

The effects of the cold winter of 1962/63 on the Helgoland population of *Branchiostoma lanceolatum* (Pallas)

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KURZFASSUNG: Wie Wirkungen des kalten Winters 1962/63 auf die Helgoland-Population von *Branchiostoma lanceolatum* (PALLAS). Über die Hälfte der Helgoländer *Branchiostoma lanceolatum*-Population starb, als die Wassertemperatur während des strengen Winters 1962/63 unter 0° C herabsank. Dies geht aus der Analyse von Fängen hervor, welche mit Van Veen und Hensen Dredgen gemacht worden waren. In der jüngsten Altersgruppe der Population stieg die Sterberate auf 100% an. Die Empfindlichkeit von Individuen verschiedener Größe gegenüber niedriger Temperatur wird diskutiert hinsichtlich ihres Verhaltens sowohl im Laboratorium als auch im Grobsand ihres Habitats. Untersuchungen über das Aktivitätsmuster von Vertretern der Helgoland- und der Neapel-Populationen in Abhängigkeit von verschiedenen Temperaturen machen wahrscheinlich, daß *B. lanceolatum* eine mediterrane Art ist, welche sich nordwärts in den Englischen Kanal und die Nordsee ausgebreitet hat. Die Tiere sind nicht in der Lage, sich bei Temperaturen unter 3° C – wie sie bei Helgoland im Winter normalerweise vorkommen – fortzubewegen.

INTRODUCTION

Branchiostoma lanceolatum is found in large numbers in coarse sand deposits at several localities in the German Bight. An important and accessible ground where these animals can easily be studied lies some 3 miles to the N.E. of Helgoland. The authors are at present investigating the life history and populations of *B. lanceolatum*, chiefly at Helgoland, with a view to comparison with a tropical species, *B. nigeriense*, from West Africa (WEBB & HILL 1958). This work is part of a wider programme of research in which it is hoped to show that the distribution of populations of lancelets can be used as a guide to the pattern of ocean currents.

The winter of 1962/63 was unusually severe and the prolonged cold weather caused high mortality among many European marine and littoral animals. It so happened that the composition of the lancelet population at Helgoland, had been determined prior to the onset of the cold weather and it was possible, therefore, in this instance to form an estimate of the effects of prolonged low temperature on this animal under natural conditions.

ENVIRONMENTAL CONDITIONS AT HELGOLAND
IN THE WINTER 1962/63

The normal temperature of the water at a depth of 20 metres over the Helgoland amphioxus ground ranges from 16°–18° C in August and September to a minimum of 3°–4° C in February and March. A curve showing these temperatures compiled from data obtained in 1960/61/62 is given in Figure 1 (broken line). In the year 1962 summer temperatures were a little lower than normal and fell during the autumn to 7° C in December, which is not greatly different from that observed at this time in previous years. At the beginning of January, 1963, however, water temperatures fell rapidly and toward the end of the month were below zero, remaining around this

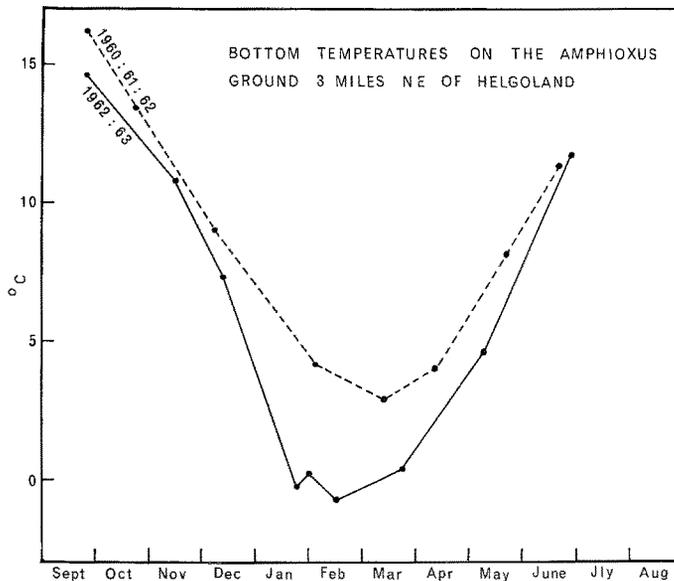


Fig. 1: Bottom temperatures on the *Amphioxus* ground three miles NE of Helgoland

level until almost the end of March, 8 weeks later. The minimum temperature recorded at a depth of 20 metres during this period was -1.3° C in mid-February as can be seen from Figure 1 (full line). Sub-normal temperatures continued until the end of June. In January, February and March ice from the shores of Denmark and the estuaries of the Weser and the Elbe drifted across to Helgoland, where it accumulated and formed a sheet of pack-ice extending out from the island for several miles and covering the amphioxus ground. This ice carried with it a large quantity of silt and organic debris from the shores where it had been formed and this was deposited on the amphioxus ground when the ice melted. The conditions arising from the cold weather, therefore, were unusual in two respects. First, in the prolonged period with temperatures 3°–4° C lower than normal and second, in the subsequent alteration of the sea bottom through the deposition of both organic and inorganic materials derived from neighbouring shores.

THE LANCELET POPULATION AT HELGOLAND IN
NOVEMBER, 1962 AND APRIL, 1963

The population of lancelets at Helgoland was sampled both with a Van Veen grab and a Hensen dredge. The bottom material brought up by these means was deposited in a tub and a quantity of formalin added. The sand was then stirred

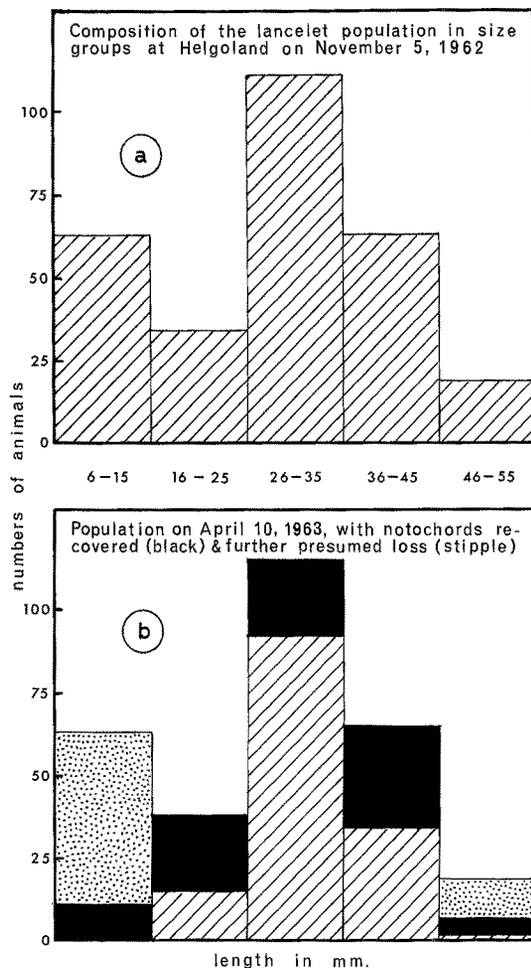


Fig. 2: Composition of the lancelet population

thoroughly so that the organisms in it were killed and floated in the supernatant fluid. The formalized sea water was then poured through a sieve which retained the dead animals and organic debris. The water was returned to the tub and the process repeated twice, which has been found sufficient to extract all lancelets present.

Branchiostoma lanceolatum metamorphoses at 5-6 mm and grows to a maximum length of 55 mm. In the sample taken on the 5th November, 1962, a complete size

range of animals was found to be present. Two-thirds of these were from 26–45 mm in length, but there was also a large number of newly metamorphosed animals 6–15 mm long. Animals above 45 mm were relatively few in number. For the purpose of comparison with later samples the animals in this sample were separated into 5 groups, each covering a size range of 10 mm. The numbers recovered in each group are shown in the histogram in Figure 2a.

On April 10, 1963, after the cold weather, a second sample was collected and analysed in a similar manner. Then it was found that 90% of living animals were in the range 26–45 mm and most of the remainder were of 16–25 mm in length. Very few animals above 45 mm were found and none below 16 mm. In the debris recovered from this sample, a large number of white filaments were found which on closer inspection proved to be notochords. These were evidently the remains of animals that had died during the cold weather. The notochords were then measured and grouped in the same way as the living animals. The catch of both living animals and notochords is shown in the histogram in Figure 2b.

The total number of living animals plus the notochords, representing dead animals, of length 16–45 mm taken in April agrees very closely with those of this size in the November sample. This agreement is undoubtedly fortuitous as considerable variation in the density of lancelets from one part of the amphioxus ground to another is known to exist and there can be no certainty that precisely the same area was sampled on each occasion. Moreover, the November sample was taken with the Van Veen grab and the April sample with the Hensen dredge, and these two methods are not quantitatively comparable. However, the close agreement of the relative numbers in each of the three size groups included in the range of 16–45 mm in the November and the April samples would appear to have significance. In the lowest of the size groups (6–15 mm) and again in the largest (46–55 mm) the April collection appears to be deficient. The group of smallest animals was well represented in the November sample, some 20% of the total catch being of this size. In the April sample, however, no living animals of this group were found and relatively few notochords of this length. A similar deficiency, though of lesser extent, is seen in the large animals of the 46–55 mm group, where the total of living animals and notochords of this length recovered in April does not equal the number of animals of that size found in the November sample.

Extent of mortality

There can be little doubt that the notochords found in April represented animals that had died during the cold spell. In no previous samples had isolated notochords ever been found which suggests that the animals to which they had belonged had not passed through the gut of a predator. A total of 143 living animals and 92 notochords were recovered from the April sample which is direct evidence of a 40% mortality. The relative numbers of notochords in each of the five size groups, however, were not the same. The 6–15 mm group was represented entirely by notochords and no living animals of this size were found. In the 16–25 mm group notochords represented 60% of dead and living animals, but only 20% in the 26–35 mm group. Among larger ani-

mals the percentage of notochords present again rose to 47% in the 36–45 mm and 70% in the 46–55 mm groups. These percentages in relation to body length are shown in Figure 3. It is clear from this graph that mortality decreases in linear form from the smallest to the middle-sized animals and then increases, again linearly, to the largest.

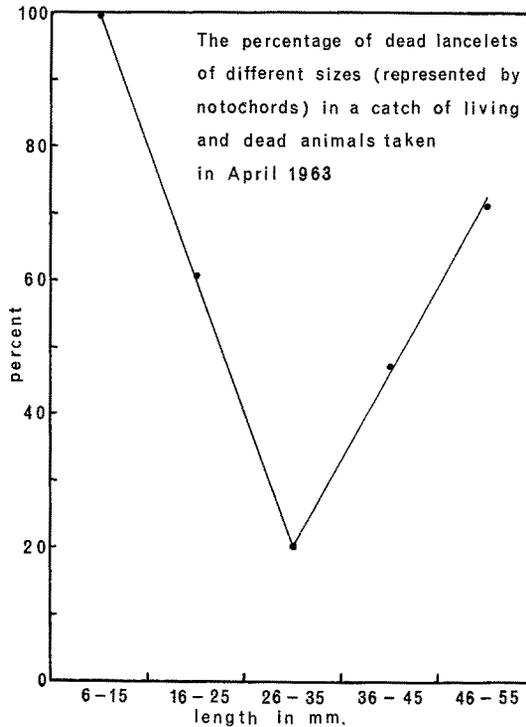


Fig. 3: Percentage of dead lancelets in a catch taken in April 1963

Although the presence of notochords is evidence of death, the period over which that mortality occurred is not known for it cannot be presumed that the notochords had persisted since the onset of cold weather. It may well be that animals killed at the beginning of this period had disintegrated entirely leaving no direct evidence of mortality at that time. Indeed a sample taken on January 30 about 2 weeks after the temperature had fallen to 0° C indicated that about two-thirds of the newly metamorphosed animals prominent in the November sample had already disappeared. On the other hand, the largest animals which by April also appeared to have suffered high mortality had in January incurred little or no loss. It is clear, therefore, that there are a number of animals evident in the November analysis which are not represented either as notochords or as living animals in the April histogram. It is probable, therefore, that further loss beyond that indicated by the presence of notochords had occurred in all size groups, but was presumably greatest among smallest and largest animals. In an attempt to estimate what this loss may have been, the April histogram has been adjusted by the addition of stippled areas to agree with the structure of the

November population. If this presumed loss is accepted then it would appear that at least a further 10%, making 50% in all, of the lancelets died during the cold period. Evidence in support of this is perhaps forthcoming from the general rate of capture of animals by means of the Van Veen grab before and after the cold weather. In 40 samples taken before December, 1962, a mean value of 6.9 animals per Van Veen grab was obtained. In 140 Van Veen samples taken since March, 1963, an average of only 3.0 animals was recovered. These figures again suggest a mortality of between 50% and 60%.

THE ACTIVITY AT DIFFERENT TEMPERATURES OF BRANCHIOSTOMA LANCEOLATUM

Branchiostoma lanceolatum is a species with a wide distribution extending from the coast of Norway through the English Channel and the Mediterranean into the Red Sea and the Indian Ocean (WEBB 1956). The different populations are thus subject to extremes of temperature almost as great as are to be encountered in any part of the oceans. In the Suez Canal, for instance, where GRUVEL (1933) found the animal to be numerous, summer temperatures rise to as much as 27° C. On the other hand, as has been shown, in the German Bight at Helgoland where *B. lanceolatum* is near the northern extreme of its range, winter temperatures normally fall as low as 3° C. A species, capable of surviving such a range in temperature presumably either has become adapted to different local conditions or has a wide temperature tolerance. To determine how far these alternatives apply in the present case experiments were conducted to show the activity of lancelets from Helgoland and from the Mediterranean (at Naples) at different temperatures.

In these experiments animals were placed in clean cold sea water in the light and the temperature first slowly raised to a little above 30° C, and then allowed to fall. When room temperature was reached the container was transferred to a refrigerator and cooled to 0° C. It was then removed and allowed to return to room temperature. Activity was measured both with regard to its type and its duration. Recognition of the type of activity was largely subjective and was classified according to the scale given in Figure 4. Duration of activity was measured in seconds and was found to follow the type of activity closely, duration being longest in the more vigorous forms. Immobile animals did not respond to touch with a bristle. The active categories are self-explanatory ranging from a single twitch through slow wagging of the tail to extremely rapid swimming of as much as 30 seconds duration.

It was found that the type of activity shown by animals from Helgoland and Naples was very similar (see Fig. 4). In both forms active or very active movements of relatively long duration occur between 9° and 20° C with a rising temperature. Above this temperature the animals were more or less inactive. When the water was allowed to cool, however, the animals became extremely active within a temperature range of 26° C to 21° C and returned to the very active state at 20° C. When the water was cooled below 8° C activity declined rapidly. The animals were incapable of progressive movement below 3° C.

The graphs shown in Figure 4 suggest that *B. lanceolatum* both from Helgoland and Naples is best adapted for life in water from 10° to 20° C and is therefore in all probability basically a Mediterranean animal. Its temperature tolerance is such, however, that it could and indeed does survive in waters up to 27° C and also between 10° and 3° C. It appears to be significant that the normal lower limit of winter tem-

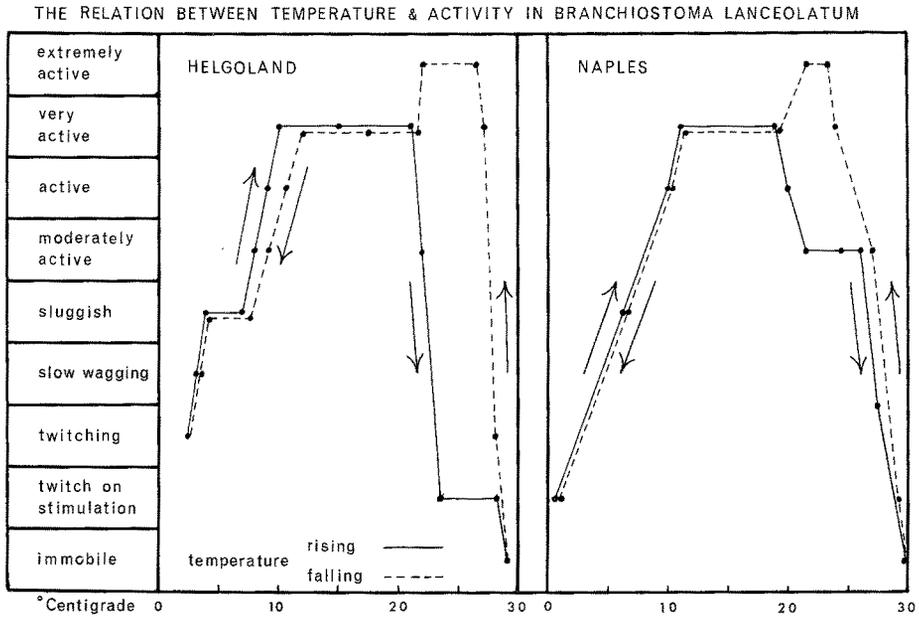


Fig. 4: Relation between temperature and activity in lancelets

peratures at Helgoland is about 3° C which coincides with the lowest temperature for progressive movement. These experiments suggest that the species probably owes its wide distribution to an ability to survive in a correspondingly wide range of temperatures.

THE EFFECT OF TEMPERATURES BELOW 3° C ON BRANCHIOSTOMA LANCEOLATUM

Lancelets frozen in seawater at -5° C and allowed to thaw became opaque. They showed extensive epithelial disintegration and the pharynx contained large amounts of mucus and cellular debris. There was no muscular movement and they did not recover when transferred to clean water at 10° C. However, at temperatures near freezing certain reversible effects were observed which disturbed the coordination of the pharyngeal mechanism.

The pharyngeal mechanism at low temperatures

One of the features of feeding of lancelets described by DENNELL (1950) in *B. bermudae* and confirmed by the authors for *B. lanceolatum* is the periodic clearing of particles blocking the mouth with a violent reverse current of water driven out of the pharynx by spasmodic contractions of the metapleural folds. In *B. lanceolatum*, contractions of the metapleura can be induced by touching the oral cirri with a needle. This reaction has been used as an indication of the responsiveness of these animals at low temperatures.

Freshly caught Helgoland lancelets were kept at 5.0° C for 5 days and then cooled over a period of 15 minutes to -1.7° C (when ice crystals were forming in the dish) and held at that temperature for a further 15 minutes. At 3.0° C the contraction of the metapleura was so slight that the lancelets failed to drive water out of the mouth and at 2.0° C and lower the response disappeared altogether. At 1.0° C the rhythm of the ciliary beat of the pharyngeal bars became disturbed and at 0.5° C the cilia stopped. On warming from -1.7° C the cilia began to twitch at 0.5° C and were beating at 1.0° C. At 4.0° C contraction of the metapleural folds on stimulation of the oral cirri was resumed and quantities of mucus were expelled through the mouth from the cavity of the pharynx. It appears, therefore, that the production of mucus by the endostyle continued at temperatures below that at which the cilia were able to function and, to judge by the quantity secreted, may even have been accelerated. On further warming to 5.0° C the lancelets swam sluggishly and at 10° C all animals were active and apparently normal in their behaviour.

These experiments show not only that *B. lanceolatum* cannot survive freezing, but also that the pharyngeal mechanism cannot operate normally at temperatures below 3.0° C. This mechanism involves three principal activities which in the normal animal are coordinated. These are the production of a mucus film and its transport along the ciliary tracts of the pharynx, the maintenance of a water current by ciliary action through the pharynx and atrium and the provision of a "cough" mechanism in which the contraction of metapleural muscles produces an outward water current which clears the mouth when it becomes blocked.

At temperatures falling slowly below 3.0° C first the "cough" response disappeared and then ciliary activity ceased, but mucus production continued. As a consequence mucus accumulated in the pharynx which at low temperatures could neither be ejected through the mouth nor transported by cilia to the intestine. Animals subjected for long periods to temperatures at or just below 0° C would thus be expected to fill the pharynx with mucus to such an extent that its removal either by ciliary or muscular activity even on return to higher temperatures might prove difficult. The chief feature of the 1962/63 winter was the prolonged period for which temperatures were around or below 0° C which should have induced this condition in the lancelets.

In addition, it has already been mentioned that pack ice over the amphioxus ground liberated large quantities of silt into the water and thus increased the turbidity. Under these conditions it would be expected that blocking of the mouth and pharynx would occur more frequently thus interrupting the inflowing water current essential not only for feeding but also in all probability for respiration. It has been observed, as

would be expected, that in small animals the mouth becomes blocked more frequently than in large and even under normal conditions they continually clear the pharynx by contractions of the metapleural folds. Failure to do this at temperatures below that at which muscular movement is possible might well lead to mortality among these smaller animals.

Differential survival at low temperatures

It has been shown that the climatic conditions of the 1962/63 winter not only resulted in an overall mortality of lancelets of some 50%, but appeared to have greatest effect on the small and the large animals in the population. This differential mortality has been demonstrated most clearly in Figure 3 where the percentage of notochords recovered is plotted against length. The reason for the survival of so many of the animals of medium length is difficult to establish with certainty as the precise conditions at the sea bottom and the reactions of the animals subjected to prolonged cold are not known and cannot readily be reproduced in the laboratory. However, the fact that the entire population was not exterminated suggests that the animals either differed in susceptibility according to length, or else that survival was due to some form of physical protection.

Low temperatures may prove lethal by causing mechanical damage to a vital system as in the formation of ice crystals in the tissues or they may inhibit the operation of a physiological process essential to the life of the animal. The failure first of the neuromuscular system at temperatures below 3° C and then of ciliary activity has already been shown to interfere with the normal method of clearing the mouth and to lead to cessation of the water current providing oxygen and food and removing waste products in animals living in coarse sand. Inability to absorb food, alone, is thought unlikely to be a prime cause of death, for animals of all sizes have been kept without food in the laboratory at higher temperatures (6°–8° C) for periods up to 6 months. However, oxygen lack or the accumulation of mucus or of waste products might well prove fatal in a relatively short period of time and could be a limiting factor. It is possible to envisage one or other of these processes becoming limiting at different times in animals at different stages of growth and so result in differential mortality related to size.

Quite apart from differential susceptibility on a physiological basis there remains the chance that physical protection of some of the individuals in the sand could have occurred. Lancelets burrow into coarse sand and commonly remain with the oral aperture above the surface. However, where permeability of the substrate is high many live completely buried at a depth of several centimetres and rarely come to the surface. The depth to which these animals burrow depends first on their ability to displace the sand grains and secondly on the freedom with which a current through the pharynx can be set up in the interstitial water. Moreover, as HAGMEIER & HINRICHS (1931) have shown, the activity in sand of lancelets causes a sorting of the substrate material so that large grains come to rest at the surface with the finer material beneath. Under these circumstances, small recently metamorphosed lancelets

could live within and may well be limited to the upper relatively large interstitial spaces of the coarse layer, whereas, larger individuals are known to be able to penetrate more deeply. It is probable that the largest animals by virtue of their greater ciliary current also require to live at or near the surface where permeability is relatively high.

When the temperature of the sea is falling the interstitial water in the substrate held to a large extent against external current movement and convection will remain warmer than the water above the sand. Moreover, with temperatures above the sand falling below 4° C, interstitial water at this temperature will be of greater density and will tend to remain undisturbed. It is possible to visualize, therefore, the situation where the water above the sand and in the coarse surface layer is at or near freezing while that beneath it in the finer sand is at an appreciably higher temperature. In such a case lancelets in the upper coarse layer, immobile through cold, might be killed, whereas those in the finer and warmer layer beneath would survive. We cannot be sure that this happened during the cold period at Helgoland, but from what is known of the behaviour of *B. lanceolatum* and both the physical conditions of the water at that time and the nature of the substrate, such a series of events was clearly possible. Thus freezing conditions penetrating the coarse surface layer of the sand would kill all lancelets in that layer and survival would be limited chiefly to those large enough to penetrate the deeper fine sand but small enough to be able to exist under the conditions of reduced permeability to be found there. As a result there would be a differential mortality such as has been observed. The linear nature of the graph in Figure 3 is perhaps explicable more easily as a result of a physical process of this kind.

The full cause of the mortality is probably a combination of both an early failure of a physiological mechanism, which might have been responsible for the early disappearance of the youngest lancelets (as shown by the sample taken in January) and a physical protective mechanism accounting for the survival of the middle-sized individuals.

The latent effects of the cold winter of 1962/63

The results presented here are those obtained from samples taken in November, 1962, and in January and April, 1963, but later samples indicated that the effects of the cold period were probably prolonged well into the summer. In catches in April and subsequently in May and July a number of the larger lancelets were found to have a pink coloration over all or part of the body. Moreover, in many specimens, there was evidence that a break down of the tissues in these pink regions was in progress and lancelets with part of the tail or with central myotomes missing were found. The cause of the colour was not known but it is considered that it may be associated with exposure to low temperatures. The fact that the pink coloration was restricted to the larger animals, and then only to certain parts of the body, suggests that they may have been lying across a temperature discontinuity in the sand and were thus exposed in part only to a cold upper layer. It is believed that further mortality among these animals so affected continued well into the summer after temperatures had returned to normal.

It has been observed that damaged lancelets and animals in poor condition are subject to attack from ciliate protozoa. A lancelet slightly damaged between the atriopore and the anus was kept in the laboratory at 8° C. The area of damage gradually increased until after 7 days all tissues in the centre of the body except the notochord had disappeared and the animal consisted of an anterior third joined to the tail by about 1 cm of notochord in its sheath. Both the pharyngeal region and the tail appeared normal, cilia were beating, a current of water was entering the mouth and both regions were capable of active independent movement. However, the exposed tissues were covered with large numbers of heterotrichous ciliates which were presumably responsible for the progressive enlargement of the wound.

The close resemblance of this specimen to those collected in the field suggests that the notochords recovered from the April sample may have had a similar origin. The fact that ciliates attack damaged lancelets but are not found on healthy animals suggests that low temperatures may have been only indirectly responsible for mortality and that animals damaged or in some way rendered attractive to ciliates by exposure to cold were unable afterward to resist invasion of their tissues and the gradual dissolution which followed. This would also account for the continued mortality, presumably in lightly damaged animals, long after temperatures had risen to normal.

SUMMARY

1. Seawater temperatures at Helgoland were subnormal for six months during the winter of 1962/63 and fell to -1.3° C. Quantities of silt from the shores and estuaries of the mainland were released into the water when pack ice around the island melted in late February.
2. In a sample dredged from a ground near Helgoland in April, 1963, 40% of the lancelets consisted of isolated notochords. From comparison with a sample taken in November, 1962, it is estimated that at least 50% of the population died during the winter. The average number of animals per Van Veen grab fell from 6.9 before to 3.0 after the cold period.
3. None of the autumn settlement of newly metamorphosed animals survived, and there was a very high death rate estimated at 90% among the largest animals.
4. An analysis of activity in relation to temperature in *Branchiostoma lanceolatum* collected from Helgoland and Naples showed a temperature tolerance of 3° to 27° C in both populations with active swimming at temperatures of 10° to 20° C. There is no muscular movement below 3° C which is the lower limit of the normal winter temperature at Helgoland. It is suggested that the species is best adapted to Mediterranean conditions.
5. The pharyngeal mechanism cannot operate normally below 3° C and although it is considered unlikely that starvation was the primary cause of death, other effects of a reduced pharyngeal current may have proved lethal.
6. It is held that the depth to which lancelets of different sizes burrow into the substrate could account for differential mortality through cold.

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Discussion following the paper by COURTNEY & WEBB

WIESER: Small animals have relatively higher energy requirements than large animals. Therefore any failure in the respiratory mechanism due to cold should affect the former more severely than the latter. This could account for the differential mortality in *Branchiostoma*. Is there any clue as to whether or not this fact could apply to your material?

COURTNEY: The total oxygen requirement of a large animal would surely be equal to or greater than that of a smaller animal. Preliminary experiments in London have shown that this is so for *B. lanceolatum*.

PROSSER: The activity-temperature curves are strikingly similar to swimming data which Dr. ROOTS and I published on fresh-water sunfish. We found marked temperature acclimation. Your curves comparing Naples and Helgoland animals suggest that there is no temperature acclimation in this species as to activity. Have you any activity data on Helgoland animals taken at a time of year when water temperatures were higher?

WEBB: Measurements of activity of Helgoland lancelets were made back in April when the temperature of the sea was 4° C and in August of the previous year when the temperature was 18° C. There was no difference in activity on these two occasions, suggesting that a significant acclimatization does not occur in *B. lanceolatum*.

BOOLOOTIAN: Have you noted any difference in the gametogenic processes, either histologically or cytochemically between the years 1962 to 1963?

COURTNEY: We have studied the gonad development for the year August 1962 to the present. We do not have data of a year with normal temperatures for comparison.