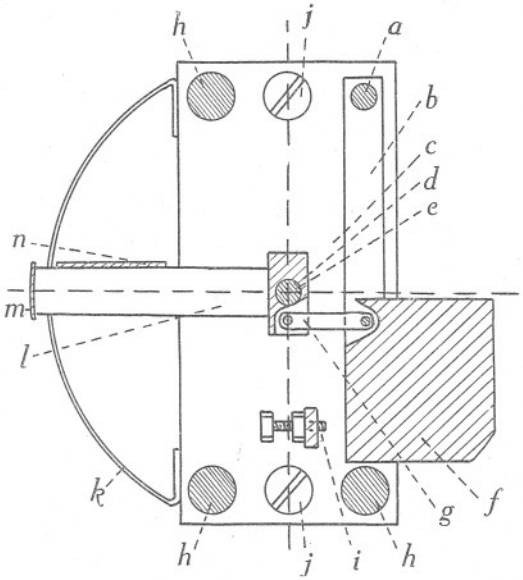

Fig. 3. Section of angle metre from side, and just above centre. The circular locking strips have been removed, as well as the outer frame plate and outer half of dial.

against the glass. In assembling the instrument the inner locking strip is inserted first and tightened into place. The framework, attached to the outer locking strip, is then dropped into place, and attached to two brackets (not shown) on the inner locking strip by two screws (3*j*) through the inner frame plate, these screws being accessible through two holes (2*a*) in the outer frame plate. The outer locking strip is then tightened up.

The glass jar is filled with castor oil to damp out too rapid swings, and closed with a glass top and rubber washer, since castor oil does not soften natural rubber. The jar is clamped shut by an outer frame which drops into slots in the wooden outer mounting, and so retains a constant relative angle. As used on the *Culver* the instrument was set to work at a constant towing angle, but the mounting could easily be modified so that this was variable. The bearings of the instrument were made of steel, and the rest, except for the lead pendulum weight, of brass. The dial and indicator were painted with ordinary water colours, which were found to stand up best to the action of the oil, but a baked enamel finish would no doubt be better.

The instrument was mounted in a wooden turret (Fig. 1) on a frame which pivoted horizontally immediately above the roller on which the towing wire passed over the stern of the ship. At the back of the frame carrying the turret was a second roller which rode on the wire, and thus tilted the apparatus with the changing wire angle. The whole apparatus was attached by clamps, and

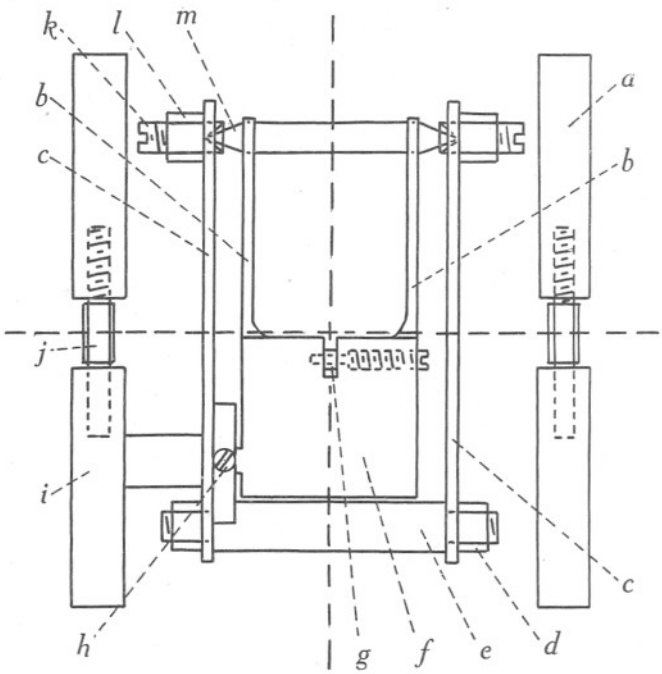

Fig. 4. Rear elevation of angle metre. The parts in front of the pendulum (central block, indicator and dial) are omitted, as well as the brackets attaching the frame to the inner locking strip (*a*).

could easily be lifted off when nets were being handled over the rollers, but it did not have to be shifted if wire was being let out or taken in.

The instrument as described proved quite satisfactory, and the nets were certainly fished at a more uniform depth after its introduction than with the old hand instrument. It could easily be modified for use with other arrangements of the towing wire, and could be made with the dial at the side or the top if this were required. Actually we had the pendulum weight set far back so that the instrument faced slightly upwards.

MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

Report of the Council for 1940–41

The Council and the Officers.

During the year the Association has suffered a great loss in the death of Prof. E. W. MacBride who, as Chairman of Council, had presided at the Council meetings since 1928. In the early days of the Association Prof. MacBride was a constant worker at the Plymouth Laboratory and throughout his life he took the greatest interest in its welfare. He had been a Governor since 1924, representing the Zoological Society of London, and in recent years was also Chairman of the Fishery Advisory Committee of the Development Commission.

The Council also regret to note the death of Dr G. Herbert Fowler, who held the post of Secretary to the Association from 1891 to 1895 and for many years gave valuable service as a Council member.

Four ordinary meetings of the Council were held during the year, two of them in the Rooms of the Royal Society, London, two, in October and January, in the Zoological Laboratory, Cambridge; the average attendance at the meetings was twelve. The thanks of the Association are due to the Royal Society and to Prof. J. Gray for allowing the use of the rooms.

The Visiting Committee of the Council inspected the Plymouth Laboratory on 13 April 1940. In June an Emergency Committee was appointed with authority to act for the Council in any urgent matters if it should not be possible to hold the ordinary meetings. At the January meeting, on the motion of the President, Prof. J. Gray was appointed Deputy Chairman of Council.

Air Raid Damage to the Plymouth Laboratory.[1]

Serious damage to the Association's property has been incurred through enemy action during the year under review. In an early raid many windows were destroyed by blast and at a later date heavy damage was sustained by fire and from high-explosive bombs. Two of the latter fell near the front gate, one of them destroying the water main, while another came down on the north side of the Director's house, blew in the wall and brought down a large brick arch, also causing much damage to the south walls of the physiological and chemical laboratories and in the aquarium. At the same time an incendiary came through the roof of the Director's house and though tackled immediately it was not possible to bring the fire under with stirrup pumps. The fire main, which could easily have subdued the outbreak, could

[1] See Additional Note, p. 433.

not be used owing to lack of water. Help was obtained from the military authorities, who sent a squad of men and a motor pump which could draw from our sea-water reservoirs, and the fire was thus prevented from spreading to the Laboratory as at one time appeared imminent. The Director's house was completely gutted and almost all its contents were destroyed. On the following night three incendiaries struck the premises but were extinguished without causing serious damage: one of these, which was put out with considerable difficulty, fell on the men's lavatories, one in the Common Room and one in the Director's garage.

As a result of the attacks practically all the windows are broken except those on the top floor of the south side of the main building, many window frames are demolished or fractured, ceilings have fallen, plaster walls are cracked and bulging and most of the doors have been shattered, split or wrenched from their fastenings. Internal glazing in fanlights and in cupboard doors is for the most part broken and considerable damage has been caused by flying glass. The small store behind the Director's house is demolished, the garage is unroofed and in the Easter Class House the roof is broken and the brickwork at the south-west corner is unsafe. The roof of the dog-fish store is destroyed and that in the constant temperature room has been lifted and the skylights broken. The Aquarium has suffered heavily; a number of the thick plate-glass fronts to the tanks on the northern side have been broken and the supply pipes fractured, most of the larger fish being lost.

The Library is completely undamaged except for the broken skylight and windows, and losses to apparatus and equipment are comparatively light in view of the severity of the attacks. In some rooms furniture has suffered and valuable material in the sales department destroyed.

In clearing the debris, which lay thickly in all parts of the buildings, much assistance was given by visiting workers—Miss N. G. Sproston, Dr M. Parke, Dr N. K. Panikkar, Mr P. G. Corbin and Mr W. J. Rees—and valuable help was also given by a party of students from the Municipal Technical College, organized by Miss F. Stanbury and Mr H. Barnes. The Library was made weatherproof in the first two days and within a fortnight the first and second floors of the main laboratory, with some research rooms in the north building, were habitable. A small amount of glass has been restored, while other windows have been boarded or covered with fibre sheeting from the ceilings of the new laboratories. In the north building old glazed frames and aquarium fronts which had been kept in store have been used to admit light, other parts of the windows being boarded or bricked.

The buildings have been inspected by Mr A. Thorpe, Architect to the Ministry of Agriculture and Fisheries. The most severe structural damage is in the Director's house. With the fall of the large arch on the ground floor of the northern elevation the structure appeared to be in some danger of collapse and the coign to the west of the arch was badly damaged by the explosion. The arch has now been shored, the windows above it strutted,

and timbers over the inner side of the windows, which had been burnt through, have been supported. The cut stone south and east elevations of the house are in good condition, though in some places the limestone round the windows will need replacement. The eastern end of the north building is extensively damaged on its southern side; the walls are deeply cracked and heavily scarred by flying debris and the whole building is shaken. The south-east corner has been shored and Mr Thorpe is of the opinion that the southern face at least will have to be rebuilt. The Easter class house will no doubt need rebuilding, and it will not be possible to re-open the Aquarium until after the war has ended.

Mr Thorpe recommends the appointment of a qualified surveyor to prepare the Association's claim for compensation and his advice in this and in other matters is being followed. During the summer a number of repairs either permanent or temporary must be carried out in order to protect the building: slating on the sides of the top floor of the main laboratory must be renewed, new guttering and rain-water pipes must be fitted, asphalt roofs will need attention in order to prevent leaks, and some at least of the outbuildings must be re-roofed.

The water supply was restored about a week after the raid, and electric supply on most circuits failed for the same period; it was, however, possible to keep the sea-water reservoirs pumped up, thus providing a supply of water for any further emergencies. An old $3\frac{1}{2}$ h.p. paraffin engine from the motor boat has now been refitted and installed in the engine room. It can be used to drive the salt-water circulation pumps, serving a small fire hose of sufficient length to reach the upper floors of the library and laboratories.

The Ship and Motor Boat.

As noted in last year's Report the *Salpa* was requisitioned by the Admiralty in December 1939. The rate of hire for the ship is still the subject of correspondence with the Ministry of Shipping, but certain interim payments have been made. All sums received on account of hire will be credited to the *Salpa* Depreciation Fund. The motor-boat *Gammarus* is permitted to work over a restricted area in the Sound and Cawsand Bay.

The Staff.

Five members of the scientific staff are away on National Service. Mr F. S. Russell has a commission as Flight Lieutenant in the Intelligence Branch of the Royal Air Force, Dr L. H. N. Cooper has an appointment in the Ministry of Supply, Dr A. Sand and Mr G. A. Steven have commissions in the Royal Naval Volunteer Reserve and Mr G. M. Spooner has an appointment at the Foreign Office. Mr E. Ford and Dr W. R. G. Atkins hold commissions in the Home Guard and Mr D. P. Wilson is giving voluntary service in the photographic department at the Police Headquarters in Plymouth.

Of the clerical and technical staff four are on National Service: J. H. Bowden (R.A.F. photographic branch), A. Stoate (balloon barrage), A. Mattacola (Royal Tank Corps) and W. H. Gladwell (Royal Marines Police). Of the others three are in the Home Guard, one in the Auxiliary Fire Service, one in the St John's Ambulance Brigade and one is an Air Raid Warden. The engineer-caretaker, E. S. Jope, has reverted to service as a naval officer.

Mr A. J. Smith.

During the year the Laboratory has suffered a heavy loss in the death on 29 January 1941 of Mr A. J. Smith, who had been a valued servant of the Association for 43 years. As Chief Laboratory Attendant he had for a long period done much to further the progress of research at Plymouth.

Occupation of Tables.

The following have occupied tables at the Plymouth Laboratory during the year:

H. BARNES, Plymouth (Hydroxylamine and nitrate degeneration in sea water).
Dr A. M. BIDDER, Cambridge (Library).
Dr G. P. BIDDER, Cambridge (Library).
W. THORPE CATTON, Chelsea Polytechnic (Haematopoiesis in Teleosts).
Mrs P. G. CORBIN, Plymouth (Library).
P. G. CORBIN, Plymouth (Mackerel research).
L. R. CRAWSHAY, Plymouth (Sponges).
Miss V. J. FOOTE, Nigeria (Shore ecology).
Dr V. FRETTER, London (Formation of the egg capsule in Stenoglossa).
A. T. GOODMAN, Exeter (Helminth parasites of fishes).
A. GRAHAM, London (Alimentary canal of stenoglossa).
R. J. HARRISON, Kent (Life-history of caprellids).
Dr T. J. HART, Discovery Committee (Phytoplankton).
P. H. T. HARTLEY, Westmorland (Library).
Dr M. W. JEPPS, Glasgow (*Polystomella*).
R. JONES, Anglesea (Museum).
F. KNOWLES, Marlborough (Colour changes in crustaceans).
A. G. LOWNDES, Rugby College (Density of aquatic organisms).
Miss M. MARE, Cambridge (Micro-fauna and -flora of mud deposits: phytoplankton).
Dr H. B. MOORE, Bermuda (Plankton).
Dr N. K. PANIKKAR, London (Osmo-regulation in marine animals).
R. PIKE, Reading (Nervous system of *Galathea*).
W. J. REES, Millport (Hydroids and hydromedusae).
P. B. RICHARDS, Truro (Oyster culture).
The Hon. MIRIAM ROTHSCHILD, London (Trematode parasites in *Hydrobia*).
Miss H. G. Q. ROWETT, Plymouth (Antifouling research).
Dr J. E. SMITH, Cambridge (Nervous system of asteroids).
Miss N. G. SPROSTON, London (Metazoan parasites of fishes).
Miss F. A. STANBURY, Plymouth (Silica uptake by diatoms).

The usual Easter Vacation Courses in Marine Biology were conducted by Mr D. P. Wilson and Mr G. A. Steven. They were attended by thirty-nine

students from the Universities of Oxford, Cambridge, London, St Andrews, Aberdeen, Edinburgh, Wales (Bangor), Birmingham, Sheffield, Bristol, Southampton and Hull.

During the Easter Vacation Mr I. T. Hamilton brought one student from Dauntsey School, Mr J. M. Branfoot three from Oundle School, Mr W. L. Francis two from Repton School, Mr A. A. M. Gardiner two from Radley College, Mr A. H. Lewis six from Wellington College and Mr G. C. M. Harris five from Monkton Combe School. The Autumn class was abandoned owing to the prevalence of air raids in the Plymouth area.

The Scientific Work of the Plymouth Laboratory Staff.

It is unfortunately necessary to record a diminution in the research activities of the staff as a result of the war. In addition to the loss occasioned by the absence of a considerable part of the staff on National Service, conditions do not favour concentration on problems in marine biology, and the auxiliary services have made demands which have inevitably interfered with work in the Laboratory. At one time it was hoped that the staff might be able to work together as a team on problems of some national importance, but efforts to bring about such an arrangement have not hitherto been successful. In a number of matters, however, the members of the staff have been able to make small contributions to the national cause, the most important, perhaps, being the assistance which Dr Atkins has been able to give to the Admiralty as well as to two Committees concerned with questions of corrosion and fouling. In addition Dr Atkins, assisted by Mr Russell and afterwards by Mr Steven, has undertaken special work for the naval authorities in the dockyard at Devonport. Under-water tests on the resistance of submarine cables to boring organisms have been carried out and some help has been given on a proposal to utilize seaweeds for purposes of national importance. Information on sea temperatures and other matters has been supplied to service departments. Work has also been carried out by Mr Crawshay and members of the staff on a growth of freshwater sponges which was obstructing the flow in water-supply mains; methods of checking or suppressing the growth have been recommended and experiments are in progress.

During the early part of the year Dr W. R. G. Atkins worked upon a meteorological instrument for the Royal Air Force and prepared for publication the tests carried out upon the preservation of ropes, trawl twines and nets since 1936. Later on he was consulted by the Admiralty Corrosion and Fouling Committee and by that of the Iron and Steel Institute. The action of sea water and of fouling organisms upon various materials was studied. Advice was also given to the Water Pollution Board's staff and an instrument required for a special investigation was re-tested and issued on loan. The Admiralty work on fouling and corrosion is still in progress. A few estimations of the phosphate content of sea water during the winter maximum period were also made.

In view of the direct relationship, found by Russell, between the abundance of young fish and plankton off Plymouth and the quantity of phosphate occurring in the water when at its maximum during the winter, experiments have been made by Dr H. W. Harvey on the quantity of phosphate set free in sea water during storage, and also when the organic phosphorus compounds in solution in it are hydrolysed to phosphate by heating with acid. The aim is to evolve a method of assessing the quantity of phosphorus in a sample of water, collected in summer when the phosphate content is low, which would in nature be converted into phosphate in course of time. It is thought that this quantity may provide an index of the 'potential fertility' of the water, and that its magnitude may differentiate one body of water from another. It is known that when sea water is stored in bottles there is a slow increase in phosphate content, often preceded by an initial decrease; the latter is due to utilization of phosphate by bacteria which multiply rapidly during the first few days of storage. Experiment has shown that phosphorus utilized by bacteria is almost entirely returned to the water as phosphate within three weeks. It appears that the quantity of phosphate liberated in stored water is less, or often less, than would be liberated in nature and that the liberation is due in part to the action of phosphatase enzymes since it takes place in water saturated with chloroform. The quantity of organic phosphorus compounds in solution which are hydrolysed to phosphate on treatment with acid have given interesting but anomalous results. Some evidence has been obtained suggesting that the rapid multiplication of bacteria, which takes place when sea water is kept in a glass vessel, is due to adsorption of organic matter on the glass surface, particularly at the meniscus, where it provides a localized concentration of food for their growth.

Last year it was noted that Mr D. P. Wilson had established clone cultures of the two main forms of *Nitzschia closterium*, the normal spindle-shaped cells and the tri-radiate cells. Cultures of both types remained pure—apart from producing the spineless forms mentioned below—through several stages of sub-culturing and for several months. Eventually a very small number of tri-radiate cells appeared in later sub-cultures of normal ones, and spindle-shaped cells in tri-radiate cultures. Investigation has thrown some light on the manner in which change in shape takes place, although the cause is not known. In cultures of *Nitzschia*, especially those approaching the end of their growth, spineless cells, more or less oval in shape, are produced by division from the spined forms. These spineless cells themselves divide and thereby increase their abundance. If a few such cells be isolated into fresh culture media they usually continue to divide for a time, sometimes giving rise to large numbers of spineless cells. In course of time spined forms are produced by a process of gradual enlargement, a phenomenon at present imperfectly understood. A striking feature is that spineless forms which have originated from tri-radiate cells give rise mainly to normal spindle-shaped forms, although there may be a small proportion of new tri-radiates. On the

other hand spineless cells produced originally by spindle-shaped cells have up to the present only been known to give rise to new spindle-shaped cells. The investigation is being continued.

Work on Polychaete larvae, particularly those of the genus *Magelona*, has made some headway but in the main has been seriously hampered by lack of material due to wartime restrictions on collecting. It has been impossible to obtain adults of the especially interesting species *M. cincta*, whose relatively large larvae are the most suitable for sectioning. A few late larvae have been obtained from tow-nettings in the Sound and it is now possible to distinguish in the plankton the larvae of all three species.

Some preliminary experiments with the larvae of a species of *Teredo* have shown that they are generally unable to metamorphose unless allowed to settle on wood, or perhaps some similar substance such as paper. The capacity of the larvae to metamorphose endures for a period of about a week; if at any time during that period they come into contact with suitable timber they are able to settle and subsequently bore into the wood. If the larvae are kept continually in clean glass vessels it appears that ability to metamorphose is eventually lost and they soon die. These experiments extend to the Mollusca principles already shown to hold good for some annelids. They are being continued whenever it is possible to obtain larvae.

During the summer of 1940 Mr Wilson assisted in a technical capacity with the rearing of oysters on a commercial scale in the tanks of Mr J. Kingcome on the River Yealm. An excellent spatfall was obtained and many thousands of young oysters are growing well. They are likely to be of real value to the fishery at a time when it is cut off from its usual sources of supply in Brittany.

Miss Lebour has finished the main part of the work which she did in Bermuda and has published part of it, the remainder being in the hands of the publishers. Two papers have appeared in the *Journal* of the Association: on the larvae of the Pandalidae and the larvae of the British species of *Spirontocaris* and their relation to *Thor* (Crustacea Decapoda). Both of these combine work done at Plymouth with Bermuda work. With Dr R. Gurney a report on Sergestid larvae has been published in the *Discovery Reports* and a further paper with him is in proof in the *Journal of the Linnean Society*. This includes an account of the larvae of Hoplophoridae, Stenopidae, Palaemonidae and others. A paper on larval crabs hatched in Bermuda and another on larval molluscs, especially those from the open waters of Bermuda, with notes on the eggs of the Bermudan Mollusca have been accepted for publication in *Zoologica*, and a paper on larval *Processa* from Bermuda, which affords interesting comparisons with the Plymouth species, and another on larval Axiids, are to be published in the *Annals and Magazine of Natural History*.

Miss Lebour has been working, whenever possible, on the larval Crustacea and Mollusca of Plymouth, continuing her previous work. She has hatched out the little-known zoeae of *Portumnus latipes* and partially reared them, and

has made notes on the early crab stages of *Portumnus* and a number of rare Brachyuran larvae. The larvae of *Porcellana* are also being studied and the life-histories of the two common shore species, *P. longicornis* and *P. platycheles*, have been differentiated and worked out. She has also observed the breeding seasons of the two common species of *Teredo* from the raft in the Sound and found that they are both capable of breeding throughout the year. The larvae and young metamorphosed forms of the small species related to *Teredo navalis* have been investigated.

Miss Lebour has also examined tow-nettings from close inshore in the Sound when it was possible to procure them, keeping an eye on the larval Crustacea and Mollusca, noting the breeding seasons and general contents of the hauls.

Up to the time when he joined the Royal Air Force Mr F. S. Russell continued his work on the Monograph of the British Hydromedusae and his routine observations of the off-shore plankton in the Plymouth area. Now that the *Salpa* is no longer available the regular collection of plankton samples has become difficult. For a time arrangements were made with one of the few remaining trawlers working out of Plymouth, and when this failed, through the kind offices of Capt. C. H. Lush, R.N., samples were for a time collected for the Laboratory by one of the mine-sweeping vessels.

Mr G. M. Spooner, while still at the Laboratory, continued the studies referred to in previous reports, relating to estuarine faunas and the special investigation on species of *Gammarus*. Further samples of gammarids have been examined but the general work on the ecology of estuaries has come to a standstill owing to the difficulties of access to these areas in wartime. The work on the systematics and distribution of species of *Marinogammarus* was completed, and a paper brought out in co-operation with Mrs E. W. Sexton. In this paper all the species have been described and figured in detail, and collections from many localities in the British Isles and Northern Europe have been examined and recorded.

Mrs Sexton is now working on another species, *Gammarus zaddachi*, first described by her in 1912, from freshwater and estuarine material. A remarkable variation was noted in the degree of development of the setose armature, and this appeared to be correlated with the degree of salinity of the water. The freshwater form was distinguished by heavier chitin and dense clusters of long setae, the saline type by thin chitin and few setae. Since 1912 both forms of *G. zaddachi* have been recorded from numerous rivers and their estuaries, and usually confused with *G. locusta* and *G. duebeni*. Owing to its abundance, wide distribution and the variation of form, it is much used in connexion with ecological problems, and for this reason it is important to re-describe and figure the two forms on a larger scale.

Detailed drawings have also been made of *Gammarus locustoides* Brandt, a species formerly considered closely allied to or synonymous with *marinus*, but now recognized as belonging to the genus *Anisogammarus*.

Mr G. M. Spooner has assisted the Air Ministry in measures to reduce the numbers of peregrine falcons, which are a danger to carrier pigeons. Previously existing and newly acquired information on the distribution and habits of the falcons has been supplied, and a team has been formed to deal with the coastal area between Plymouth and Torquay.

Mr G. A. Steven continued his work on the mackerel of the western approaches to the Channel up to the time when he left on National Service. The data for a comprehensive paper embodying the results of three years' observations have now been assembled, but it is doubtful whether it will be possible for him to complete his work while the war continues. It is hoped that the collection of data on the mackerel stocks can be continued during his absence.

A paper by Mr E. Ford on vertebral variation in the herring has been published in the *Journal*. The attributes of the species *Clupea harengus* being plainly visible in every element of the herring backbone throughout its length, it is suggested that corresponding 'hallmarks' of sub-specific forms of herring should be looked for. The discovery of such direct indicators of identity would greatly facilitate the study of local populations of herrings and their migrations. In the meantime it is necessary to persevere in the indirect method of assessing and comparing the extent of vertebral variation among the individuals of statistical samples, using counts along the vertebral series as the bases of comparison. Great caution is needed in the interpretation of the data, since individual variation is very pronounced and bilateral asymmetry of common occurrence, even among backbones having the same total number of vertebrae. An interesting alternative method of counting the keeled scales along the ventral edge of the body from the throat backwards to the anus is given, which is in accordance with the marked degree of meristic agreement between the vertebral elements, the myocommata and fin-radials. In the absence of Mr F. S. Russell, Mr Ford is subediting the *Journal*.

Under arrangements made through the Development Commission Dr Mary Parke, who has hitherto been engaged on algological research at Port Erin, has been working at the Plymouth Laboratory. She has recently been studying the life-histories of certain little-known flagellates which have proved valuable as food for oyster larvae. It is long since any continuous work was undertaken on the algae of the Plymouth area and the new arrangement, by which Dr Parke's services are transferred to the Laboratory for a short term of years, is therefore very welcome to the Council.

The Library.[1]

The thanks of the Association are again due to numerous Foreign Departments, and to Universities and other Institutions at home and abroad for copies of books and current numbers of periodicals presented to the Library,

[1] See Additional Note, p. 433.

or received in exchange for the *Journal*. Thanks are also due to those authors who have sent copies of their books or papers, which are much appreciated. Accessions to the library, particularly in foreign serial publications, have naturally diminished under war conditions; it appears probable that the normal grant for library purchases, now considerably reduced, will have to be increased when the war is over in order to make up arrears.

Published Memoirs.

Vol. XXIV, No. 2 of the *Journal* of the Association was issued in August 1940, and Vol. XXV, No. 1 in February 1941.

The following papers, the outcome of work done at the Laboratory, have been published elsewhere than in the *Journal* of the Association:

GURNEY, R. & LEBOUR, M. V., 1940. Larvae of Decapod Crustacea. Part VI. The genus *Sergestes*. *Discovery Reports*, Vol. XX, pp. 1–68.

HOLMES, W., 1940. The colour changes and colour patterns of *Sepia officinalis* L. *Proc. Zool. Soc.*, A, Vol. CX, pp. 17–35.

HOLMES, W., PUMPHREY, R. J. & YOUNG, J. Z., 1941. The structure and conduction velocity of the medullated nerve fibres of Prawns. *Journ. Exp. Biol.*, Vol. XVIII, pp. 50–4.

KIRTISINGHE, P., 1940. The myenteric nerve-plexus in some lower chordates. *Quart. Journ. Micros. Sci.*, Vol. LXXXI, pp. 521–39.

LÖWENSTEIN, O. & SAND, A., 1940. The mechanism of the semicircular canal. A study of the responses of single-fibre preparations to angular accelerations and to rotation at constant speed. *Proc. Roy. Soc.* B, Vol. CXXIX, pp. 256–75.

LÖWENSTEIN, O. & SAND, A., 1940. The individual and integrated activity of the semicircular canals of the elasmobranch labyrinth. *Journ. Physiol.*, Vol. XCIX, pp. 89–101.

METTEN, H., 1940. Studies on the reproduction of the dogfish. *Phil. Trans. Roy. Soc.* B, Vol. CCXXX, pp. 217–38.

MOORE, H. B. & SPROSTON, N. G., 1940. Further observations on the colonization of a new rocky shore at Plymouth. *Journ. Anim. Ecol.*, Vol. IX, pp. 319–27.

PANIKKAR, N. KESAVA, 1940. Osmotic properties of the common prawn. *Nature*, Vol. CXLV, p. 108.

PANIKKAR, N. KESAVA, 1940. Influence of temperature on osmotic behaviour of some Crustacea and its bearing on problems of animal behaviour. *Nature*, Vol. CXLVI, pp. 366–7.

SAND, A., 1940. The mechanism of acustico-lateral sense organs in fishes, with special reference to problems in the physiology of semicircular canals. *Proc. Roy. Soc. Med.*, Vol. XXXIII, pp. 741–50.

SMITH, J. E., 1940. The reproductive system and associated organs of the Brittle-star *Ophiothrix fragilis*. *Quart. Journ. Micros. Sci.*, Vol. LXXXII, pp. 267–309.

STEVEN, G. A. & CORBIN, P. G., 1939. Mackerel investigation at Plymouth. *Rapp. Proc. Verb., Cons. Int.*, Vol. CXI, App. 2, pp. 15–18.

WEBB, D. A. & YOUNG, J. Z., 1940. Electrolyte content and action potential of the giant nerve fibres of *Loligo*. *Journ. Physiol.*, Vol. XCVIII, pp. 299–313.

Membership of the Association.

Vice-Presidents. During the year the Association has suffered the loss of two of its Vice-Presidents, the Duke of Bedford and Lord St Levan. At the

BALANCE SHEET 1941

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

BALANCE SHEET 31ST MARCH, 1941

	£ s. d.	£ s. d.
SUNDRY CREDITORS		76 13 1
PROPORTION OF SUBSCRIPTIONS RECEIVED IN ADVANCE	19 18 0	
GRANT RECEIVED IN ADVANCE	150 0 0	
		169 18 0
INCOME TAX SUSPENSE ACCOUNT		53 9 8
AQUARIUM GUIDE PRINTING FUND:		
As at 31st March 1940	21 0 0	
Add: Sale of Aquarium Guides	1 19 0	
	22 19 0	
Less: Expenditure	17 6	
		22 1 6
SPECIAL APPARATUS FUND:		
As at 31st March 1940	8 16 11	
Add: Grant received from the Royal Society ...	45 0 0	
Transfer from Constant Temperature Rooms Fund	1 8 0	
		55 4 11
MACKEREL RESEARCH FUND:		
As at 31st March 1940	93 17 0	
Add: Sundry Receipts	12 10 0	
	106 7 0	
Less: Expenditure	49 17 4	
		56 9 8
BUILDINGS EXTENSION FUND:		
As at 31st March 1940	232 4 10	
Less: Expenditure 267 19 6		
Amount transferred to E. T. Browne Bequests Fund, as per Statement annexed ... 18 8 6		
	286 8 0	
BALANCE DUE TO GENERAL FUND, as per contra	£54 3 2	
E. T. BROWNE BEQUESTS FUND, as per Statement annexed		5774 5 8
"SALPA" DEPRECIATION FUND:		
As at 31st March 1940	2655 8 9	
Add: Amount received from Ministry of Shipping on account of Hire	300 0 0	
Interest on Investments, less Tax	47 3 3	
Income Tax recovered	59 19 9	
		3062 11 9
COMPOSITION FEES FUND:		
As at 31st March 1940		157 10 0

	£ s. d.	£ s. d.
BOATS AND EQUIPMENT, at valuation as estimated by the Director at 31st March 1941:		
S/S "Salpa"	2000 0 0	
Motor Boat "Gammarus"	100 0 0	
Nets, Gear and General Equipment	30 0 0	
		2130 0 0
LABORATORY APPARATUS, ENGINES AND PUMPS: At valuation as estimated by the Director at 31st March 1941		4000 0 0
LIBRARY: As per valuation of Mr Ridgill Trout in January 1941		15750 0 0
STOCK OF SPECIMENS, CHEMICALS AND JOURNALS, as valued by the Director:		
Specimens	1100 0 0	
Chemicals	150 0 0	
Journals	400 0 0	
		1650 0 0
SUNDRY DEBTORS: Sale of Specimens and Journals		267 9 9
INCOME TAX RECOVERABLE		54 13 4
PREPAYMENTS		44 11 2
GENERAL FUND INVESTMENT at market value as at 31st March 1931: £352. 2*s.* 3*d.* Local Loans 3% ... ... (Market value at date £320. 8*s.* 6*d.*)		232 7 10
"SALPA" DEPRECIATION FUND INVESTMENTS at Cost:		
£590. 6*s.* 0*d.* Local Loans 3%	506 10 9	
£2474. 4*s.* 1*d.* Conversion Loan 3%	2506 1 0	
(Market value at date £3054. 13*s.* 6*d.*)		3012 11 9
REPAIRS AND RENOVATIONS FUND INVESTMENT at Cost: £153. 1*s.* 0*d.* Conversion Loan 3% ... ... (Market value at date £155. 14*s.* 7*d.*)		153 18 7
COMPOSITION FEES FUND INVESTMENTS at Cost:		
£18. 8*s.* 6*d.* Local Loans 3%	15 15 0	
£139. 2*s.* 11*d.* Conversion Loan 3%	141 15 0	
(Market value at date £158. 6*s.* 11*d.*)		157 10 0
BROWNE BEQUESTS INVESTMENTS as per Statement annexed		5774 5 8

REPAIRS AND RENOVATIONS FUND:										
As at 31st March 1940					205	5	8			
Add: Transfer from Income and Expenditure Account					50	0	0			
Interest on Investment					1	14	11			
Income Tax recovered					1	3	8			
								258	4	3
CONSTANT TEMPERATURE ROOMS FUND:										
As at 31st March 1940					4	1	2			
Less: Expenditure		2	13	2						
Transfer to Special Apparatus Fund		1	8	0						
					4	1	2			
								–	–	–
M. PARKE FUND:										
Grant received during the year					208	0	0			
Less: Expenditure					318	19	2			
BALANCE DUE TO GENERAL FUND, as per contra					£110	19	2			
CAPITAL RESERVE ACCOUNT:										
Arising from revaluation of Library and other assets								17311	8	2
SURPLUS:										
As at 31st March 1940					7095	19	4			
Less: Deficiency for the year as per Income and Expenditure Account		122	6	6						
Income Tax recovered transferred to Special Funds ...		100	13	0						
					222	19	6			
								6872	19	10
								£33,870	16	6

Note: The above Surplus will be supplemented by receipt of the balance of compensation due from H.M. Government for Hire of S/S "Salpa", the amount of which has not yet been ascertained.

CASH AT BANK AND IN HAND:										
Coutts & Company—Current Account					101	0	10			
Lloyds Bank Limited—Current Account ...					328	16	0			
Cash in Hand					48	9	3			
								478	6	1
RECOVERABLE EXPENDITURE:										
Building Extension Fund as per Contra... ...					54	3	2			
M. Parke Fund as per Contra					110	19	2			
								165	2	4
								£33,870	16	6

E. J. ALLEN
L. A. HARVEY } *Members of Council.*

TO THE MEMBERS OF THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM:

We report that we have examined the above Balance Sheet with the books of the Association and have obtained all the information and explanations we have required. Capital expenditure on erection of Buildings on Land held on Lease from the War Department is excluded. Subject to this remark we are of opinion that the Balance Sheet is properly drawn up so as to exhibit a true and correct view of the state of the Association's affairs according to the best of our information and the explanations given to us and as shown by the books of the Association.

Shinners Bridge, Totnes, S. Devon.
2nd June, 1941.

PRICE, WATERHOUSE & Co.

E. T. BROWNE BEQUESTS

STATEMENT OF FUNDS AND INVESTMENTS 31ST MARCH, 1941

	£	s.	d.
BUILDING FUND INVESTMENTS at Cost:			
£1069. 9s. 7d. 3% Conversion Stock	1050	8	5
(Market value at date £1088. 4s. 0d.)			
LIBRARY FUND INVESTMENT at Cost:			
£1049. 8s. 7d. 3% Conversion Stock	1030	19	7
(Market value at date £1067. 15s. 11d.)			
SPECIAL APPARATUS FUND INVESTMENT at Cost:			
£2146. 2s. 7d. 3% Conversion Stock	2110	4	5
(Market value at date £2183. 14s. 9d.)			
SCIENTIFIC PUBLICATIONS FUND INVESTMENT at Cost:			
£1609. 11s. 11d. 3% Conversion Stock	1582	13	3
(Market value at date £1637. 15s. 3d.)			
	£5774	5	8

		£	s.	d.	£	s.	d.
BUILDING FUND:							
By Balance as at 31st March 1940		996	2	2			
„ Interest on 3% Conversion Stock, *less* Tax	18 5 2						
„ Income Tax recovered ...	17 12 7						
		35	17	9			
„ Transfer from Building Extension Fund ...		18	8	6			
					1050	8	5
LIBRARY FUND:							
By Balance as at 31st March 1940		995	14	10			
„ Interest on 3% Conversion Stock, *less* Tax	17 18 3						
„ Income Tax recovered ...	17 6 6						
		35	4	9			
					1030	19	7
SPECIAL APPARATUS FUND:							
By Balance as at 31st March 1940		2039	14	11			
„ Interest on 3% Conversion Stock, *less* Tax	35 16 7						
„ Income Tax recovered ...	34 12 11						
		70	9	6			
					2110	4	5
SCIENTIFIC PUBLICATIONS FUND:							
By Balance as at 31st March 1940		1529	16	2			
„ Interest on 3% Conversion Stock, *less* Tax	26 17 4						
„ Income Tax recovered ...	25 19 9						
		52	17	1			
					1582	13	3
					£5774	5	8

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31ST MARCH 1941

	£ s. d.	£ s. d.
To Salaries, including the Association's Contributions to Superannuation		7249 12 0
„ Laboratory Wages, including National Insurance and the Association's Contributions to Superannuation		2555 12 4
„ Depreciation of Library, including Valuation Fee		368 13 3
„ Scientific Publications, *Less* Sales		591 12 4
„ Upkeep of Laboratories and Tank Rooms:		
Buildings and Machinery	208 19 8	
Electricity, Gas, Coal, Oil and Water ...	353 4 7	
Chemicals and Apparatus	194 16 0	
Rates, Taxes and Insurance	109 3 7	
Travelling Expenses	104 13 8	
Stationery, Postages, Telephone, Carriage and Sundries	194 7 5	
Specimens	131 10 1	
Reinstatement of War Damage	133 11 6	
Air Raid Precautions	12 12 0	
		1442 18 6
„ Maintenance and Hire of Boats:		
Wages, including Diet Allowance, National Insurance and Casual Labour	976 15 8	
Coal, Water, Oil, Petrol, etc.	5 3 7	
Maintenance and Repairs, with Nets, Gear and Apparatus	87 15 9	
Purchase of Material for Nets for Sale, excluding Labour	13 5 3	
Boat Hire and Collecting Expenses	2 19 6	
Insurance	108 13 8	
		1194 13 5
„ Bank Interest (net)		2 19 9
„ Transfer to Repairs and Renovations Fund		50 0 0
		£13,456 1 7

	£ s. d.	£ s. d.
By Grants:		
Ministry of Agriculture and Fisheries Grant from Development Fund	10687 2 5	
Fishmongers' Company	600 0 0	
Royal Society	50 0 0	
British Association	50 0 0	
Cornwall Sea Fisheries Committee	10 0 0	
		11397 2 5
„ Subscriptions (excluding Subscriptions received in advance)		432 10 3
„ Donations		56 14 3
„ Sales:		
Specimens	808 19 4	
Fish	– – –	
Nets, Gear and Hydrographical Apparatus ...	49 12 11	
		858 12 3
„ Table Rents (including Universities of Oxford £52. 10*s.* 0*d.*, Cambridge £105, Bristol £25, London £105, Sheffield £5, Birmingham £15. 15*s.* 0*d.*, Manchester £10. 10*s.* 0*d.*, Leeds £10. 10*s.* 0*d.*; Trustees of Ray Lankester Fund £20)		446 0 0
„ Tank Room Receipts		126 2 9
„ Interest on Investments, *Less* Tax		9 0 0
„ Income Tax Recoverable		4 14 4
„ Sale of M. V. Lebour's Book		16 10
„ Sale of "Marine Fauna" of Plymouth ...		2 2 0
„ Balance being Deficiency for the Year ...		122 6 6
		£13,456 1 7

CONTENTS

The Council of the Marine Biological Association wish it to be understood that they do not accept responsibility for statements published in this *Journal* excepting when those statements are contained in an official report of the Council.

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