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THE ECOLOGY OF LAGOS LAGOON
IV. ON THE REACTIONS OF *BRANCHIOSTOMA NIGERIENSE* WEBB TO ITS ENVIRONMENT

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Estimations have been made of the salinity tolerance of the larva and adult of the lancelet *Branchiostoma nigeriense* and the effects of fluctuating salinities on its ciliary feeding mechanism. The results obtained have been correlated with the times of occurrence and the rates of growth of these lancelets in the Lagos area.

The reactions of larval and adult lancelets to changes in temperature have been observed and the range of temperature within which these animals can survive has been determined.

The behaviour of larval lancelets in salinity gradients has been studied. It is shown that the reactions of the larva to salinity and temperature restrict the animal to waters of high salinity and temperature.

The distribution of adult lancelets in the Lagos area has been shown to coincide with that of sand deposits containing not more than 25 % of fine grains passing a 90-mesh to the inch sieve or 1·5 % of silt. Experiments have been performed confirming the preference of lancelets for this type of sand and indicating the factors which lead to its selection by the animal through a process of trial and error.

The behaviour of *B. nigeriense* is discussed. The distribution of this lancelet is shown to be a result of its reactions to environment.

Introduction

In a previous paper (part III) on the life history of *Branchiostoma nigeriense*, it was noted that the distribution and seasonal incidence of this lancelet in Lagos Harbour followed a distinct pattern. Since the lancelet was not found everywhere in the region nor at all seasons, it seemed clear that its distribution must have been governed by factors of which salinity tolerance was certainly one and the nature of the bottom deposits another. It was decided to determine how far these factors were responsible for distribution and in what ways the behaviour of the animal and its reactions to different environmental conditions led to the congregation of adults in definite areas.

The lancelet is peculiar in so far as it combines a feeding mechanism of a type usually associated with sedentary animals with powers of locomotion comparable to those of macrophagous forms. The behaviour of such an animal, therefore, is likely to be of interest in showing how the apparently opposed requirements of ciliary feeding and active swimming are met. The sensory reactions of lancelets have been studied most comprehensively by Parker (1908), who also gives a full bibliography for the subject prior to that date, but work on their behaviour is limited to a few observations on their mode of swimming and burrowing. In the present paper the behaviour of the lancelet and its physiological reactions are related to the differences in environment likely to be met in nature.

THE SALINITY TOLERANCE OF LARVAL AND ADULT LANCELETS

Both the larva and the adult of *B. nigeriense* in Lagos Harbour and Lagos Lagoon are subject to frequent and, at times, drastic changes in salinity (see parts II and III). In order to survive, therefore, these lancelets must have some degree of tolerance to salinity change and some powers of adaptation to waters of low salinity. The degree of change they can withstand and the limits of salinity in which they can survive must be important factors determining first the time at which invasion of the harbour by larvae takes place and, second, the extent to which the adults can penetrate the brackish lagoons. Very little work on the salinity tolerance of lancelets has been reported in the literature. Parker (1908) showed that, in *B. caribaeum*, dilution of sea water with up to an equal volume of fresh stimulated this lancelet to vigorous swimming. He also confirmed observations made by Bert (1869) and Johnston (1905) that lancelets quickly die in fresh or very dilute sea water. Chin (1941), however, working with *B. belcheri*, measured the salinity range within which that species can live normally. He found that lancelets in water of salinity below 19·2 or above 29·0 parts per thousand* all died, but that death occurred in a matter of minutes rather than days at salinities of 14 p./10^3 or lower and at salinities above 40 p./10^3. He concluded, therefore, that the salinity range of *B. belcheri* was 19·2 to 29·0 p./10^3. It is known, however, that *B. nigeriense* tolerates a wider range of salinity than this (see part III), so it is evident that the powers of osmoregulation are not the same in the various lancelet species. In order to relate the distribution of this species to the salinity of the water in which it is found it is necessary to determine precisely the limits within which it can survive.

A series of experiments on both larvae and adults of *B. nigeriense* was carried out to find the lower limit of salinity at which survival was possible. Larvae and adults which had been kept in sea water of salinity between 25 and 30 p./10^3 were placed in batches in waters of different salinity covering the range from 0 to 25 p./10^3. At the intervals from 1 to 120 h shown in figure 1, lancelets from each batch were returned to the original sea water in which they had first been kept to determine whether the effects of immersion in water of low salinity were permanent, or whether recovery took place. These experiments were designed to show first the lowest salinity or threshold value to which the larva and the adult could become accommodated after prolonged immersion, second, the periods for which the larva and the adult could withstand immersion in water of salinity below that threshold value and still recover on return to a higher salinity, and, third, the effects of osmotic change above the threshold salinity. All experiments were repeated several times. The results obtained are summarized in figure 1.

These experiments showed that five 'zones' representing the effects on lancelets caused by different salinity ranges in conjunction with time of immersion could be recognized. The zones are labelled *A* to *E* in figure 1. The salinities in zone *A* caused almost immediate death of the lancelets, while at those in zone *B* the animals were badly affected with respect to muscular movement and ciliary action, showed signs of swelling and loss of transparency and, even after return to water of higher salinity, did not recover and eventually disintegrated. Zone *C* represents salinities at which the animals were affected and

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* For this paper the words 'parts per thousand' will be contracted to p./10^3.

would have eventually died, but recovered on return to water of higher salinity. At the salinities in zone *D*, the animal was affected by the change from a higher salinity, but, after a length of time varying with the salinity, recovered and behaved normally. Zone *E* represents salinities at which the animals, on transfer from a higher salinity, were not badly affected and remained active and comparatively normal throughout long periods of immersion. Changes of salinity within this zone produced some effect usually causing slight reduction of activity and cessation of ciliary beat on parts of the gills or in the ilio-colonic region of the gut, but recovery was always more or less rapid and complete. The line of demarcation between zones *C* and *D*, therefore, represents the threshold value for salinity or the lowest salinity to which the animals became accommodated after prolonged immersion.

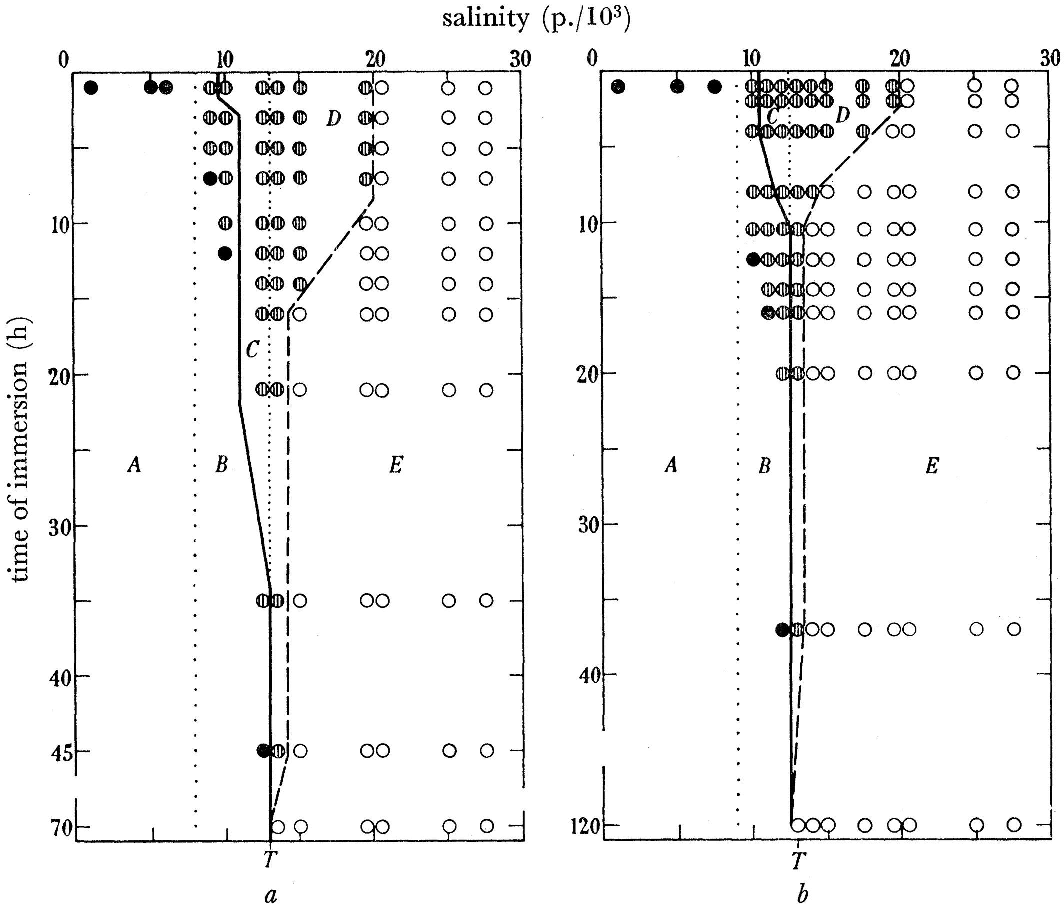

FIGURE 1. The salinity tolerance of: *a*, the larva; and *b*, the adult. ○, muscular movement, response to touch and translucency unaffected by reduced salinity; ◍, muscular movement, response to touch or translucency affected by reduced salinity; ●, death caused by low salinity; *T*, threshold value 13 p./10^3. Zone *A*: rapid death due to low salinity, ... Zone *B*: no recovery possible even after transfer to a higher salinity, ——. Zone *C*: recovery on transfer to a higher salinity, ... Zone *D*: recovery by adjustment to the low salinity; - - - -. Zone *E*: relatively unaffected by reduced salinity.

In the case of the larva, it is seen from figure 1 that death followed immediately on immersion in water of salinity 8 p./10^3 or less, and that the larvae inevitably died, though not immediately, after immersion at salinities from 8 to just below 10 p./10^3 (zones *A* and *B*). In salinities from 10 to 13 p./10^3, death occurred if the larvae were not returned to a higher salinity (zone *C*), but toward the upper limit of the range in this zone they were able to tolerate immersion for 20 to 30 h and still recovered in water of higher salinity. At salinities above 13 and below 20 p./10^3 the larvae became accommodated, although adjustment after transfer from a higher salinity in some cases took over 50 h at the lower end of the range (zone *D*). Transfer from sea water to water of salinity above 20 p./10^3 had little effect upon the larvae. The effect of changes around 20 p./10^3, however, was remarkable in so far as the transition from zone *E* to zone *D* was not gradual as might have been expected. Larvae transferred from sea water to water of salinity of 20·5 p./10^3 were only lightly affected if at all, while larvae transferred to water at 19·5 p./10^3 became immobile. It is evident from these results, therefore, that the threshold salinity for the larvae between zone *C* and zone *D* lies at or a little above 13 p./10^3.

The analysis of the experiments on the adult (see figure 1) shows a similar pattern to that for the larva, although there are some differences. In the adult, rapid death occurred at salinities below 9 p./10^3 and a slow death at 10 p./10^3 or a little above (zones *A* and *B*). Zone *C* for the adult, however, is more restricted, particularly in the time component, than has been found for the larva. At salinities of 10·5 to 12·5 p./10^3, adults could not tolerate immersion for more than 10 h without return to a higher salinity, and after this period were beyond recovery. Zone *D*, as in the larva, extends from 12·5 or 13 to 20 p./10^3, but is also reduced in the time component. Adjustment to the salinity at the lower end of this range, though not quite so slow as in the larva, took over 40 h. Adjustment at the higher salinities of the range, however, was much more rapid than in the larva, pointing to more efficient osmoregulatory mechanisms in the adult. As in the larva, transfer from sea water to water of salinity above 20 p./10^3 had a comparatively slight effect on the adult compared with transfer to a salinity below this figure. Thus the transition from zone *E* to zone *D* was equally abrupt. The threshold salinity for the adult, therefore, is between 12·5 and 13 p./10^3 and is similar to, though perhaps a little lower than, that for the larva.

Measurements on the upper limit of salinity which *B. nigeriense* can withstand have been made on the adult but not on the larva. As lancelets will only be subject to salinities above that of sea water where they are cut off from the sea in salt pools or lakes concentrating by evaporation, it is desirable that experiments to determine the upper limit of salinity should imitate those conditions as closely as possible. Accordingly, 20 adult lancelets were confined in a brevet of sea water open to the air to permit evaporation. The behaviour of these lancelets was in every way normal for a period of about 2 months, until one morning all were found lying on the surface of the sand in the bottom of the jar incapable of movement and showing distinct signs of opacity. A sample of the water was taken and found to be of salinity 58·9 p./10^3. Aerated distilled water was then added to the brevet to reduce the salinity to about 40 p./10^3. The majority of the lancelets showed no signs of recovery and were removed, but three remaining did recover and were still active and normal in their behaviour a month later. It is clear, therefore, that the adult of *B. nigeriense* can become adapted to salinities approaching 60 p./10^3 and that the upper limit of 58·9 p./10^3 is quite sharp. A similar experiment was not possible with larvae as they could not be kept alive in the laboratory for so long a period.

It would appear from these comparisons of salinity tolerance that the adult is rather better adapted to withstand changes in salinity in brackish waters than the larva and so is more suited for life in Lagos Harbour and Lagos Lagoon. Nevertheless, during certain periods of the year, diurnal fluctuation in salinity in the harbour is severe (see part II) and one of these periods from May to June coincides with the presence of adults in those waters. Thus further experiments were carried out to show the effect of repeated salinity changes on the adult. Adult lancelets, which had been kept in water of salinity 31 p./10^3, were transferred to water of salinity 23 p./10^3 for 8 h and then returned to the higher salinity for a similar period. This process was repeated for eight changes, after which the animals appeared to be unaffected. The range of fluctuation was then increased, the animals being placed alternately in water of 31 and 14·5 p./10^3. At the end of a series of five changes the lancelets had become rather opaque, but were still active. Thus changes equivalent to a diurnal fluctuation in salinity of 8 p./10^3 were tolerated by the animal, but fluctuations of 16·5 p./10^3 were evidently a little beyond its powers of adjustment. These findings did not appear to be in accordance with the results of the previous experiments on salinity tolerance in so far as the range of fluctuation to which the animals were exposed would have been expected to have had more serious effects. Detailed observations, therefore, were made on the process of adjustment to salinity change. A number of young adult lancelets which had been kept in water of salinity 25 p./10^3 for 7 days were transferred to water of salinity at the threshold value of 13 p./10^3. At various intervals from 10 to 90 h after the transfer some of the animals were returned to their original water and their condition observed over the period of the experiment. The results obtained are given in figure 2.

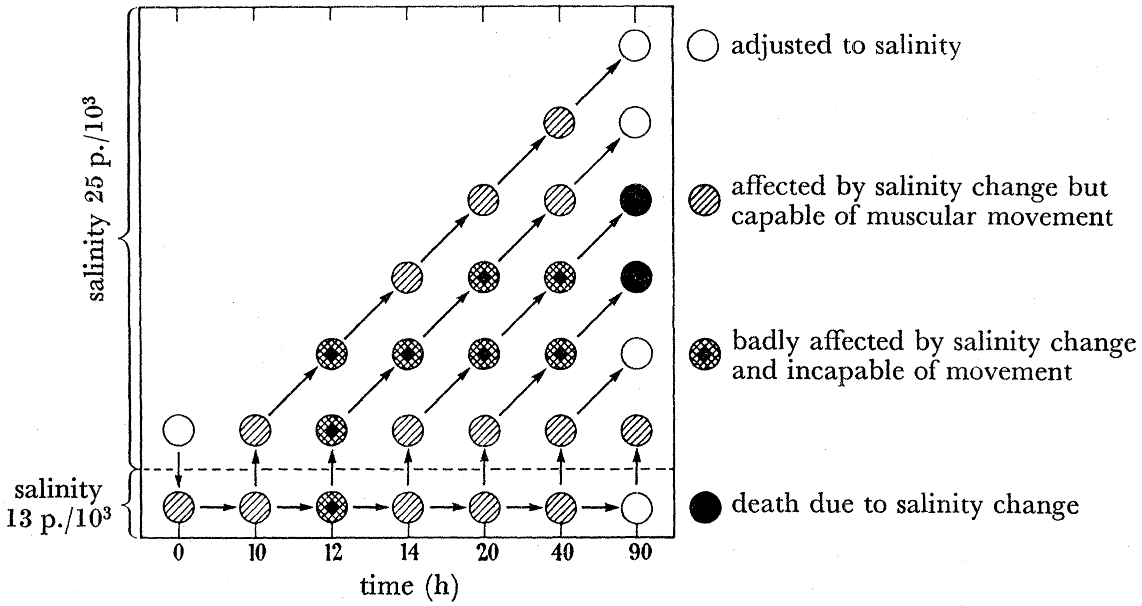

FIGURE 2. Diagram showing the rate of accommodation of young adult lancelets to salinity changes between 25 and 13 p./10^3.

On first transference to the lower salinity the lancelets became rather opaque and muscular movement was impaired. Their condition deteriorated until, by 12 h, they had become incapable of movement. At the 14 h observation, however, they were again able to move and their condition continued to improve until, after 90 h immersion, the animals had returned to normal. Thus accommodation to the low salinity was accomplished in 40 to 90 h. Those lancelets which were returned to the higher salinity after 10 h immersion at the lower, however, did not recover immediately and 2 h after this second transfer became immobile. They then slowly recovered to normality. The immobile animals returned after 12 h remained immobile at the higher salinity for a much longer period than those kept at the low salinity, but by the 40 h observation were recovering and had become normal before the 90 h observation was made. On the other hand, those lancelets which had been 14 and 20 h respectively in the water of low salinity and were recovering from a period of immobility, when transferred once more to a high salinity, did not continue to recover, but again became immobile and finally died. In contrast, those animals which had been kept at low salinity for 40 h before transference to the high salinity became normal by the end of the experiment. When the lancelets which had become fully adjusted by 90 h immersion in the water of low salinity were returned to a high salinity they were affected in the same way as they had been by the reverse change at the beginning of the experiment. These results not only give a measure of the time needed for adjustment to the change in salinity to be accomplished, but also show that, during the process, there is a critical period between about 14 and 20 h at the low salinity when a further osmotic shock due to a rise in salinity, far from hastening recovery, in fact may cause death. It is very unlikely that such conditions as these would ever obtain in nature and certainly not in the sea. Fluctuations in salinity even greater than that used in this experiment occur in the harbour at Lagos and in Five Cowrie Creek at the end of May (see part II), but as the periodicity of the fluctuation is only about 6 h, the critical period of adjustment will not be reached and, under these circumstances, as shown previously, the animal is able to survive. Moreover, the salinity could fall below the threshold value of 13 p./10^3 for a short period without causing death (see figure 1), so that until salinities in the harbour fall to 10 p./10^3 the lancelets there are not likely to be destroyed.

These findings can be checked against the salinities of the waters at the times of appearance and disappearance of lancelets in the harbour and lagoon. The first appearance of adults at Onikan and in the lagoon depends not only on the salinity tolerance of the adult but also on that of the larva. Settlement of lancelets occurred at Onikan on 2 January 1954 when the salinity at low tide was 16·6 p./10^3 and therefore minimal. Settlement in the lagoon occurred on 20 January 1954, when the salinity at low tide was 16·1 p./10^3. This salinity has been shown to be sufficiently above the threshold value to permit survival of the larva. The disappearance of lancelets in May or June, on the other hand, depends on the salinity tolerance of the adult alone. Unfortunately it is not possible to determine the time of disappearance of adults with the same accuracy as that of their arrival. At the end of May and in June the lancelet population is reduced more or less gradually until only the odd specimen, with perhaps greater tolerance than the average, can be found with difficulty. Moreover, the salinity figures available are those for the water above the sand and not for the water in the sand in which the lancelets live. Nevertheless, the salinity values taken at Onikan and in the lagoon at the time of disappearance are of interest. At Onikan in the middle of May 1954, adults were common when the salinity at low tide was 16·8 p./10^3 and at high tide 25·7 p./10^3. Two weeks later the low tide salinity had fallen to 5·9 p./10^3 and the high tide salinity to 20·2 p./10^3. At this time lancelets were rare. In mid-June the low tide salinity was 5·3 and the high tide 15·8 p./10^3 and on this occasion the last specimen was found with great difficulty. It is impossible from these figures to give an accurate end-point, but the salinity was clearly appreciably lower than that at which settlement took place. It is evident also from these figures that the salinity of the water in the surface layers of the sand must have been considerably higher than that of the water above the sand at low tide. In the lagoon, the diurnal fluctuation is not so great as at Onikan and a better estimate is possible. At Ikoyi Jetty in Lagos Lagoon on 3 April 1954, adults were common when the low tide salinity was 17·9 and the high tide 19·6 p./10^3. Two weeks later the salinity had fallen to 16·2 at high tide and 13 p./10^3 at low tide and adults were rare. On 18 May, one specimen was taken, the last to be seen, when the salinity at both high tide and low tide was 11·4 p./10^3. Allowing for the fact that, with a falling salinity, the water in the sand is likely to be at a slightly higher salinity than the water above the sand, this figure is in very close agreement with the experimentally determined threshold value for the adult of between 12·5 and 13·0 p./10^3.

THE EFFECT OF SALINITY FLUCTUATION ON CILIARY FEEDING

In a previous paper (part III) it was shown that the rate of growth of lancelets in Lagos Lagoon is about six times more rapid than at Onikan and considerably faster at both these localities than in the sea. These differences in growth rate could be due to the quantity of food available to the animals or their ability to absorb it. Both at Onikan and in the lagoon north of Ikoyi Island the nature and the organic content of the deposits in which lancelets are found is essentially similar (see parts II and III), thus suggesting that the rate of flow of the water and the organic material it carries is also not very different at the two localities. It is difficult, therefore, to account for the difference in growth rate observed on grounds of lack of food at Onikan as compared with the lagoon. In the case of the marine populations living in the clean sand east of the harbour, however, the slow rate of growth can probably be attributed to the limited food supply in the sea (see part III).

The chief difference between Onikan and the lagoon area lies in the salinity of the water. In the lagoon the salinity is lower than at Onikan, but the diurnal fluctuation is slight. At Onikan the fluctuation in salinity is always greater than in the lagoon and can reach considerable proportions at certain times of the year. Although osmoregulation accounts for part of the metabolism of animals living under conditions where the salinity is constantly changing, the energy involved in achieving adjustment to the change is relatively small and could have little effect on growth rates. On the other hand, it is possible that salinity fluctuations interfere with the ability of the animal to take up food by causing an interruption of the ciliary feeding mechanism. With this in view the effects of changing salinity on the action of the gill cilia in both larval and adult lancelets were examined.

Larvae that had been kept in water of salinity 33 p./10^3 were subjected to a series of lower salinities and the effect of each change in concentration on the beating of the gill cilia was observed. The larvae were placed in Petri dishes in the water of high salinity. To avoid undue disturbance this water was removed with a pipette before that of a lower salinity was added to the dish. The immediate effect of the change was a temporary cessation of the ciliary beat of a duration dependent upon the magnitude of the osmotic shock to which the animal had been subjected. Replacement of the water in the dish by water of the same salinity in certain cases produced a stoppage of ciliary beat for 2 or 3 min. This was evidently due to mechanical disturbance and has been allowed for in the results of the experiment given in figure 3. These results show that there was a gradual lengthening of the period of interruption of the beat from about 6 min after transfer from a salinity of 33 to 31 p./10^3 to about 12 min after transfer to a salinity a little above 19 p./10^3. Thereafter the period of cessation greatly increased, transfer from 33 to 17·7 p./10^3 causing the cilia to stop beating for 130 min and transfer to 14·5 p./10^3 for 150 min. The graph in figure 3 indicates that there is a critical salinity of about 19 p./10^3. Larvae accustomed to a higher salinity could not tolerate immersion in water of salinity below this concentration without severe effect on the ciliary action of the gills.

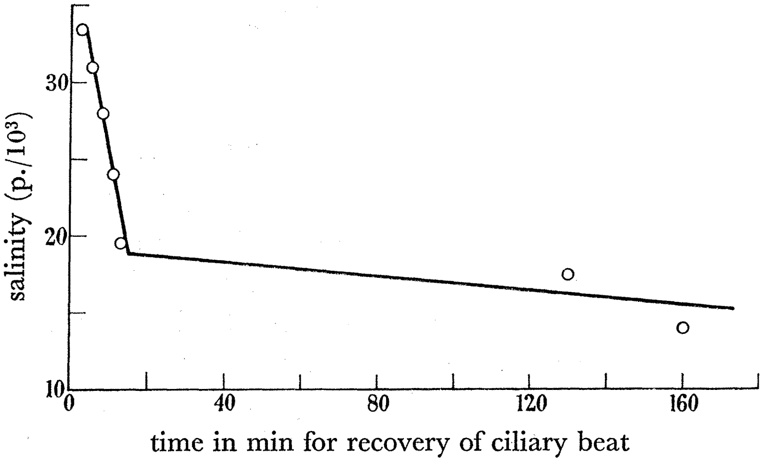

FIGURE 3. Graph showing the periods of immobility of the gill cilia of lancelet larvae following different reductions in salinity from 33·5 p./10^3.

A second series of experiments was conducted to confirm these findings and to show in addition the effect of changes from both low and high salinities to water of about 20 p./10^3. Again a slow increase in the period of ciliary immobility followed an increase in the degree of change from a high to a low salinity as shown in figure 4, but this period greatly increased when the change involved a passage to salinities below 19 p./10^3. It was also found that larvae accustomed to a salinity of 13·5 p./10^3, when placed in water of salinity 21 p./10^3, suffered a severe ciliary check of 40 min, while others transferred from 28·5 p./10^3 to a salinity of 21 p./10^3, a reverse change of similar magnitude, suffered a ciliary check of only 15 min. It is evident, therefore, that the rise in salinity to 21 p./10^3 had a greater effect on the larva than the corresponding fall to that figure. Thus, although the threshold salinity for the larva is a little above 13 p./10^3, salinity changes across the value of 19 p./10^3 appear to have a far greater effect on the larva than changes in other regions of the salinity range.

Similar experiments were carried out on the adult and the results are shown in figure 4. Again there appeared to be a critical salinity at about 20 p./10^3 at which adjustment was most difficult, but there was a considerable difference in effect according to the direction in which the change was made. Adults accustomed by long immersion to a salinity of 19 to 20 p./10^3 when transferred to water of either higher or lower salinity showed no appreciable stoppage in the beat of the gill cilia. On the other hand, transfers from either high or low salinity to a salinity of about 20 p./10^3 caused an interruption of the ciliary beat of 50 or 60 min. Similarly, transfers from salinities above to salinities below this critical figure, or transfers in the reverse direction, brought about as great or even greater interruptions in the feeding mechanism. The small reduction in salinity from 33·5 to 31 p./10^3 caused a 5 min cessation of beat.

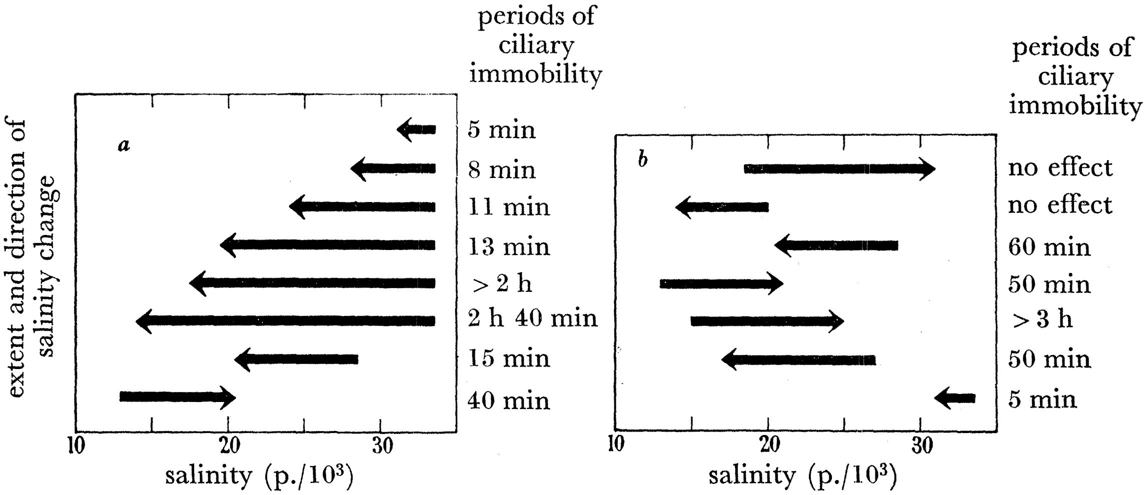

FIGURE 4. The effect of salinity change on the ciliary beat in *a*, larval, and *b*, adult, lancelet gills.

The results of these experiments show that considerable interference with the feeding mechanism in both the larva and the adult is likely to follow certain salinity fluctuations rather than others and thus, by affecting the quantity of food absorbed, give rise to differences in the rate of growth. Where adult lancelets have become accustomed to a salinity of 19 to 20 p./10^3, even quite considerable fluctuations have little effect on the ciliary feeding mechanism. It would appear to be significant, therefore, that these are the salinity conditions which obtain in Lagos Lagoon in the months of mid-January to mid-May when lancelets are to be found in the sand deposits, the mean salinity for this period being about 19 p./10^3 with a total fluctuation of ± 4 and a diurnal fluctuation at no time greater than $\pm 1{\cdot}5$. In Five Cowrie Creek at Onikan, on the other hand, the mean salinity at this time is 25 p./10^3, well above the critical value of 20, the total fluctuation is ± 7 and the diurnal fluctuation as much as ± 5 p./10^3 (see part II). The salinity fluctuations at Onikan, therefore, are such as would interfere with the ciliary feeding mechanism of lancelets for appreciable periods each day, whereas, in the lagoon, lancelets adjusted to the critical salinity should suffer no ciliary check. It is difficult to estimate the total effect of fluctuation in salinity at Onikan on the growth of lancelets there, but it may well have been sufficient to account for their small size as compared with those from the lagoon. The periods of immobility given in figure 4 represent the times of total stoppage, but this was followed by only partial recovery, often for a much longer period of variable extent, so that the interference in feeding caused by fluctuation was likely to be greater than is apparent from the times given. Salinity fluctuation in Five Cowrie Creek, therefore, appears to be the most likely cause of the slow rate of growth of lancelets at that locality.

THE TEMPERATURE TOLERANCE OF LARVAL AND ADULT LANCELETS

As lancelets occur in both tropical and temperate seas, different species would be expected to show different degrees of temperature tolerance according to their distribution. Certain species, notably *Branchiostoma lanceolatum* and *B. belcheri*, are found in both temperate and tropical regions (see Webb 1956*a*, *b*, *d*, 1957) and thus would be expected to tolerate a wide range in temperature, but most species are more limited in their distribution. *B. nigeriense* is known to occur only on the Nigerian coast (see Webb 1955, 1956*c*) and it is of interest to determine how far the restricted range of this species is governed by reaction to different temperatures. Parker (1908) investigated the effects of different temperatures on *B. caribaeum*, a tropical species living in water at about 30 °C. He found that an increase in temperature up to 37 °C caused the lancelet to become more active, but that at 40 °C death occurred. At temperatures below 30 °C these lancelets swam energetically, but at 10 °C and lower they became quiescent. It is evident that these are the reactions of a tropical species which could not live in temperate seas where temperatures below 10 °C are common. Chin (1941), working with *B. belcheri* from Amoy on the Chinese coast, recorded that the temperature of the water in which these lancelets live ranges from 12·0 to 30·5 °C, but he did not suggest that these were the extremes tolerated by that species. The fact that *B. belcheri* can live at temperatures above 30 °C no doubt accounts for the survival of this species in the tropical waters of the East Indies and East Africa (Webb 1956*a*, 1957) as well as in the cooler seas of China and Japan. In this respect the temperature tolerance of *B. belcheri* appears to differ somewhat from that of *B. caribaeum*. The temperature requirements of other species are not known.

Although the water of Lagos Harbour and Lagos Lagoon is always at a relatively constant and high temperature of 25 to 30 °C, lancelets living in the intertidal zone may be subject to even higher temperatures at low tide when the shore is exposed to the sun (see part II). To determine the limits of temperature which *B. nigeriense* can withstand, experiments were carried out on both the larva and the adult and the effects of progressive changes of temperature observed.

Larvae were placed in a beaker of sea water of salinity 28 p./10^3 and at a room temperature of 26 °C. They were almost transparent and were mostly lying on the bottom except for an occasional rapid excursion to the surface. These larvae were considered to be of normal behaviour and in good condition. On lowering the temperature to 24 °C all larvae became active and swam rapidly to the surface where they remained swimming more or less continuously as the temperature fell still further. The rate of the swimming movements, however, decreased with the temperature until, at 12 °C, the movements were so slow as to be insufficient to counteract the effects of gravity and the larvae then sank to the bottom of the beaker. At 8 to 9 °C none of the larvae showed movement and none responded to the stimulus of touch. The reduction in temperature from 26 to 9 °C occupied a period of 2 h. The water in the beaker was then allowed to return slowly to room temperature. At 10 °C a few larvae showed signs of movement, but most remained inactive and none showed the continuous swimming movements characteristic of their behaviour during the fall in temperature. This condition continued until 22 °C was reached, when most larvae responded to touch, but only with rapid movements of short duration.

When the temperature had risen to 28 °C, however, spontaneous swimming occurred in some larvae, but not all. At 30 °C, 3 h later, all larvae were normal, capable of active spontaneous movement and responded vigorously to touch.

To determine the effect of low temperature for more prolonged periods, larvae were cooled to 9 °C, showing in each case the characteristic pattern of behaviour during cooling, and held at that temperature for periods of 5 and 24 h respectively. During these periods all remained immobile and unresponsive to touch. On return to room temperature, however, all larvae recovered and behaved normally.

The effect of an increase in temperature was also observed. Larvae, mostly quiescent at 26 °C, were gradually warmed. At 30 °C they remained quiescent but reacted very violently to touch. At 32 °C some showed violent spontaneous activity which increased until 36 °C was reached. At 37 °C many became inactive, but responded to touch. As the temperature rose further, both activity and the degree of response to touch decreased until at 39·5 °C all were inactive and unresponsive and were becoming opaque. It was clear that a further rise or prolonged immersion at that temperature would have caused death. The beaker was then allowed to cool to room temperature and, $1\frac{1}{2}$ h later, when the temperature had reached 31 °C, all larvae behaved normally and were responsive to touch.

Similar experiments were repeated with adults. In this instance, as the temperature fell, the animals behaved normally until 17 °C was reached, when they began to perform slow, more or less continuous swimming movements, quite unlike the normal rapid, sporadic swimming of the adult, and did not respond to the stimulus of touch. These movements became more sluggish with falling temperature until, at 13 to 14 °C, movement was very slight and finally ceased at 10 °C. The temperature was held at 10 °C for 4 h and then allowed to rise. The animals showed immediate signs of recovery and passed through the same pattern of behaviour with slow swimming movements until 17 °C was reached, when normal behaviour was resumed, swimming movements ceasing and the animals reacting very sharply to touch.

When the temperature was raised above the normal of about 30 °C adults showed increasing activity, but otherwise behaved normally until a temperature of 37 °C was reached. They then showed a lack of muscular co-ordination followed at 38·5 °C by immobility except for an occasional muscular quivering. The temperature was held at 38·5 °C for 4 h and then lowered to 34 °C when the animals began to show signs of recovery which was found to be complete 15 h later. Adults raised to 40 °C became immobile and opaque and did not recover on transfer to a lower temperature. Thus the temperature range for *B. nigeriense* and the reaction of the adult to temperature changes appears to be similar to that described by Parker (1908) for *B. caribaeum.*

In these experiments the reactions of the larva and the adult to change of temperature were different. Although falling temperature stimulated both the larva and the adult to continuous activity, the temperature at which this reaction occurred was not the same in each case. Moreover, whereas the same sequence of behaviour was repeated in the adult on return to a higher temperature, this was not so in the larva. The temperature range each can withstand is about 12 to 37 °C, and within this range they can survive for long periods without apparent harm, both larvae and adults having been kept for days near the extremes of the range. Whether the species could survive indefinitely at these extreme temperatures, however, is not known. It is clear that the reaction of *B. nigeriense* to low temperatures is likely to restrict this lancelet to warm tropical seas, while the ability of the adult to withstand temperatures up to 37 °C for long periods is important in determining its distribution in the intertidal zone where temperatures as high as this occur (see part II).

THE EFFECT OF SALINITY AND TEMPERATURE GRADIENTS ON THE VERTICAL DISTRIBUTION OF LANCELET LARVAE IN PLANKTON

Observations made on plankton taken in Lagos Harbour have indicated that, where a vertical salinity gradient exists, the larva may be able to react to a change in salinity in such a way as to maintain itself in waters with a salt concentration high enough for survival (see part III). Salinity gradients ranging from near-fresh water at the surface to salt water in the depths occur in Lagos Harbour at the beginning and end of periods of rain (see part II). On 26 November 1954, larvae were very numerous in the flood-tide water at all depths at the harbour mouth, but, except for a few dead or dying specimens, were absent from the surface water at the ebb tide of the following day when the salinity had fallen to 9 to 10 p./10^3. This showed that larvae caught by the flow of brackish water, which is very considerable at this time of the year, did in fact die.

Two weeks later, on 13 December, larvae were absent from a horizontal trawl taken on the ebb tide near the surface when the water was brackish with a salinity subsequently found to be 13·35 p./10^3. Active larvae were present, however, in large numbers in a vertical haul from the bottom, but, on transfer from the net to jars of surface water, rapidly became opaque and inactive. Thus, larvae in good condition were present in the deeper water, which could be presumed to be of high salinity. The larvae were kept in the surface water of 13·35 p./10^3 for subsequent observation. The following day jars of sea water of salinity 28·4 p./10^3 were obtained. Larvae were still absent from the surface water on the ebb tide but present in the deeper layers. Some of these larvae collected in a vertical haul were placed in the jars of high-salinity sea water and others in jars of the surface harbour water which, on this day, was of salinity 12·85 p./10^3. The larvae in sea water were transparent and active and were still in good condition after 16 days, while those in the jars of surface water of salinity 12·85 p./10^3 rapidly became opaque and inactive and did not recover. On the other hand, the larvae taken the previous day and placed in surface water at 13·35 p./10^3 did recover, and a few were still alive 16 days later. These observations confirm the findings of the previous experiments on the salinity tolerance of the larva that the lower threshold salinity is about 13 p./10^3 and show that the larvae can remain in large numbers in water of high salinity overlaid by a stratum of brackish water in which they could not survive. Two weeks later, on 28 December, the salinity of the surface water at the harbour mouth had risen to well above 20 p./10^3 and larvae were present in both horizontal and vertical plankton hauls. Thus it may be presumed that the absence of larvae from the surface waters, when the salinity was low but slightly above the threshold value, was not due to vertical movement in response to light.

The lancelet larva sinks quite rapidly in still water and the larger the size of the larva the more rapid the rate of fall. From measurements made on larvae of different sizes in sea water, it was found that very small larvae at about the 8-gill-pouch stage sank at the rate of 1 cm in 20 s, larvae of medium size with from 12 to 16 gill pouches sank 1 cm in 8 to 10 s and large larvae at the 22-gill-pouch stage or above in 4 s. Clearly the rate of fall under natural conditions will also depend on the specific gravity of the water and hence the salinity, the degree of turbulence and the activity of the animal. Observations on larvae kept in the laboratory showed that activity is sporadic, bursts of swimming alternating with long periods during which the animal remained quiescent, resting on the bottom of the container. Thus, except in turbulent or strongly flowing waters, the larva will sink to the bottom when inactive and will only rise by swimming. In the very young larva and also in the late larva approaching metamorphosis the body is straight, but in all other stages the body is shaped rather like a boomerang with the posterior part of the body bent sharply downward at an angle of about 60 degrees. This deflexion of the posterior region is evidently responsible for the fact that such larvae when swimming always rise toward the surface of the water, whereas the very young and the fully grown larvae with a straight axis do not necessarily do so. Rice (1880) also noted that lancelet larvae swim upward to the surface of the vessel in which they are contained. Thus young larvae will tend to remain at the bottom in the neighbourhood of the spawning grounds and larvae about to metamorphose will also tend to settle. Larvae in which the posterior region of the body is deflected, on the other hand, will become planktonic and leave the spawning grounds for the surface waters of the sea. This is evidently the reason why larvae younger than the 6- to 8-gill-pouch stage are not found in plankton samples except from turbulent waters and then only rarely (see part III).

To test the reaction of the larva to a salinity gradient, a burette was filled a quarter to a third full of water of high salinity containing larvae. Water of progressively lower salinities was then added, thus establishing a falling gradient from the bottom to the top of the burette. Salt water being heavier than fresh, the gradient remained stable in the narrow tube for a long period of time. Experiments on the temperature tolerance of the larvae have shown that a fall in temperature to 24 °C induces continual swimming movements until the temperature becomes so low that inactivity follows. By transferring the burette with the larvae to a refrigerated room, the temperature of the apparatus was brought below 24 °C and the larvae, stimulated to activity, swam steadily upward. It was noticed, however, that, when the larvae reached a certain point on the graduated scale of the burette, movement ceased and they sank back to the bottom. The movements of a number of different larvae and also the successive excursions of one larva were observed, and it was found that swimming ceased always at the same point in a given gradient. A change of gradient produced by adding different volumes of water of different salinity to the burette altered the point at which swimming stopped. Moreover, in a burette filled with sea water in which there was no gradient, the larvae swam to the surface of the water before sinking to the bottom. There was, therefore, nothing inherent in the apparatus or in the swimming powers of the larvae to prevent a rise throughout the length of the burette. Thus the point at which activity ceased must have been in response to a certain salinity in a given gradient. To determine this salinity, water was run off to within 1 cm of the level to which the larvae rose and then the next 2 ml were taken for salinity estimation. The figure obtained gave the average salinity for the region 1 cm on either side of the point where movement ceased and should, therefore, be a close estimation of the salinity at that level.

With larvae acclimatized to water of 25 p./10^3 in a salinity gradient extending from 25 to 12·5 p./10^3, activity ceased on reaching water of 20 p./10^3. Similarly, with larvae taken from water of 35 p./10^3 and subjected to a salinity gradient of 35 to 14·5 p./10^3, activity again ceased at 20 p./10^3. Thus it was evidently this salinity which was important and not the extent of the gradient through which the larva passed. In this last experiment it was noticed that activity tended to be resumed before the larvae reached the bottom of the burette. By drawing off water 5 cm above and 5 cm below the 20 p./10^3 level, an estimation of the slope of the gradient was obtained which showed that resumption of activity occurred at a salinity a little above 25 p./10^3. The region between 20 and 25 p./10^3, therefore, appeared to have a critical effect on behaviour such that the larva was restricted to waters of salinity above 20 p./10^3. This last figure was found to have particular significance in previous experiments testing the salinity tolerance of the larva and also the effects of salinity change on the beating of the gill cilia.

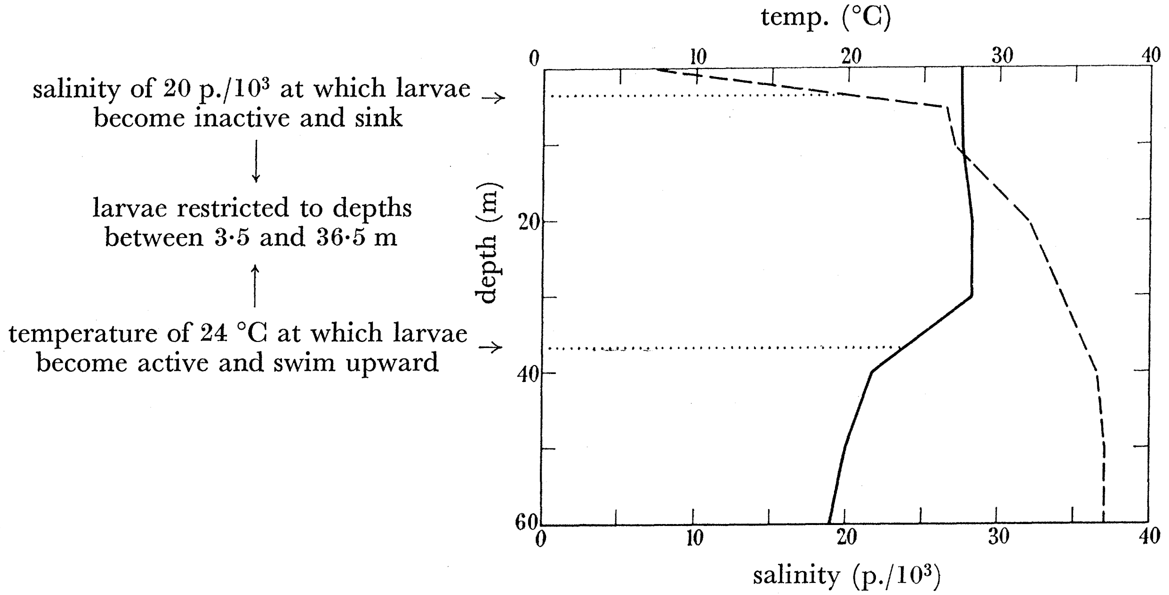

Figure 5. Diagram showing the changes of salinity (– – –) and temperature (——) with depth off the Nigerian coast and the water layer to which lancelet larvae are restricted.

This reaction to a salinity gradient must be considered in relation to the behaviour of the animal toward changes of temperature. In the waters off the Nigerian coast, the salinity of the sea increases with depth to about 35 p./10^3 at 33 m. According to the time of the year and the rate of flow of water from the rivers, the surface waters near Lagos Harbour may be of comparatively low salinity (communication from the West African Fisheries Research Institute). Conversely, the temperature of the water decreases with depth from 27·5 °C at the surface to 19 °C at 60 m (see figure 5). As the activity of the larva ceases on reaching surface waters of salinity 20 p./10^3, but is resumed either when the salinity rises above 25 p./10^3 or more certainly when the temperature falls to 24 °C, it is shown in figure 5 that the larva will be restricted to waters of depths between about 3 m (when the surface waters are brackish) and a maximum of 37 m, at which depth the temperature is 24 °C. It is to be expected, however, that the distribution of the larvae in the plankton will not be limited at its lower level by the salinity when the surface water is above 25 p./10^3. In Lagos Harbour, on the other hand, vertical salinity gradients occur at certain times of the year without a corresponding temperature gradient (see part II) and here the larvae will be restricted to the deeper waters of high salinity by their reaction to salinity alone.

THE RELATION BETWEEN THE DISTRIBUTION OF *BRANCHIOSTOMA NIGERIENSE* AND THE DEPOSITS IN LAGOS HARBOUR AND LAGOS LAGOON

All records of the occurrence of different species of the genus *Branchiostoma* giving details of the deposits in which the animals were found show that lancelets are restricted to bottoms of a sandy or shell-gravel type. The only work on the composition of deposits in relation to the distribution of lancelets, however, has been carried out by Chin (1941) who analyzed the sands in which *B. belcheri* is found at Amoy. From these analyses Chin concluded that lancelets are not found on muddy bottoms and he suggested that, as the pH tolerance of *B. belcheri* is between pH 7·4 and 8·6, the reason for this might lie in the low pH of the mud (pH 7·2) as compared with that of the sand (pH 8·4). However, his tables of analyses of various deposits in which lancelets were present or absent show that no lancelets were found where a high proportion of fine sand or silt was present, although lancelets were also absent in a few cases where this proportion was low. On the other hand, lancelets tended to be most numerous where the bottom was predominantly coarse sand. Thus there is evidently some relationship between the physical nature of the deposits and the occurrence of lancelets, although it is not clear from Chin's analyses what the relationship is or why it should exist.

The distribution of *B. nigeriense* in the harbour and lagoon at Lagos and in the sea immediately outside the harbour is not uniform, the animal occurring in the sand in certain definite regions but not in others (see part III). The seasonal distribution of these lancelets has been shown to be related to the extreme salinity changes which occur in the harbour and lagoon during the year, but the spatial distribution of the adults in the months of January to May must be governed by other factors, as the salinity at this time is above the threshold value for survival. It has already been shown (see part II) that the deposits in the Lagos area are varied, being derived from sands from the barrier beach and riverine silt mixed in proportions determined by the force and direction of the water currents and by wave action on the shores. When the distribution of lancelets (see part III) is compared with the distribution of the different deposits (see part II), it is clear that the animal occurs only where there are relatively undisturbed sands in which the proportion of fine grains passing a sieve of 90-mesh to the inch is not greater than 25%, or the proportion of silt not greater than 1·5% (see figure 6). The deposits over most of the area shown on the map in figure 6 are either of mud or of sand with a relatively high proportion of silt and are shown in black. In limited regions in the lagoon and harbour, but in more extensive areas along the coast at sea, sand deposits occur in which there is very little silt, but a high proportion of fine sand passing a 90-mesh to the inch sieve is present. These areas on the map are marked with diagonal lines. Sand disturbed by wave action occurs along the coast and on certain shores of the harbour and lagoon facing open stretches of water exposed to the wind. These shores have been marked with a series of undulating lines. The areas of sand with less than 25 % of fine grains and low silt content are shown in figure 6 in white, and it can be seen that there is a close agreement between the distribution of this last type of sand and that of the lancelets. There are, however, two areas where the deposits contain less than 25 % of fine sand or 1·5 % of silt and yet harbour no lancelets. The first of these is a strip of sand to the seaward side of Kuramo Water, and the second a small area immediately to the east of the harbour mouth (see figure 6). The absence of lancelets from Kuramo Water can be attributed to the fact that this lagoon communicates with Five Cowrie Creek only by a narrow and tortuous channel between 1 and 2 miles in length with a restricted water flow, and it is therefore doubtful whether lancelet larvae ever reach Kuramo Water. In the case of the sand to the east of the harbour, this area is used as a discharging point for the harbour dredger, the sand is continually disturbed and lancelets evidently do not remain under these conditions. There is little doubt, therefore, that, as Chin (1941) has suggested, the nature of the deposit plays an important part in limiting the distribution of lancelets. The reasons for this require to be investigated.

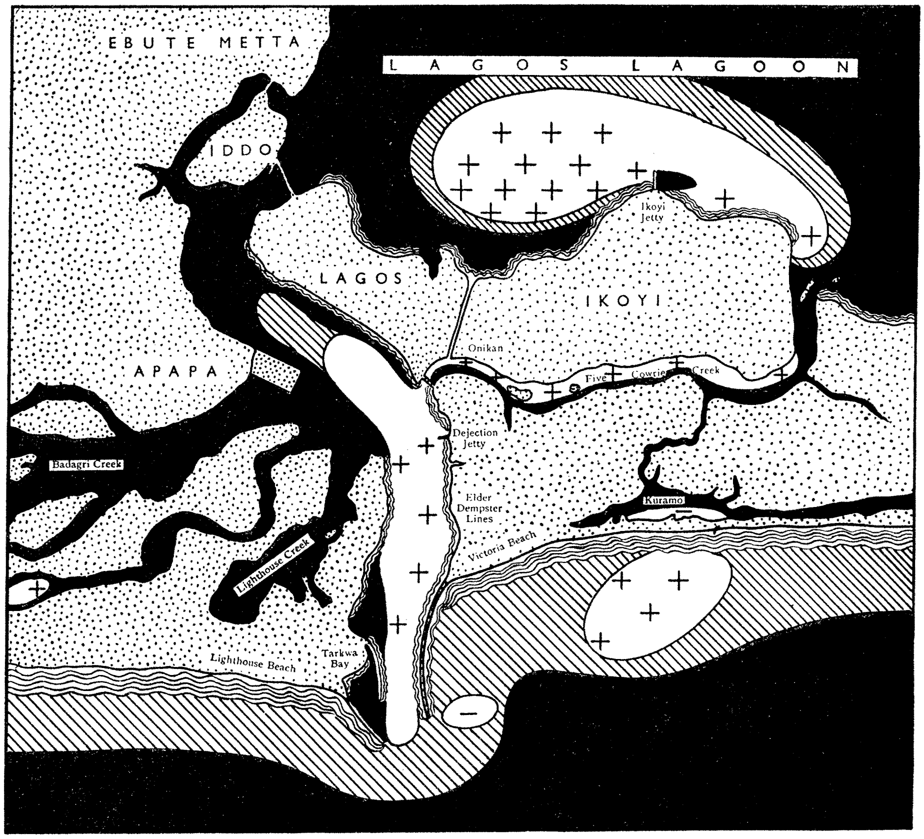

FIGURE 6. The effect of wave action and different grades of sediment on the distribution of lancelets. ≋, wave-beaten shore; ▭, sand with fine sand < 25 % and silt < 1·5 %; ▨, sand with fine sand > 25 % and silt < 1·5 %; ■, sand with silt > 1·5 % or mud; +++, lancelets present; ———, lancelets absent.

The reaction of lancelets to sands of different particle size

As the proportions of grains of different sizes in a sand seem to be important in determining its suitability for lancelets, a preliminary test was carried out to show the preference of lancelets for sands of different grain size. Accordingly graded sands were prepared by sieving and the following fractions obtained:

(1) Grains retained by a 30-mesh to the inch sieve (2·0 to 0·6 mm in diameter).

(2) Grains passing a 30-mesh but retained by a 60-mesh sieve (0·6 to 0·3 mm in diameter).

(3) Grains passing a 60-mesh but retained by a 90-mesh sieve (0·3 to 0·2 mm in diameter).

(4) Grains passing a 90-mesh but retained by a 100-mesh sieve (0·2 to 0·18 mm in diameter).

(5) Grains passing a 100-mesh to the inch sieve (0·18 to 0·1 mm in diameter).

As sieving was carried out with wet sand in fresh water, the fractions were dried and then placed in bowls each containing 500 ml. of sea water. Six lancelets of different sizes were placed in each bowl and left for 24 h. After this period it was found that, whereas in the coarser sands of grades 1, 2 and 3 the animals were active and swam rapidly when touched by a camel-hair brush, those in the sands passing the 90- and the 100-mesh sieves (grades 4 and 5) did not respond readily to touch, the body curving to an S-shape and only slowly recovering. Moreover, in sands of grades 2 and 3, the animals sometimes burrowed and remained for long periods either completely buried or with the oral end projecting above the surface, while in the fine sands (grades 4 and 5) there was little attempt to burrow and finally the animals lay inert on the surface of the sand. In the coarser sands, although the animals were very active they seemed to have some difficulty in burrowing. The experiment was repeated for a period of 48 h with similar results except that, at the end, the lancelets in the finest sand (grade 5) were moribund with an accumulation of sand particles in the gut and with the oral aperture blocked by a mass of grains held together by mucus. Large numbers of ciliates surrounded the bodies. The lancelets in sand of grade 4 were only slightly less affected. From these experiments it is concluded that lancelets cannot tolerate sands consisting entirely of grains small enough to pass a 90-mesh to the inch sieve. The most suitable sands appeared to be intermediate in grain size, passing the 30-mesh but being retained by the 90-mesh sieve. Burrowing did not appear to be essential to the animal as lancelets lying on the surface of the coarse grains or in a glass dish without sand remained fully active and behaved normally. Thus death of the animals in fine sand cannot be attributed to their failure to burrow and was more likely to have been due to occlusion of the oral aperture and the atriopore and interference with the respiratory function of the ciliary currents of the pharynx.

In these experiments lancelets were confined above the different grades of sand without the opportunity to seek other sands of a more suitable nature. To provide a choice of sand, a Perspex tank 24 cm × 24 cm × 10 cm was used in which the bottom was subdivided into nine equal trays 7·5 cm square and 2 cm deep as shown in figure 7. The four corner trays and the centre tray were filled with the five different graded sands and the tank then filled with sea water to a depth of 5 to 7 cm above the sand. Lancelets were distributed equally among the blank trays. After 24 h the trays were emptied and the number of animals found buried in each grade of sand was counted. The number of lancelets found lying in each tray, but not buried, was also counted. This experiment was performed four times. The experiments were started at approximately the same time of day and the box rotated through an angle of 90 degrees on each occasion to eliminate differences in illumination due to uneven lighting from windows. There was no evidence that the position of the source of light relative to the tank influenced the final distribution of the animals. The results of these experiments are given in composite form on a plan of the tank in figure 8. From these results it seems that sand with grains passing the 60-mesh but retained by the 90-mesh sieve was the most suitable for lancelets as the greatest number were found buried in this fraction. On the other hand, sands passing the 90-mesh sieve tended to be avoided and the numbers found buried in these grades were small. In sands retained by the 60-mesh and 30-mesh sieves respectively, a moderate number of lancelets was found. Thus some degree of selection of the more suitable fractions took place. This may have been either by an active choice of the most suitable sand, all others not fulfilling limiting conditions being rejected, or by a process of trial and error in which the length of time for which a lancelet remained buried increased with the suitability of the sand. The second method would

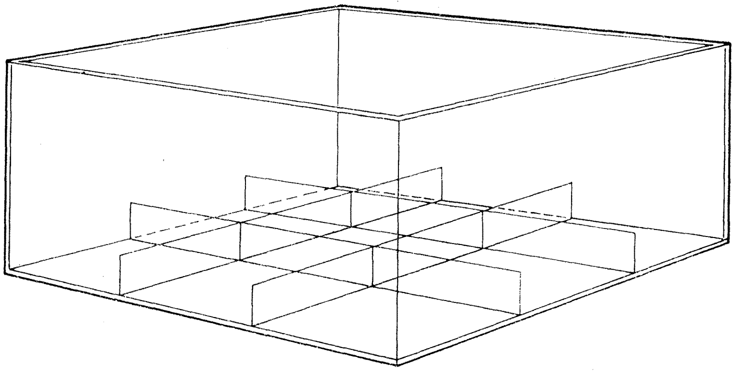

FIGURE 7. Perspex tank with compartments to hold different grades of sand.

Distribution in the tank of graded sands retained by or passing 30-, 60-, 90-, or 100-mesh to the inch sieves.

passing 30-mesh sieve, retained by 60-mesh sieve		passing 60-mesh sieve, retained by 90-mesh sieve
	retained by 30-mesh sieve	
passing 90-mesh sieve, retained by 100-mesh sieve		passing 100-mesh sieve

Numbers of lancelets found buried in each grade of sand

10		20
	8	
3		2

Numbers of lancelets found lying on the surface in each compartment

4	2	5
2	2	2
6	10	7

FIGURE 8

result in the congregation of individuals in the most suitable sands with relatively few in the least suitable. The fact that lancelets occurred at all in the finest sands suggests that the trial and error method probably obtained. Thus the relative numbers of animals found in each sand should represent the proportionate time for which the animal remained buried in those sands, and is a measure of their suitability.

The results of this experiment are in accordance with those obtained when no choice of sand was given and the animals were confined above the different sand fractions. Nevertheless, the experiment was not entirely satisfactory as in none of the sieved sand fractions were the animals fully settled. The lancelets showed much activity in passing from one sand to another and, although many buried themselves, an equal number did not and remained lying either on one or other of the sands, or in the blank compartments in the tank (see figure 8). However, the greatest numbers lying on the surface were on or between the two finest grades of sand indicating that, although none of the sands provided a completely satisfactory medium, the fine sands were the least suitable.

Several points were noted with regard to the behaviour of the lancelets during this experiment. Lancelets settling on sand of the coarsest fraction, that retained by the 30-mesh sieve, frequently swam away as though stimulated to action by contact with the coarse grains. Moreover, burrowing in this fraction seemed to be more difficult than in the sands of the 30- to 60-mesh and the 60- to 90-mesh grades. The behaviour of the animals in the sands also differed according to the size of the grains. Once the lancelets had entered the coarse sand, they usually remained completely buried. In the sands of intermediate grain size, on the other hand, they tended to rest with only the oral region projecting, while, in the finest sands, the entire body anterior to the atriopore was usually clear of the sand. The difference in posture in the different sands was evidently due to interference with the ciliary currents of the pharynx, caused by a relative restriction in the rate of flow of water between the grains according to the grade of the sand.

The relative suitability of natural sands

The preference of lancelets for sand of the 60- to 90-mesh grade suggested that grain size may be partly responsible for the differential selection of natural sands by the animal. To check the relative suitability of natural sands in which lancelets were either common, rare or absent, sand was taken from north of Ikoyi Island (see figure 6) where the animal was very common, from Onikan and from the east side of the harbour where lancelets were moderately common, from the west side of the harbour where lancelets were not plentiful and from Lighthouse Creek where no lancelets had been found. These sands were arranged in the four corner trays and the centre tray of the Perspex tank and covered with sea water. Twenty-five lancelets were evenly distributed among the four vacant trays and left for 3 days. The sands were then removed and the number of lancelets in each counted. The experiment was repeated with the tank rotated through 180 degrees to counteract any effects due to uneven illumination. The results obtained in the two experiments were closely similar and have been combined. The distribution of the lancelets was clearly unaffected by the rotation of the tank. The position of the sands in the tank, their composition, an estimate of the frequency of lancelets found in them under natural conditions and the total number of lancelets taken from each sand after the experiments, are given in figure 9. In this experiment, unlike that involving washed and graded sands, all lancelets burrowed into the sand and none was lying either on the surface of the sand or in the vacant trays. It has been remarked that washed and sieved sands seem to be unsuitable for lancelets and this is evidently so, as in the natural sands the animals burrowed rapidly and, on leaving a sand, quickly burrowed elsewhere.

Distribution in the tank and composition of natural sands in which lancelets are common, rare or absent (>30 = grains retained by a 30 mesh sieve, 30–60 = grains passing a 30- but retained by a 60-mesh sieve, 60–90 = grains passing a 60- but retained by a 90-mesh sieve, <90 = grains passing a 90-mesh sieve)

Onikan > 30 28·75 % 30–60 46·00 % 60–90 12·75 % < 90 11·00 % silt 1·50 % lancelets + +		East Harbour > 30 8·40 % 30–60 77·75 % 60–90 13·00 % < 90 0·75 % silt < 0·10 % lancelets + +
	West Harbour > 30 < 0·10 % 30–60 6·50 % 60–90 77·80 % < 90 15·5 % silt < 0·1 % lancelets +	
Lighthouse Creek > 30 2·3 % 30–60 8·4 % 60–90 15·8 % < 90 68·0 % silt 5·5 % lancelets –		North Ikoyi > 30 3·0 % 30–60 32·0 % 60–90 54·4 % < 90 9·5 % silt 1·1 % lancelets + + +

Number of lancelets recovered from each sand

11		12
	6	
1		20

FIGURE 9

It is seen from figure 9 that the sand most favoured by the lancelets was that from north of Ikoyi Island, from which a total of 20 lancelets was recovered. The sands from Onikan and the East Harbour were rather less suitable and contained 11 and 12 lancelets respectively. The West Harbour sand with six animals and Lighthouse Creek sand with only one were the least suitable. There was, therefore, a close agreement between the estimated numbers of lancelets occurring naturally in these sands and the numbers recovered after the experiment. A comparison of the composition of these sands showed that the sand from Lighthouse Creek differed from all others in the very high proportion of sand grains passing the 90-mesh sieve and also in the high proportion of silt present. It was clear from the previous experiments that the failure of lancelets to remain in this sand for any length of time could be attributed to the high proportion of fine particles. Among the remainder, the West Harbour sand had two features of note. First the proportion of fine grains was relatively high and, secondly, the sand was very uniform with a high proportion of grains passing a 60-mesh sieve but retained by a 90-mesh sieve and very few grains larger than this. This was an unstable or quick sand which did not form a firmly compacted layer. The Onikan and East Harbour sands had one feature in common; they were relatively coarse, with the dominant grain size in the 30- to 60-mesh range. Onikan sand was a better mixture of grain sizes than the East Harbour sand, but, on the other hand, it contained a higher proportion of fine grains and an appreciable quantity of silt. The North Ikoyi sand was also a good mixture of grain sizes, but, unlike the Onikan sand, the dominant grain size was in the 60- to 90-mesh range which, from previous experiments, appeared to be the most suitable for lancelets (see figure 8). In addition, the proportions of fine sand and particularly silt were less than in the Onikan sand. It seemed likely, therefore, that the North Ikoyi sand became heavily populated with lancelets because it did not possess features which caused the animal to leave the sand once burrowing had taken place. The comparison with the other sands tested suggests that these features rendering North Ikoyi sand more attractive to lancelets may have been partly connected with the type of mixture of grains of which the sand is composed. The evidence indicated that a mixed sand, that is a sand with a wide range of grain size, was preferred to one in which the majority of grains were of similar size. In mixed sands preference was again given to one in which the majority of grains fell in the fraction passing a 60-mesh but retained by a 90-mesh sieve. These features, however, are evidently not limiting in nature since sands of uniform grains not in the 60- to 90-mesh size range will support a population of lancelets presumably because more suitable sands are not available in the immediate vicinity. The factors which are likely to determine whether or not the animal can survive in a sand are first the proportion of fine grains passing a 90-mesh sieve and, secondly, the quantity of silt present. It is clear that some fine sand and silt can be tolerated by lancelets, for both are present in appreciable quantities in the North Ikoyi and Onikan sands; it was, therefore, important to determine the maximum proportions of these fine particles the animal can withstand.

An experimental analysis of North Ikoyi sand

On the evidence of the tests to show the preference of lancelets for different natural sands, it was clear that sand from North of Ikoyi Island was the most suitable of the series and that the proportions of both fine sand passing a 90-mesh sieve and silt were likely to prove factors limiting the colonization of a sand deposit by lancelets. To demonstrate the extent to which fine sand and silt are important, experiments were carried out to show how far the removal of these fractions from North Ikoyi sand, on the one hand, and an increase in their proportions, on the other, rendered the sand either more or less attractive to the animal. In preparing modified sand samples, the silt was removed where necessary by repeated gentle agitation in a small quantity of sea water followed by decantation after the sand grains had been allowed to settle. The silt was recovered by filtration for addition to other sands. After the removal of silt, the fine sand fraction was separated from the coarser grades by sieving wet through a 90-mesh to the inch sieve using sea water for the purpose. The fine sand also was collected for addition to other sands. Modified samples of the North Ikoyi sand were thus prepared in which first the silt, then the fine sand and then both were removed. In other samples the normal fine sand content of a little under 10% was increased to 20 and 30% respectively. In another sample the silt content was doubled. As, in the process of sieving, it was impossible to avoid considerable disturbance of the bulk of the sand, an artificial mixture was prepared from sieved fractions and silt as a guide to the effect of sieving on the sand. It was believed that disturbance by sieving of the microfauna and microflora might result in the sand becoming less attractive to lancelets. The natural unmodified North Ikoyi sand was used as a control. These seven variants of the North Ikoyi sand and the control were arranged in the trays of the Perspex tank, leaving the centre tray empty. The arrangement of these sands in the tank is given in figure 10.

After the sands had been placed in the tank and covered with sea water, lancelets were liberated in the vacant centre tray. The tank was examined 3 or 4 times daily and the number and position of the lancelets seen with the oral end projecting from the sand was noted. After 4 days the sands were removed from the tank and the number of animals recovered from each counted. The experiment was repeated with the tank rotated through 180 degrees to eliminate differences in illumination. The results of the two experiments were essentially similar, showing again that light did not affect the distribution of the animals. In figure 10 the combined number of lancelets recovered from each sand is given,

The distribution of modified Ikoyi sands in the experimental tank

Ikoyi sand with grains passing a 90-mesh sieve removed	natural Ikoyi sand control	Ikoyi sand with the passing-90-mesh grains increased to 20 %
Ikoyi sand with both silt and grains passing a 90-mesh sieve removed		Ikoyi sand with the passing-90-mesh grains increased to 30 %
Ikoyi sand with the silt removed	artificial Ikoyi sand containing equivalent amounts of sieved grains and silt	Ikoyi sand with twice the normal quantity of silt

Numbers of lancelets recovered from each sand

14	12	11
16		3
32	5	7

Average number of lancelets seen surface feeding at each examination

6·7	8	10
6		2·8
12	2·6	6·4

Average number of lancelets surface feeding as a percentage of the total number found

48%	66%	91%
37%		93%
37%	52%	91%

FIGURE 10

together with the average number of animals seen at the surface in each sand at each interim examination. This number is also expressed as a percentage of the number of animals recovered. For the purpose of comparison of the effect of each sand on the animal, the relative preference of lancelets for each of the sands and the percentage of lancelets feeding at the surface in each sand is shown diagrammatically in figures 11 and 12 respectively.

Considering first the total number of animals found in each sand at the conclusion of these experiments, it is clear that the natural sand from north of Ikoyi Island, though preferred to other natural sands from the harbour and creeks (see figure 9), by no means provided optimum conditions for lancelets. Removal of the silt from the sand greatly increased its attractiveness to the animal. Out of 100 lancelets used in the experiment, 32 were found in the sand without silt as compared with only 12 in the untreated control. Conversely, in the sand containing twice the normal quantity of silt, the number of animals recovered was only seven. It may be assumed, therefore, that the proportion of silt present in the sand, though found to be only 0·8% (see part II), is an important factor affecting the attractiveness of sand to lancelets. The removal of the fine grains appears to have improved the attractiveness of the sand slightly as compared with the control, although it is doubtful how far the difference between the number of lancelets recovered from each is significant. The removal of both the fine sand and silt, however, did not give a result commensurate with that obtained by the removal of silt alone, although more lancelets were found in this sand than in the control. This may have been due to the fact that, whereas the removal of silt was accomplished with the minimum of disturbance to the sand mixture, the separation of the fine sand fraction from the mixture by sieving resulted in a gross disturbance of the sand structure. The low value obtained for the sand lacking both fine grains and silt may in some way have been associated with sieving. This effect can also be presumed to have operated in the sand lacking only the fine grains which should probably have given a higher reading than was obtained in this experiment. The effect of sieving is also confirmed by the low number of lancelets taken from the sand prepared entirely from sieved fractions as compared with the natural sand control. Thus, any sands which had undergone sieving appear to have given a lower reading than would have been expected on theoretical grounds. The addition of fine grains to the sand to increase the proportion of this fraction to 20% and 30% respectively, however, did not involve sieving of the bulk of the sand and thus the mixture was relatively undisturbed. The results obtained with the control, in which there was about 10% of fine grains, and the sand with fine grains increased to 20% were similar, 12 animals having been recovered from the control as compared with 11 from the sand with 20% fine grains. However, when the proportion of fine grains was increased to 30%, the sand appeared to become far less attractive to the animal as only three lancelets were recovered from it, the lowest figure for any of the samples tested.

The various modifications of the North Ikoyi sand can be arranged in order of attractiveness to lancelets according to the number of animals recovered from each. This has been done in figure 11, which shows that there was a preference for sands with reduced content of fine grains and silt as compared with samples in which these fractions had been increased. The relative effect of the fine grains and silt, however, was different, small quantities of silt having a greater influence in reducing the attractiveness of the sand than larger amounts of the fine sand. In fact very little difference was found between samples containing no fine grains, 10% or 20% of fine grains. It was only when the proportion of this grade was increased to 30% that the sand became unattractive. Evidently some factor came into operation between these concentrations which rendered the sand unsuitable.

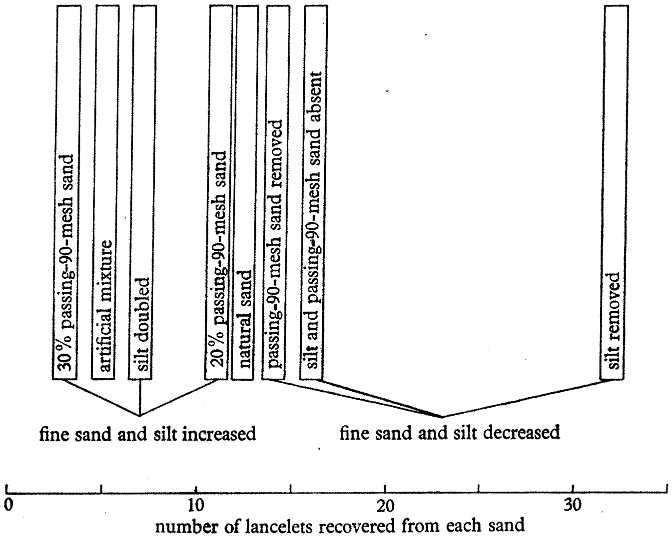

Figure 11. Diagram showing the relative preference of lancelets for modified sands as compared with natural Ikoyi sand.

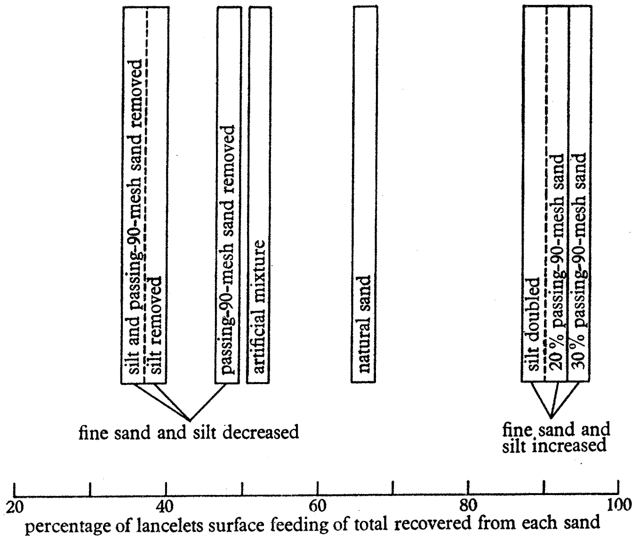

Figure 12. Diagram showing the percentage of lancelets surface feeding in modified sands as compared with natural Ikoyi sand.

With silt, on the other hand, the range of concentrations tested was much smaller. The silt fraction of the natural sand was 0·8% and of this about 20% was organic matter (see part II). Thus the doubling of the silt content gave a concentration of 1·6%. At this concentration of silt the sand was less attractive than that containing 20% of fine grains and 0·8% of silt. It appears, therefore, that there are limiting concentrations of fine sand and silt and that lancelets cannot tolerate amounts in excess of these. These effects cannot be due to mechanical blocking of the feeding mechanism or to the entry of fine particles into the gut for, were this so, the animal would be unable to tolerate the presence of any fine sand or silt, which is far from the case. It is evident, therefore, that the increase in fine particles beyond a certain concentration causes a change in the physical properties of the sand sufficient to interfere with the normal requirements of the animal. The nature of this change in the physical properties of sand will be considered elsewhere (see part V).

In these experiments it was impossible to avoid some degree of interference with the normal structure of the sand as it exists in the natural state. The addition or removal of fine grains or silt altered the proportions of all fractions present, while sieving disturbed not only the integral relationship existing between grains of different sizes, but also the microfauna and microflora of the sand. The effects of sieving on the nature of the sand, therefore, required further investigation.

The reaction of lancelets to sharp sand grains and to the presence of living micro-organisms and decaying organic remains in sand

The effect on sand of sieving was investigated in a further experiment. It was thought that the mechanical action of sieving, apart from disturbing the mixture of grains of different sizes, would tend to remove the fauna and flora of the sand and any colloidal layer which might normally coat the grains. Sieving might also cause abrasion or chipping of the grains themselves. The failure of sieved sands to attract lancelets to the same extent as natural sands of similar composition, therefore, might be accounted for either by the presence or absence of living or dead organic matter or by the rough nature of the surface of the grains with which the animals came into contact. Accordingly, a series of sands was prepared to demonstrate the possible effects of these factors on lancelets. Sand from north of Ikoyi Island was treated in the following ways. A sample was heated in an autoclave and stored in a sterile jar, thus killing all living organisms but retaining the dead organic remains. A further sample was treated with formalin, neutralized with ammonia and stored in a sterile jar, again killing all living organisms but retaining the organic remains. Three samples washed to remove silt were taken. Two of these were incinerated to remove all organic matter, while the third was air dried and stored in the dry condition. One of the incinerated samples was stored dry, while the other was stored under unfiltered sea water for 4 weeks. In these three samples, the air-dried sand contained dead organic matter on the surface of the grains, the organic content of the silt having been removed; the incinerated sample stored dry contained no organic matter on the grains, while that stored under sea water had an opportunity to regenerate its microfauna and microflora. Finally two samples of sand were prepared from sieved fractions to imitate the natural Ikoyi sand. One of these was stored dry while the other was stored under unfiltered sea water for 4 weeks to allow the regeneration of the microfauna and microflora. The natural sand which had been stored for a similar period in a moist condition in an unsterilized jar was also tested. These 8 sands were arranged in the peripheral trays of the Perspex tank in the order shown in figure 13 and covered with sea water. Lancelets were added to the centre tray, which was vacant. After 3 days the sands were removed and the number of animals recovered from each counted. The experiment was repeated with the tank rotated through 180 degrees to eliminate the effects of uneven illumination. The results of the two experiments were essentially similar and composite numbers of the animals recovered from each sand are given in figure 13. On the conclusion of the experiments, the sands were tested by smell to determine the presence or absence of decaying organic matter and examined under the microscope to assess both the extent of the growth of living organisms on and between the grains and the presence or absence of abrasion due to sieving. It was found that grains that had been passed through a sieve could be recognized by the presence of conchoidal fractures on their surfaces, by sharp edges and by the presence of numerous flakes in comparison with the unsieved lagoon sands. Sands stained with iodine and eosin showed organic growth where present in patches on the surface of the grains. The extent of the organic growth, the nature of the grains (whether sharp or rounded) and the presence or absence of organic decay in each sand tested is given in figure 13. Thus, in the natural sand and in the autoclaved and formolized sands, organic growth was present in varying degree and the sand grains were rounded, but in all cases there was considerable organic decay. Although two of these sands had been sterilized, the period of the experiment was sufficient for decay of the dead organic matter present to occur. The fact that the natural sand had been stored in a moist condition for over 4 weeks was evidently responsible for organic decay in that sample. The artificial mixtures of sieved sands were free from decay and, in the case of the sample stored beneath sea water, a rich growth of organisms was present, but in both these sands the grains were sharp. The air-dried sample from which silt had been removed had developed a rich organic growth during the experiment, but there was decay of the residual organic remains left on the grains after drying. In the two incinerated sands there was no decay, but a rich organic growth was present in that stored beneath sea water while very little growth had occurred on the grains of the sand stored dry. From figure 13 it is seen that, of the eight sands tested, only the sand which had been incinerated and allowed to regenerate a fauna and flora beneath sea water was also free

Distribution in the tank of Ikoyi sands modified with regard to living and dead organic content and sharpness of grains

Ikoyi sand autoclaved and stored in sterile jar	Ikoyi sand stored in an unsterilized jar	Ikoyi sand formolized, neutralized with ammonia and stored in a sterile jar
artificial mixture of sieved and graded grains equivalent to Ikoyi sand stored under sea water for 4 weeks		Ikoyi sand washed to remove silt, dried and incinerated to remove all organic matter and stored dry
artificial mixture of sieved and graded grains equivalent to Ikoyi sand stored dry	Ikoyi sand washed to remove silt and dried (not incinerated)	Ikoyi sand washed and incinerated and stored under sea water for 4 weeks

Numbers of lancelets recovered and the growth of micro-organisms, the sharpness of grains and the organic decomposition in each sand

2 organic growth very rich: rounded grains: decay present	2 organic growth rich: rounded grains: decay present	3 some organic growth: rounded grains: decay present
3 organic growth rich: sharp grains: no decay		4 very little organic growth: rounded grains: no decay
2 very little organic growth: sharp grains: no decay	2 organic growth very rich: rounded grains: decay present	13 organic growth rich: rounded grains: no decay

FIGURE 13

from decay and had rounded grains. In this sand 13 lancelets were found as compared with numbers not greater than four in any of the other sands. This experiment indicates that, quite apart from the proportions of fine particles present, sands are only attractive to lancelets when living micro-organisms are present on and between the grains and the sand is relatively free from decaying organic matter. Sharp edges to the grains, such as are produced by sieving, repel the animal. This is presumably due to a highly developed sense of touch. Just as lancelets can be stimulated to rapid swimming movements by the touch of the hairs of a brush, so the touch of sharp grains will cause the animal to leave a sand which has been passed through a sieve.

The behaviour of lancelets in different sands

The experiments that have been described also give information on the behaviour of lancelets in the different sands tested. During the course of the experiments, observations were made on the number of animals resting with the oral aperture open to the water above the sand. As these numbers taken at any one time never equalled the number of animals known to be present in the tank, it is evident that some of the lancelets were completely buried. It was of interest to know whether the numbers feeding at the surface, as compared with the numbers completely buried, were in the same proportion in all sands irrespective of composition, or whether, in certain sands, surface feeding was more prevalent than in others. The nearest approach that could be obtained to an estimate of these figures was a comparison of the average number of lancelets seen at the surface in each sand per observation compared with the total number recovered at the end of the experiment. These comparisons are subject to error in view of the fluctuations in numbers of lancelets in the different sands known to have occurred, as, for instance, when on occasion more animals were seen at the surface of a particular sand during an experiment than were recovered from that sand at the end. In such a case migration away from that sand clearly took place during the experiment. Nevertheless, the results obtained in the experiment with modified North Ikoyi sands show that the percentage of animals feeding at the surface was significantly greater in sands containing a high proportion of silt or fine sand grains than in those in which the silt or fine sand had been removed (see figures 10 and 12). Where the quantity of silt had been doubled or the percentage of fine sand increased to 20 or 30 %, it was estimated that over 90 % of the animals in these sands rested with the oral aperture above the surface as compared with 66 % in the natural unmodified sand used as a control. Removal of the fine sand fraction resulted in a fall in this incidence, the percentage at the surface in this case being 48 % of the total number of animals present. The effects of removal of the silt fraction alone, or the silt and the fine sand fractions together, gave similar results, and here only 37 % of the animals were observed at the surface in each instance. In the artificially prepared mixture, the percentage was of the same order as in the control, but this artificial sand harboured so few lancelets that the figure of 52 % obtained must be considered inaccurate. These figures seem to establish that, however other factors may operate, the presence or absence of fine grains or silt in a mixed sand is a major factor governing the burrowing behaviour of the animal.

Observations on the behaviour of lancelets in various types of sand and in mud have been made. It has been found that lancelets characteristically adopt different positions relative to the deposit according to the sizes of particle present. These positions are illustrated in figure 14. In coarse sand the animal usually remains completely buried and the body is thrown into a sinuous curve. In a mixed sand with particles covering a wide range in size and with a moderately high proportion of fine sand or silt, the animal rests with the oral aperture at the surface of the sand. The body when fully settled is straight and at such an angle that the subterminal oral aperture is more or less level with the sand surface. A similar body position is adopted in fine sand except that the animal rests in a funnel-shaped depression with the atriopore at the base of the funnel, but clear of the sand. In mud, the lancelet does not burrow at all and lies with the body held straight on the surface of the deposit. In fine sand or mud, where the greater part of the body is exposed, lancelets, which are extremely sensitive to touch, are readily disturbed and swim rapidly away from the bottom, only to settle once more at a new location. It is evident, therefore, that settlement on mud or fine sand is likely to be transient compared with settlement in a medium or coarse sand in which the animal can remain almost completely buried and thus undisturbed. In this way the behaviour of lancelets in response to different deposits automatically ensures that they congregate at localities where the nature of the sand permits burial for the longest period, such localities being found by a process of trial and error. It is, therefore, not necessary to postulate active powers of selection in lancelets, but merely a definite reaction to a rather limited set of environmental circumstances. The behaviour of the animal in different deposits suggests that the factor causing lancelets to leave sands of fine texture or to remain completely buried in sands of coarse grain is the maintenance of a water current through the pharynx adequate for the supply of oxygen and the removal of waste products. Where the flow of water between the sand grains is relatively unrestricted, the animal can remain completely buried, but, where it is partly or wholly restricted by the presence of different concentrations of fine particles, then it becomes necessary either for the oral aperture alone or for both the oral aperture and the atriopore to be clear of the sand. It is likely that the stimulus causing the animal to leave a sand in which the rate of flow of water between the grains is inadequate is the accumulation of carbon dioxide which must inevitably occur. If this is so, then it is also probable that the

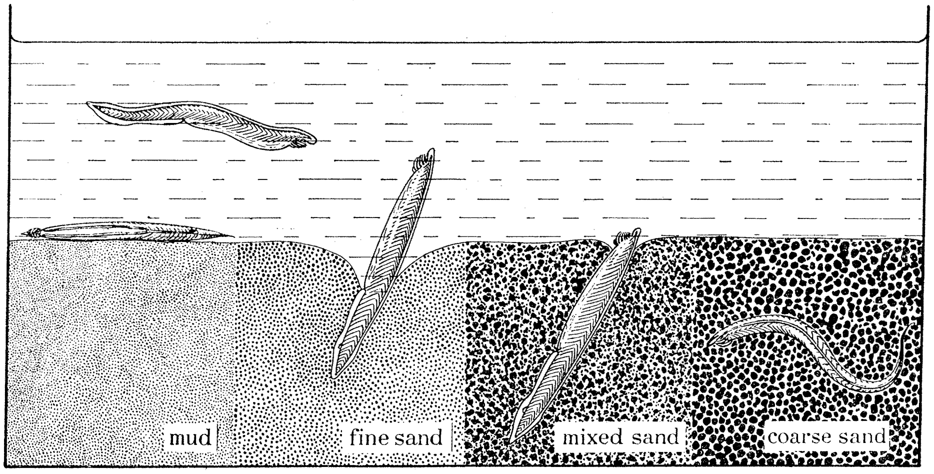

FIGURE 14. The behaviour of lancelets in deposits of different particle size.

proportions of fine grains or silt in a sand which can be tolerated by lancelets will change with the size of the animal. A small immature lancelet should be able to live in sand with a higher proportion of fine particles than the mature fully grown lancelet. This may be the reason why the large lancelet, *B. lanceolatum*, occurs, for instance, in the coarse shell-gravel of the Eddystone beds, while *B. nigeriense*, a smaller lancelet, is found in finer deposits.

TABLE 1. THE VARIATIONS IN NUMBERS OF LANCELETS SEEN FEEDING AT THE SURFACE OF MODIFIED NORTH IKOYI SANDS

type of sand	minimum number of lancelets seen at surface at one time	maximum number of lancelets seen at surface at one time	number of lancelets recovered from each sand per experiment
natural North Ikoyi sand	0	13	6
Ikoyi sand minus silt	2	14	16
Ikoyi sand minus silt and grains passing 90-mesh sieve	0	11	8
Ikoyi sand minus grains passing 90-mesh sieve	1	6	7
Ikoyi sand with double silt	1	7	4
Ikoyi sand with 20% grains passing 90-mesh sieve	0	10	6
Ikoyi sand with 30% grains passing 90-mesh sieve	0	3	2
artificial mixture	0	4	3

The congregation of adults in suitable sands by a process of trial and error suggests that lancelets show a degree of activity which has not previously been suspected. The intensity of this activity has been demonstrated in the experiments on the reactions of lancelets to the various modifications of the North Ikoyi sand (see figure 10). In these experiments it was observed that the numbers of lancelets in each sand were by no means constant, and that there was a continual movement of individuals from one type of sand to another, the animals counted at the surface in each sand varying considerably from one interim observation to the next. In table 1 the maximum and minimum numbers counted in each sand are compared with the average numbers recovered at the end of the experiments. The maximum and minimum numbers show first that the number of lancelets present in each sand fluctuated considerably and, second, where the maximum number exceeded the total recovered, that migration away from the sand took place during the course of the experiment. These figures, however, take no account of the fact that a higher percentage of animals at the surface was to be expected where fine sand or silt was present than in those sands in which fine particles were absent. Thus the natural sand, and the sands in which the silt or the fine grains and silt had been removed, must at times have harboured considerably larger numbers of lancelets than were recovered at the conclusion of the experiment. When a large number of lancelets are congregated in a comparatively small volume of sand, it is likely that a rapid exhaustion of the oxygen supply in the water permeating the sand and a corresponding accumulation of carbon dioxide may render the sand temporarily unattractive to the animal and result in a migration away from that sand. It is evident, from the figures given in table 1, that this occurred in the natural sand and possibly also in the sands in which fine grains had been removed. In the sands containing a high proportion of silt or fine grains and also in the mixture of sieved fractions, it is equally clear that at no time during the experiments were large numbers of animals present. This was confirmed by the reactions of the animals to these sands. Lancelets were seen to bury the anterior end of the body in the sand and then in most cases to leave almost immediately. In sands lacking fine grains there was evidence of considerable activity beneath the sand, the oral apertures of lancelets visible at one point on the surface disappearing only to reappear elsewhere. It is clear, therefore, that congregations of adult lancelets are not static, movements taking place almost continually from one place to another either by swimming or by burrowing in the sand itself wherever conditions are not entirely satisfactory to the animal. However, where conditions are suitable, lancelets have been observed to remain buried in the same position for days and to show no signs of movement.

THE BEHAVIOUR OF *BRANCHIOSTOMA NIGERIENSE* IN RELATION TO ITS DISTRIBUTION

The experimental data already given on the salinity and temperature tolerance of the adult and larva, and the composition of the bottom deposits in which the adult is found, give the conditions under which this animal can live. These conditions may now be set down as follows.

(1) The salinity of the water must not be less than 13 p./10^3 nor greater than 58 p./10^3.

(2) The temperature of the water should not fall below 24 °C for the larva or 17 °C for the adult as at these temperatures normal behaviour is disturbed. Both the larva and the adult are virtually inactive at 13 to 14 °C and again at 38 to 39 °C.

(3) The adult is limited to deposits of sand in which the proportions of grains passing a 90-mesh to the inch sieve or of silt do not exceed 25 or 1·5% respectively. The sands must be undisturbed by wave action, relatively free from organic decay and composed of rounded grains on and between which there is a rich growth of micro-organisms.

It is clear from this list that the limits to distribution are set by the physiological reactions of the animal to salinity and temperature, salinity determining the extent of penetration into brackish waters and temperature the wider distribution by ocean currents. Within these limits, however, *B. nigeriense* shows a preferential selection of habitat resulting from the pattern of behaviour of the animal and its reactions to the particular environmental conditions in which it finds itself.

The deposits in which the adults are found, although often very limited in area, are usually very heavily populated, as many as 30 or 40 lancelets being present on occasion in one handful of sand. As already indicated (part III), the young larva, in which the axis of the body is straight, is probably a bottom-living form more or less confined to the same ground as the adult and it is not until a little before the 6-gill-pouch stage, when the hind region of the body becomes deflected downward, that the swimming activities of the larva carry it up to the surface waters. When the larva becomes planktonic, its reaction, first, to waters of salinity of 20 p./10^3 in which swimming activities cease and, second, to either a rise in salinity to 25 p./10^3 or a fall in temperature to 24 °C when swimming recommences, tends to maintain the larva in the planktonic state. At this stage the larvae become widespread in the sea and cover an area far greater than that of the adult. The late larva loses the downward deflexion of its posterior third and the axis of the body once more becomes straight. This larva is very active and presumably remains planktonic because of this activity since the quiescent larva sinks rapidly. At metamorphosis, however, the larva becomes less active and sinks to the bottom. For this reason lancelets in the later stages of metamorphosis have rarely been taken in plankton samples, although larvae commencing metamorphosis and sometimes young post-metamorphic forms have been netted. It seems, therefore, that the larval excursion from the bottom to begin a planktonic life which ends with a return to the bottom at metamorphosis can be related to the differential activity of the animal in conjunction with a change of shape of the body controlled, first, during larval life, by a reaction to changes in salinity and temperature and, secondly, at metamorphosis, by physiological change. When larvae in the process of metamorphosis sink to the bottom, the chance of settlement on a substratum suitable for adult life is small. Thus, if there was no mechanism ensuring that a proportion of the post-metamorphic lancelets settling on unsuitable deposits found their way to sands in which they could live, it is unlikely that the spawning grounds would become sufficiently densely populated to permit reproduction. In the absence of any sexual behaviour in lancelets, it seems that the crowding together of mature adults in a small area is essential for fertilization and this is unlikely to obtain if the wide distribution of the larvae at metamorphosis is maintained. The congregation of adults which seems to be necessary for the continuance of the species is evidently achieved through the behaviour of the post-metamorphic lancelets and their reaction to environment.

The most characteristic feature of lancelet activity is the alternation between swimming and burrowing. Although the adult lancelet spends most of its life buried in sand, it is capable of very rapid swimming movements of an undulatory character. The earliest reference to these movements, as Gudger (1945) points out, relates to an alleged observation of chain swimming of a number of lancelets progressing end to end made at Algiers in December 1837, first published by Wilde (1840), repeated by Yarrell (1844) and then figured by Lydekker (1896). The original observation has never been confirmed and, although almost certainly due to a mistaken impression, perhaps points to the difficulty of making accurate observations on an animal which moves with great rapidity and has little distinction between the anterior and posterior ends. It is easy to imagine how such an impression might be given by a large number of lancelets leaving a small patch of sand on being disturbed. The difficulty of distinguishing between the anterior and posterior ends of a swimming lancelet is probably responsible for the controversy on whether lancelets swim forward or backward or are capable of both types of movement and also whether they burrow with oral end or tail foremost. Rice (1880), working with lancelets from the east coast of America, remarked that they swim only forward, while Parker (1908) and Arey (1915) found that the direction of swimming in *B. caribaeum* depended on the region of the body stimulated, the movement being such as to produce the most rapid avoidance of the stimulus. Thus stimulation of the anterior fourth of the body by touch or by a narrow beam of intense light caused a backward spring, while stimulation of the posterior region produced forward movement. However, Arey found that backward movement was never continued for more than a few centimetres. This has been confirmed in *B. nigeriense*, but it is also found that forward swimming is otherwise invariable, as can be seen in lancelets in which the rapidity of movement has been reduced by cooling. External stimuli also affect the direction of swimming. Franz (1923, 1924) has shown that *B. lanceolatum* while swimming is negatively phototactic and that the direction of movement is changed to avoid a bright source of light. As Drach (1948) has pointed out, normal swimming movements are not only rapid and of short duration, but also are never spontaneous. It seems, therefore, that movement always requires a definite stimulus, the reaction to which is one of avoidance. This certainly appears to be the case in *B. nigeriense* except, perhaps, as has been shown, when the lancelet is subjected to low temperatures.

With regard to burrowing, Parker (1908) and Arey (1915) agree that *B. caribaeum* burrows tail first and Hagmeier & Hinrichs (1931) suggest that *B. lanceolatum* behaves similarly. On the other hand, Gage (1905) observed that Bermuda lancelets usually enter sand oral end foremost while Franz (1924), working with *B. lanceolatum*, suggests that either method may be used and gives figures to show the positions taken up by the body in the sand following each type of entry. There is, therefore, a difference of opinion expressed which is evidently not due to the fact that more than one species has been studied, but rather to the difficulty of making such observations on lancelets. When *B. nigeriense* is disturbed from sand it darts away and usually reburies itself almost immediately. If, however, the grade of sand is not suitable, being too fine or too coarse, entry is either much slower or else having become partially buried the animal retreats. It can, on these occasions, be seen that burrowing takes place with the anterior end foremost. Among many hundreds of living specimens of *B. nigeriense* observed, only one clear case of tail burrowing has been seen. In this instance the animal, settling tail first and supported by the side of the container, proceeded to burrow, but the action was very much slower than in normal burrowing.

Burrowing is almost always complete in the first instance and it is not until later that the oral aperture may be projected to lie above the surface of the sand, although, as has already been shown, this does not always occur and the animal may remain completely buried in coarse sands. Moreover, there is evidence of considerable activity beneath the sand, lancelets passing from one place to another. Hagmeier & Hinrichs (1931) have commented on this activity and have studied the effect of the presence of *B. lanceolatum* on the stratification of sand. They have found that, in a mixed sand, the surface layers are altered by the lancelets so that the finer grains come to occupy the lower strata, leaving the coarse particles at the surface. This layering of the sand is evidently due to the activity of the lancelets while buried and is of particular significance in view of the preference shown by lancelets for relatively coarse sands free from fine particles.

Much of the evidence for tail burrowing has been based on the position of the lancelet in the sand as it has been assumed that certain positions can only be achieved if the animal burrows tail foremost and that, once having burrowed, it remains inactive (see Parker 1908; Franz 1924). It has been shown in *B. nigeriense* that the position adopted in sand, where the oral aperture is either flush with the surface or at the bottom of a small depression and the body is held straight, may be maintained, if the animal is not disturbed, for several days. Such a position, however, is not assumed immediately after burrowing as it requires some degree of body movement to displace the sand grains which would otherwise hold the body in the sinuous curves necessary for progression both in water and in sand. It appears from observations that a rapid quivering of the body which occurs periodically is chiefly the means whereby the sand grains are displaced and the straight position achieved. Thus it is evident that the behaviour of *B. nigeriense* in sand passes through three phases, first the process of burrowing oral end foremost into the sand, second a period of activity beneath the sand and third a period of quiescence in which the body is held straight and the oral aperture is usually above the sand surface. This period is terminated when some stimulus causes the animal to move and either retreat beneath the sand or leave the sand to swim freely in the water above. Thus, where it has been remarked by Parker (1908) and Franz (1924) that lancelets buried in sand preserve a sinuous body position, it is likely that they are not fully settled and are still in the second phase of activity.

As activity in lancelets does not appear to be spontaneous but always in response to a stimulus, it is necessary to consider the stimuli which will evoke a response. The reactions to changes in salinity and temperature have already been described in this paper, while Parker (1908) has given the reactions to various chemicals, most of which are not appropriate to the natural habitat. The important chemical stimuli to which the animal will react are probably pH (see Chin 1941) and the staling products of organic decay. The reactions to mechanical stimuli and light are also clearly important. Lancelets appear to be relatively insensitive to light. *B. nigeriense* shows little reaction to normal light changes or differences in illumination and is stimulated to movement only by an intense beam such as that from a high-intensity microscope lamp. Parker (1908) carried out a number of experiments with *B. caribaeum* and found this species also to be relatively insensitive. He examined the phenomenon known to Willey (1894)and Hesse (1898) that large numbers of lancelets kept together in a jar in the dark show great activity when illuminated and showed that, in such a case, a slight disturbance due to reaction to light in one or two individuals was communicated by touch from one lancelet to the next until all were in a state of great activity. This behaviour, therefore, is a demonstration of a well-developed tactile sense rather than a reaction to light. Parker did, however, find that lancelets in an aquarium came to rest in the darkest corner. In investigating the light-sensitive regions of the body, he found that the entire body was sensitive in the region of the nerve cord except the anterior end, a fact which may be of significance as it is the anterior end which is most frequently above the sand.

The tactile sense of lancelets is most highly developed and the lightest touch of a camel-hair brush causes a vigorous reaction, the animal being stimulated to rapid swimming. Parker again found, when investigating the relative sensitivity of the different parts of the body that, unlike the reaction to light, the anterior end and in particular the oral hood was the most sensitive to touch. The tail region was also found to be sensitive, but the central region of the body was less so. The order of tactile sensitivity is such that lancelets readily respond to vibration caused by a light tap on the side of their container. It is evident, therefore, that the tactile sense in lancelets is of far greater importance than light sensitivity. Lancelets burrow freely whether they are illuminated or not. Thus, as Parker remarks, burrowing is evidently not due to a negative reaction to light. Parker suggests that lancelets are thigmotactic, but it is doubtful how far this is true. Lancelets appear to remain quiescent either when they have settled in a suitable sand or when they are lying in a glass dish free from sand. It does not seem that contact on one side with the smooth glass surface causes a reaction, as is the case when the lancelet is lying on sand. When the animal burrows, the anterior end touches the sand first and a vigorous swimming movement follows which drives the body into the sand. It is probable, therefore, that the sudden burst of vigorous movement which occurs as soon as the oral region touches the sand is a normal response to the differential stimulation of one part of the body. When the entire body is buried, contact stimuli affect all parts, the differential is greatly reduced and the animal tends to remain quiescent. This reaction, together with the fact that lancelets normally swim for short periods only and then sink to the bottom, should automatically result in burrowing.

This behaviour can now be related to the experimental results showing the preference of lancelets for different deposits. If burrowing is due to the tactile stimulation of the oral region by sand grains, the failure of lancelets to burrow in mud or very fine sand is readily understood as the surfaces of these deposits are soft and smooth and do not induce a burst of activity. In the same way lancelets in a glass container free from sand are never seen to attempt to burrow into the glass. Similarly, the avoidance of sands with sharp grains and, to a lesser extent, sands with rounded grains in which the organic covering has been removed by incineration can also be explained on the grounds that tactile stimulation by the sharp or rough surfaces is too severe. The preference shown by *B. nigeriense* for sands in which the dominant fraction consists of grains passing a 60-mesh to the inch sieve but retained by a 90-mesh sieve rather than those in which the grain size is larger may, perhaps, be related to the behaviour of the animal in the sand. It is probable that a lancelet of the size of *B. nigeriense* can more easily achieve a straight body position by displacement of grains in a 60- to 90-mesh sand than in one in which most of the grains are larger and thus pass from a state of activity in the sand to one of quiescence. Thus, apart from the physical features of the sand such as the silt and fine sand content which evidently limit the water supply to the buried animal, the sand most favoured by *B. nigeriense* strikes a balance between a size of grain sufficiently large to provide the initial stimulus to burrow and one small enough to permit a rapid attainment of the quiescent state when the lancelet is buried. In addition, smoothness of grain surface, due both to rounded contour and a coat of living micro-organisms, and absence of the products of organic decay seem to be essential in preserving the quiescent state. The congregation of adults in patches of suitable sand, which appears to be necessary for reproduction, is, therefore, evidently a direct consequence of the behaviour of the lancelet and its reactions to all other deposits not fulfilling the limiting conditions imposed chiefly by a highly developed tactile sense.

SUMMARY

1. The lower threshold salinity for both the larva and the adult of *Branchiostoma nigeriense* has been determined and found to be 13 p./10^3. It was also found that the adult in sea water slowly concentrating by evaporation survived at salinities up to 58·9 p./10^3.

2. It has been shown experimentally that adults of *B. nigeriense* can withstand diurnal fluctuations of salinity within a range as great as 14·5 to 31 p./10^3. Further experiments on the rate of adjustment of lancelets to salinity change showed that lancelets transferred from 25 to 13 p./10^3 underwent a process of accommodation to the lower salinity which became critical at 14 to 20 h from the time of transfer. Lancelets returned to the high salinity water at this time died while others returned earlier or later, or remaining at the low salinity, recovered.

3. The times of appearance and disappearance of *B. nigeriense* in Lagos Harbour and Lagos Lagoon are governed by the salinity tolerance of the animal.

4. It has been found that salinity fluctuations from a high salinity to a low or in the reverse direction cause a stoppage of the ciliary beat in larval and adult lancelet gills for a period dependent upon the magnitude of the change and the salinity range in which it occurs. A salinity of 20 p./10^3 appears to be critical in this respect as fluctuations involving passage across this salinity in either direction cause prolonged cessation of ciliary beat as compared with others of equal magnitude within a range either above or below 20 p./10^3. In lancelets accommodated by long immersion to a salinity of 20 p./10^3, changes to either higher or lower salinities cause no cessation of ciliary beat. It is believed that periodic interruption in the ciliary feeding mechanism due to salinity fluctuation in the range above 20 p./10^3 is chiefly responsible for the slow rate of growth of lancelets in Five Cowrie Creek from January to May as compared with those in Lagos Lagoon. In Five Cowrie Creek at this time the mean salinity is 25 p./10^3 and fluctuation is considerable, whereas in Lagos Lagoon the mean salinity is 19 p./10^3 and fluctuation is slight.

5. The temperature range of the larva and the adult has been found to be about 12 to 37 °C. The reactions of the larva and the adult to changes in temperature have been observed. In the larva kept at the normal temperature of 26 to 30 °C, a fall to 24 °C induced continuous swimming, which became reduced in activity as the temperature fell further, until the animal became immobile and unresponsive to touch at temperatures below 12 °C. A gradual return to 30 °C did not cause continuous swimming, the larva remaining unresponsive until 22 °C was reached. In the adult, continuous swimming commenced at 17 °C and ceased at 13 °C when the animal became unresponsive. Unlike the larva, however, on a slow return to a higher temperature, continuous swimming was resumed until 17 °C was reached. At temperatures above 30 °C both the larva and the adult showed increased activity and response to touch until 37 °C was reached. A further rise caused inactivity, death occurring at 39 to 40 °C.

6. The presence of larvae in deep water of high salinity and their absence from surface waters of low salinity in Lagos Harbour has been noted. It has been suggested that the downward deflexion of the posterior region of the body characteristic of the larva except when very young or approaching metamorphosis is responsible for the fact that such larvae always swim upward. Quiescent larvae sink in salt water at a rate dependent upon their size. Experiments carried out to show the reaction of larvae to a salinity gradient at temperatures below 24 °C indicated that larvae stimulated by cold water swam upward until a salinity of 20 p./10^3 was reached, when they became immobile and sank. Swimming was resumed at a salinity above 25 p./10^3. It is suggested that this reaction to salinity and temperature restricts vertical distribution of the larva to waters of salinity above 20 p./10^3 and temperatures above 24 °C.

7. The distribution of adult lancelets in the Lagos area has been shown to coincide with the occurrence of sands undisturbed by wave action in which the proportion of fine grains passing a 90-mesh to the inch sieve does not exceed 25 % and the proportion of silt is not more than 1·5 %.

8. It has been demonstrated that lancelets exposed to sands graded by sieving show preference for a sand passing a 60-mesh but retained by a 90-mesh sieve. They cannot

tolerate sands passing a 90-mesh sieve and die within 2 or 3 days if left in contact with them. None of the graded sands provided an entirely satisfactory medium for lancelets.

9. The preference of lancelets for various natural sands was determined experimentally and found to agree with their incidence in these sands under natural conditions. An experimental analysis of the sand in which lancelets were most numerous, that from north of Ikoyi Island, was carried out to discover the reasons for its attractiveness. Removal of silt and grains passing a 90-mesh sieve from the sand increased its attractiveness while the addition of fine grains and silt rendered the sand repellent to lancelets. However, the relative effects of silt and fine sand were different, the presence of 1·5 % of silt repelling lancelets to the same degree as 20 to 30 % of fine sand. It is suggested that the suitability of the sand for lancelets depends upon its physical properties which change with the addition of fine particles.

10. Further experiments determined that lancelets tended to avoid sands in which the grains were sharp or rough or where the micro-organisms living on and between the grains had been removed. Sands in which there was appreciable organic decay also repelled lancelets.

11. An analysis of the experimental results showed that the proportion of lancelets resting with the oral aperture above the sand, and thus not completely buried, varied according to the composition of the deposit. In coarse sands free from fine particles the majority of lancelets remained completely buried; in medium sands of mixed composition they tended to rest with the body straight and the oral aperture at the surface of the sand; in fine sands, when the lancelets burrowed at all, a similar position was adopted except that the animal rested in a funnel-shaped depression with the atriopore at the base of the funnel; in mud the lancelet did not burrow and rested with the body held straight on the surface of the deposit. It was demonstrated that lancelets may show considerable activity beneath the sand, burrowing from place to place.

12. The behaviour of the lancelet is discussed in relation to the environmental conditions under which it lives. It is suggested that the congregation of adults in patches of suitable sand necessary for reproduction is a direct consequence of the reaction of the animal to all other deposits not fulfilling the limiting conditions required by the animal.

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