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The application of new techniques to the study of planktonic organisms.

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The Application of New Techniques to the Study of Planktonic Organisms

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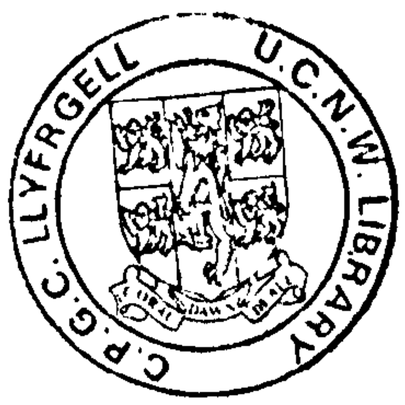

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Summary

A device, utilising the suction produced by a disposable syringe, was developed to restrain a range of very small organisms in flowing seawater. The technique was developed during an investigation of the swimming response of barnacle nauplii to changes in temperature. The combination of beat frequency and the proportion of time spent active was held constant for B.balanoides and B.hameri but steadily increased with temperature for C.montagui and B.amphitrite. E.modestus showed an intermediate response.

Methods for utilising video-tape recordings and a micro-impedance pneumograph for analysing limb beat activity, were developed during an investigation of the feeding behaviour of nauplii. The feeding mechanism was reappraised and an increase in the volume of water handled by the larvae noted in the presence of food organisms. This increase was shown by E.modestus nauplii in response to dissolved organic substances, indicating that the nauplii could determine when to feed, and what was edible, from the shell of dissolved material surrounding food particles.

A quantitative study of the grazing of E.modestus nauplii showed that the ingestion rate of algal cells increased up to concentrations of 150 - 200 cells/ul, then remained steady. Larger algal cells were taken from algal mixtures, in preference to smaller ones.

Restraint techniques and video-recordings of free-swimming cypris larvae showed them to be prodigeous swimmers, with considerable control over the direction and magnitude of the thrust produced.

The close observation of larvae allowed by the restraint technique enabled mechanical stimulation of particular sense organs. The function of these organs had been interpreted from their structure by previous authors. In some instances, a mechano-receptive function was confirmed, but in others, doubt was cast.

Restrained cyprids responded to complex sound fields by swimming less often. A dual mode sound chamber was used to differentiate between sound pressure and displacement, but negative results were obtained. The cyprids did, however, respond to substrate vibrations, at low frequencies, by swimming off the substrate.

All the developed techniques were further employed to show that the classical copepod feeding swirls were artefacts. Temora produced only a posteriorly flowing current for feeding and swimming, when restrained in larger volumes of seawater. The amount of water handled by Temora was also shown to increase when food algae were present.

Section 1

The Observation of Planktonic Larvae:

Conditions for Behavioural Analysis

The Observation of Planktonic Larvae: Conditions for Behavioural Analysis.

Introduction

The study of planktonic larval behaviour has always presented zoologists with problems. Normally such larvae are very small, requiring considerable care in rearing or in maintaining animals previously sorted from plankton hauls. In analysing the behaviour of such animals it is important to take note of their condition and past experience. The advantage of studying animals reared in the laboratory under controlled conditions lies in the certain knowledge and reproducibility of the conditions up to the time of observation. The disadvantage is the possible discrepancy between laboratory rearing conditions and those of the natural environment. However, those animals taken straight from the plankton, although reared naturally, undergo the experience of transfer to the laboratory environment. It may therefore be just as dubious to relate the laboratory behaviour of wild larvae to the natural situation as it is for laboratory reared ones.

Previous studies of behaviour have used both wild and reared larvae depending either on laboratory rearing facilities or the available seasonal occurrence of larvae in the plankton. Observations of animal behaviour should be related to the natural environment by considering the past experience of the organism in the widest sense possible. The choice between a reared and a wild experimental animal is more dependant on facilities and conditions than any inherent difference between the two which would invalidate consideration of the environmental consequences of observed behaviour.

Plankton research in general has concentrated on group movements of animals in response to various environmental stimuli. Light, for instance, is an important stimulus in the marine environment and both photopositive and photonegative behaviour in planktonic organisms has been noted and investigated from an early date (Loeb and Groom 1890). Analysis of such behaviour became more exacting as time progressed. Spooner (1933) attempted to classify the types of directed behaviour in response to light exhibited by various species and to explain the usefulness of such responses in the natural situation. The quality of the light stimulus (wavelength etc.) has also been investigated, earlier by Visscher and Luce (1928) and more recently in a more refined manner, for example in cirripede larvae, by Barnes and Klepal (1972) and Crisp and Ritz (1973). These authors were interested in the group response to minimal light intensities as a measure of sensitivity. Combinations of factors have also been studied, for example the response to light related to the nutritional state of barnacle nauplii was investigated by Singarajah, Moyse and Knight-Jones (1967). Such studies clearly show the importance of light in the marine environment but the techniques used do not allow detailed analysis of individual movements.

Likewise pressure, as another physical factor in the marine environment, has come under close scrutiny. Baroreception in zooplankters has, however, a much shorter history in the literature than the investigation of photoreception. Hardy and Bainbridge (1951) attempted one of the earliest investigations of a pressure sense in planktonic animals after hints of its existence from earlier work (Hardy and Paton 1947). Since then much work has been carried out in this field by Knight-Jones and Qasim (1955,1967) and Rice (1964) and reviews on the subject have been published by Knight-Jones and Morgan (1966), and Morgan (1972). The

experimental work was performed with groups of organisms by noting their movement with respect to changing conditions. Other large scale movements of plankton have intrigued zoologists. The much discussed phenomenon of vertical migration, for instance, is well documented. The interpretation of group behaviour is always difficult and vertical migration has been explained in many ways. Clarke (1933) stressed the importance of the physical factors involved in the control of migration (see also Verwey 1966), whereas others proposed complex ideas of the involvement of vertical migration in larval transport in estuaries (Wood and Hargis 1971) or on a larger scale, with the movements of Krill in Antartic currents (Hardy and Gunther 1935). McLaren (1963, 1970) proposed that many of the observed vertical migrations could help plankters to conserve energy by spending part of their time in deeper, colder waters. The studies of Hardy and Bainbridge (1954) on vertical migration using the 'plankton wheel', were an attempt to analyse the behaviour of an individual plankter under experimental laboratory conditions as near to the prevailing environmental conditions as possible.

As can be seen from the above, the methodology of previous experimental work has relied on the fact that in any one group of planktonic organisms the majority will react to various stimuli by movement. In such experiments the animals can be confined in a suitable vessel, pressurised or exposed to light, reduced salinity, increased oxygen tension etc. and the animals' response measured as a movement in one direction or another. Though experimental design has advanced considerably since the early days, both in counting methods and in the precise presentation of the stimulus, the animals' response is almost always measured by a directed movement. The study of how the response is effected, how the change in direction comes

about, whether the movement is faster or slower than normal etc., requires a different approach. It is, moreover, possible that observations of group behaviour may miss subtle changes related to individual conditioning or physiology. Individual swimming speeds can be measured (e.g Cragg and Gruffydd 1975) and while such an approach has a worthwhile place in plankton research, the information it yields is limited.

To ascertain correctly how an animal feeds, a detailed examination of the animal under appropriate conditions of food supply is necessary. Such a task, with a large plankter such as a copepod, may seem a relatively simple operation employing a wide field binocular microscope and confining the animal to a small volume of water. Initially the feeding currents of copepods were deduced from consideration of the anatomy of the limbs involved and from observations of the feeding currents in small volumes of water (Cannon 1928). The physics of water movements in very small volumes, however, is most unlike that of the open sea. If it is necessary to look more closely at the limb movements, or if the organism is considerably smaller than a large copepod, then more drastic constraints are necessary. For instance, Cannon (1928), measuring the limb beat frequencies of Calanus, had to constrain the animals by pressing them on a microscope slide in a small drop of water with a coverslip supported by blobs of wax at each corner. The idea of this 'compressorium' was to allow limb movement of the animal and at the same time trap it within the field of view of the microscope. Another method, based on similar principles, was used by Rainbow (1975) whereby cirripede nauplii were held individually in small drops of water floating on paraffin oil under a coverslip. I have used both methods, finding Cannon's to be suitable for very short observations on copepods (of the order of 5 mins) but quite impractical for small larvae.

The paraffin oil technique works reasonably well for small larvae but the procedure is difficult to standardise and the animals' behaviour in such very small volumes of water is often unpredictable. Daphnia have been restrained by impaling them on pins through the brood pouch (Ringelberg 1964) and it was suggested to me that small larvae could be restrained in a manner similar to that employed by entomologists to hold flies and locusts etc., i.e. sticking the animals to splints of wood with non-toxic glue.

Much of our knowledge of limb movements, feeding, and individual larval behaviour has come from studies using crude restraining devices, through interpretation of anatomy, or through chance observations and fortuitous pieces of ciné film. For instance, the feeding of cirripede nauplii has been investigated largely from anatomical considerations combined with observations of the type described above (Lockhead 1936, Norris and Crisp 1953, Rainbow 1975). Observation and handling of small larvae is difficult and convenient restraint of the animal is essential unless the observer wishes to spend a long time staring down a microscope waiting for an animal (a) to come fully into view and (b) at the same time perform the particular behaviour pattern that is of interest.

What then are the criteria of a reasonable system of restraining a small animal and thereby facilitate the full observation of the mechanisms of behaviour? Obviously the primary object is to restrain the animal without damage. Sticking pins through parts of the carapace or through a thin shell, no matter how unimportant these parts may appear to the experimenter, is always liable to influence natural movement even though it may be perfectly valid in some cases. Pin pricks in large animals, even man, cause some degree of shock and in microscopic organisms, such as Daphnia

or invertebrate larvae, must always leave the experimental result open to some doubt. 'Compressorium' techniques such as Cannon's, if applied with care, probably cause no damage. However, the chances of damage are quite high and such damage might be undetected. The biggest drawback to this type of restraint is the small volume of water surrounding the animal and the restraints imposed on the water movements themselves.

Free water movement must be the second criterion for a satisfactory restraining technique, implying that the animal must be in as large a volume of water as possible. Small volumes of water, the essence of 'compressorium' techniques, are not only unable to flow normally but are also subject to large fluctuations in temperature, oxygen tension and very often, salinity changes. For long periods of observation the task of controlling these variables in such small volumes would become almost impossible. Apart from these considerations the fact that the animals are bounded on all sides by a glass slide or a water/paraffin interface and will probably spend a great deal of time trying to escape, thereby continually brushing their limbs against a surface, makes observational interpretation in terms of natural behaviour very difficult.

Yet another criterion for a suitable restraining system must, therefore, be that all the limbs are free to move with no chance of hitting an obstacle. The suggestion of sticking animals on splints with glue is a reasonable one as it works well with larger terrestrial/aerial animals. It seems likely, however, that such a technique would involve removing the animal from the water and drying it out before it can be stuck and transferred back to the water. The procedure would greatly increase the chances of damage with physiological and behavioural disturbance to the

animal resulting again in difficulties of interpretation. The range of organisms likely to be amenable to such rough handling would limit the application of such a glueing restraint technique, while other problems such as the choice of an appropriate glue are readily apparent.

The criteria for a useful restraining technique for planktonic organisms can be summarised as follows:-

1. The animal must sustain no damage and little or no disturbance during the process of restraint.
2. The method must be simple to operate and applicable to a wide range of planktonic organisms.
3. The system must allow for manipulation of the animal within the field of view to obtain the best angle of observation and allow electrical or photographic recording of behaviour.
4. The volume of water around the animal must be large enough to allow control of all environmental factors and unrestricted water movement.
5. The system must have the capacity for the presentation of various stimuli to the organisms such as current changes, food particles, temperature changes etc.

The system described in the following pages fulfills these conditions.

METHODOLOGY

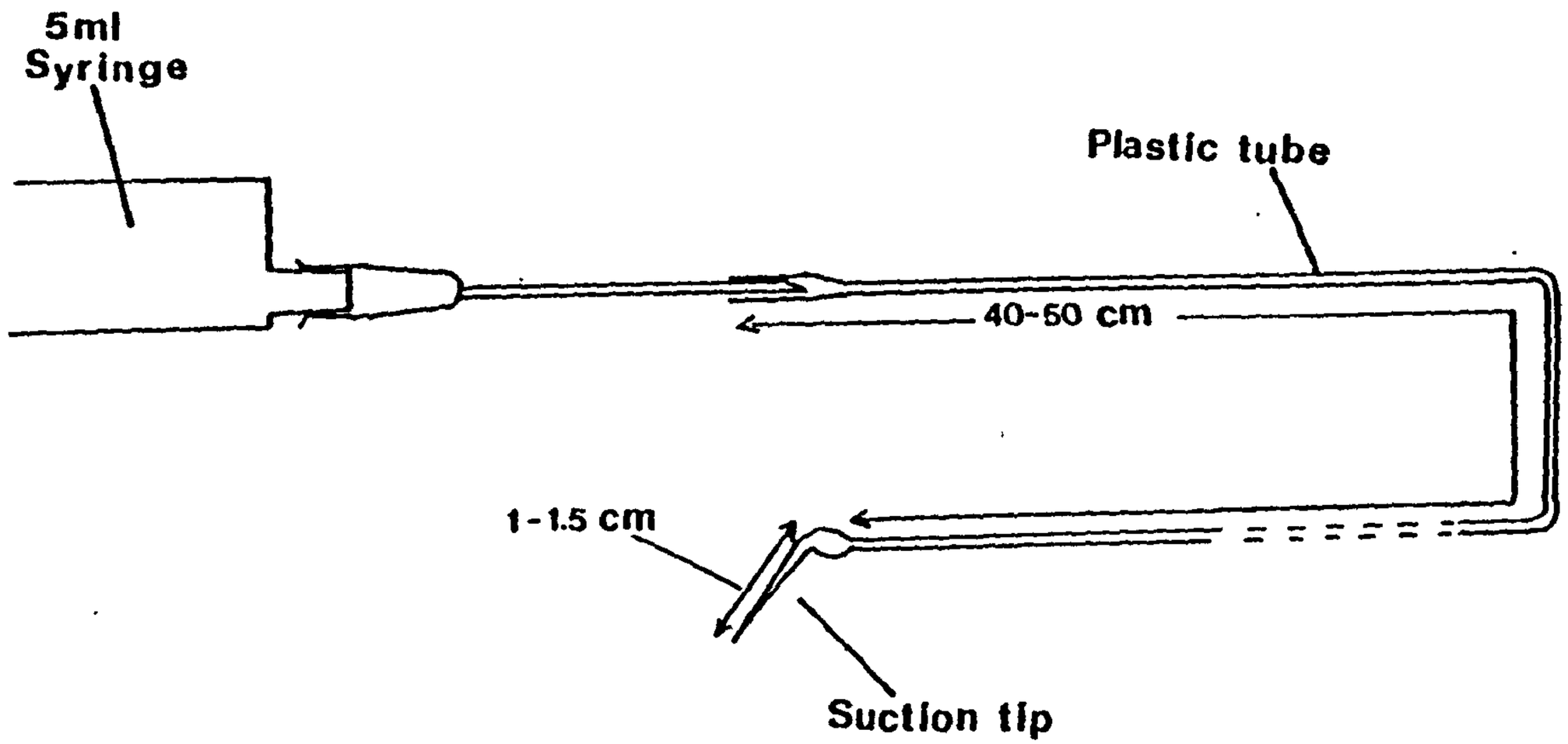
The techniques to be described here are those having a general application. More specialised methods will be given under the appropriate results section.

In essence the individual larva was held within the field of view of a microscope in such a way that met the criteria listed above. The central idea was an adaptation of the suction electrode technique used in electrophysiology for many years. Fig 1.1 shows a diagram of a general type suction tube suitable for restraining many types of planktonic organisms.

The suction tube was made from plastic capillary tubing approximately 1mm in diameter, normally about 40-50 cm long (Fig 1.1). One end of the tube was attached to a syringe needle while the other end was drawn into a very fine tube (Fig 1.1). The success of the device relies on the care and precision employed in producing the tip. The best results were obtained by heating the capillary tubing over a small flame in a draught free place and allowing the plastic to melt slowly. By exerting a small pull and then relaxing it, a small bubble of molten plastic could be formed. By then raising the tube away from the flame and gently pulling, a thin tube normally about 1.5 - 2 cm long could be made. At this stage, while the plastic was still hot, the angle of the suction tip was decided by bending the tip in the required manner. Bending the tip to a specific angle helps with the attachment of animals and with the manipulation to the desired viewing angle under the microscope. Straight tubes were often found useful for work with copepods whereas tips bent at 45^{o} to the longitudinal axis of

FIG 1.1

Diagram of a suction tube



the main tube, similar to that shown in Fig 1.1, were better for bivalve veligers.

Using a sharp scalpel, it was possible to cut the thin tube leaving only about 0.75 - 1.0 cm of small bore tube attached to the larger capillary tubing. By this method, fairly rigid tubes of 80 - 120 µm external diameter and terminating in a pore diameter of 40 - 70 µm could be made. The size of the tip depends entirely on that of the animal to be held. Normally, the larger the animal the larger the tip, but there was no hard and fast rule. The shape of the animal was also important; for instance, it was necessary to use a smaller tube than one might imagine for some copepods, because of the highly convex shape of the carapace. The nature of the surface of the animal to which the tip is applied must also be considered. Generally the technique worked best with animals that have some form of hard exterior, the shells of well advanced mollusc larvae or the carapace of late stage barnacle nauplii for instance, though it was possible, with care, to attach some softer forms such as stage II nauplii of Balanus hameri which have a flexible cuticle. In cases where soft bodied animals are to be attached, the smallest possible tips should be used and even then great care must be taken to avoid damaging the animal; such cases must, however, admit to some risk of undetected damage.

The suction tube, once prepared, was mounted on a micromanipulator under the microscope. The type used here was a Research Instruments TVC500 providing a manipulator on each side of the microscope with extensive finger-tip control of coarse and fine movement in three planes. A good manipulator makes the attachment process a good deal easier. The suction force holding the animal was provided by an ordinary plastic syringe which

could be conveniently clamped in the 'suction mode' by a large bulldog clip. The capacity of the syringe was of little consequence, but a 5 ml syringe was the easiest to handle and provided sufficient long term suction. In some instances a 10 ml syringe proved more effective, particularly for the larger copepods, but was slightly more awkward to handle.

Using a suction device, the volume of water surrounding the animal was dependant only on the physical limitations of the microscope position, allowing considerable scope for control of the environment. For cirripede larvae the arrangement shown in Fig 1.2 was found to be convenient to use. A petri dish (p) with an inlet (i) and an outlet (o) made a convenient observation chamber holding about 40 ml of seawater without flow and 50 ml when the flow was turned on. The chamber (p) was gravity fed from a 6 litre header tank (h) which was maintained at a constant level by another tank (t). This two tank header system ensured that a constant flow rate was maintained with minimal supervision or risks of propogative disturbances reaching the observation chamber during topping up. The outlet from the header tank (h) passed through a heat-exchanger (c) which was connected to a regulated water-bath. The water temperature in the chamber (p) could thus be controlled within $\pm$ 0.5°C. The temperature was checked by thermistor for nearly all the experimental work described below. Flow rates of the order of 0.25 - 1.0 cm s^{-1} were normally used but since the chamber had a larger outlet than inlet pipe, flow rates up to 5.0 cm s^{-1} could have been obtained.

Fig 1.2

Diagram showing the apparatus used to observe restrained larvae in flowing seawater.

c = condensor tube.

h = lower header tank.

i = observation dish inlet.

I = injection point (re Section 4).

m = micromanipulator.

o = observation dish outlet.

p = observation dish.

'scope = binocular microscope.

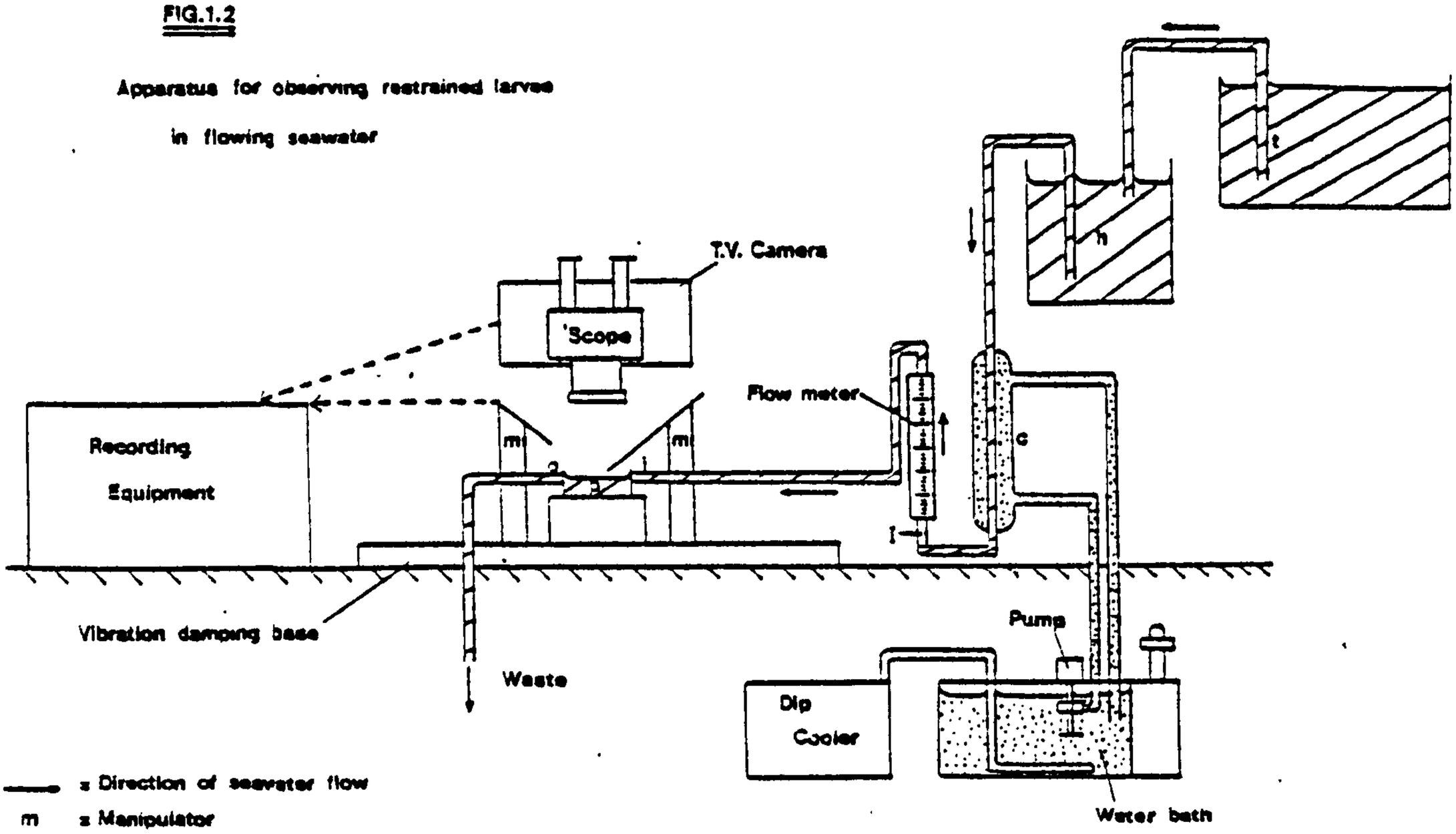
t = top header tank.

Fig 1.2(a) Side view of the observation dish (p) showing the water movements within the dish and the approximate position of the restrained larva (A).

Fig 1.2(b) Plan view of the observation dish.

FIG.1.2

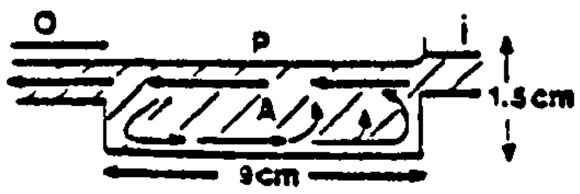
Apparatus for observing restrained larvae in flowing seawater



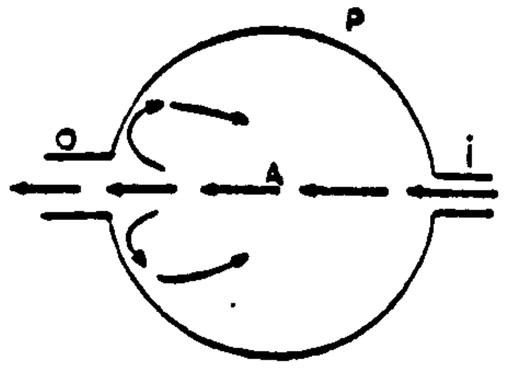
= Direction of seawater flow

m = Manipulator

1.2(a)



1.2(b)



The pattern of flow through the chamber (p) was somewhat complex. Some of the seawater flowed directly from inlet to outlet while some was mixed within the chamber. The direction of water flow is illustrated in Figs. 1.2a and 1.2b, drawn from dye experiments. If the animal was placed at position A (Figs. 1.2a and 1.2b) it then experienced a unidirectional flow. It might be desirable to employ a hydrodynamically designed tank with its flow accurately controlled and equipped with baffles and screens to smooth out the eddies. However, this simple system was considered adequately characterised for the observations made. Small modifications were made to the flow system as time progressed and only the basic system is described in Fig 1.2, a later version is described in section 10 Fig 10.1.

There may be difficulties when vigorous, fast swimming plankters are being attached to the suction tip. It was desirable to attach the organisms as quickly and as gently as possible but this could often be achieved only after anaesthetising them. 8% magnesium chloride in seawater could be used to anaethetise cirripede larvae, but it was found to take too long and the recovery time was rather unpredictable. The best anaesthetic was found to be MS 222 Sandoz used at concentrations of 20 ppm or lower in seawater. On average, the larvae took 10 - 15 seconds to cease movement and 40 - 60 seconds to recover fully in flowing seawater. 30 seconds was a reasonable time for the attachment procedure so this anaesthetic allowed ample time. Presumably the shorter the time between placing the larvae in the anaesthetic and their recovery, the smaller was the chance of permanent damage to the organism.

The procedure was as follows:- a larva was removed from culture by pipette and placed in the anaesthetic until it ceased to move. It was then transferred to the flow chamber and placed in the centre of the microscope field of view. The suction tip was used to align the larva before the tip was brought into close contact with the carapace. The suction was then increased by pulling out the syringe plunger until the animal was firmly secured. Next, the animal was moved to the appropriate position in the chamber and the seawater flow turned on. The animal was checked to ensure that it was at the correct angle for observation and experiment. If the angle was incorrect, the capillary tube was turned in the micromanipulator holder until the position was corrected. The animals normally recovered quite quickly thereafter.

Immediately after attachment, the behaviour of cirripede larvae tended to be very erratic and included more than the normal frequency of interruptions to swimming. The nauplius, for instance, showed an increase in the amount of folding of the limbs, an activity to be discussed below. However, after about 30 - 60 minutes, its behaviour became more regular and the animal appeared to habituate to the presence of the suction tip. Normally at least 45 minutes were allowed after each attachment before any observations were made. Under such controlled conditions the behaviour of individual larvae could be studied in a novel way. Since the animal cannot move, the limb movements could be seen in detail. Changes in limb frequency and amplitude are important elements in the behaviour of many crustacean plankters and a quick and accurate method of determining both was necessary.

The impedance pneumograph

The impedance pneumograph utilises the change in seawater impedance between two wires, as may be caused by a body of different resistance to seawater moving between the wires. An electrode made from two suitable wires is connected through the impedance measuring circuit and the output, representing impedance fluctuations, is recorded on a chart recorder. The wires are placed around the subject of the activity measurement in such a way that the animal's movement is not obstructed, yet the wires are close enough to measure significant changes in impedance. The deflections recorded by the chart recorder are thus due to the movement of the animals' member. The frequency of signals corresponds to rate of beat and the amplitude of the signal is approximately proportional to the distance or angle through which the member moves. The technique is relatively easy to employ, although suffering from the usual problem associated with electrical measurement in seawater, that of 'electrical noise'. Pioneered by Trueman(1967), such devices have often been used on a macroscopic scale for measuring heart beats of marine animals, in situ, and also for adult limb movements such as scaphognathite movements of crabs or the cirral beat of adult barnacles (Davenport 1976).

This basic system was scaled down for use with cirripede larvae and other small planktonic organisms. The electrode wires could be conveniently made from plastic coated bathythermograph wire and directly connected to a classical impedance unit coupled to a George Washigton chart recorder. The bathythermograph wire was too thick (0.1mm) to be positioned around nauplius limbs and had to be thinned down, in most instances, to at least a quarter of its original size. This could be readily achieved by

electrolysing both wires in a salt solution. The electrolysing current was supplied from 6 - 9 volt calculator batteries or sometimes a 6 volt, 1 Hz output from a Levell oscillator was used. The wires were often thinned unevenly and so no standard timing of the electrolysis was possible. Consistent results were obtained by keeping a constant check on the wires during electrolysis. Once the electrodes were of the required thickness and scraped clear of deposits, it was found useful to soak them in dilute citric acid solution, followed by a thorough wash in seawater, before use. Electrodes treated in this manner tended to give better signals and the possible toxic effects of any deposits left on the electrodes were avoided. Once the electrode tip had been prepared the electrode could be bent to any desired angle and mounted on another micromanipulator and placed around a limb. Normally the electrodes were kept close together and placed around the basal part of the limb thus reducing the chances of interference with limb movements. The resultant chart recorder output was usually very clear once any electrical 'noise' problems had been overcome by suitable earthing of the observation chamber and reservoir tanks. The amplitude measure, however, was often shown to be unreliable and could be seen to vary more on the trace than the actual limb beat amplitude. This variability was caused by accidental movements of the electrodes either by the operator, the animal, or the water current. Small changes in the electrode position sometimes had large effects on the signal amplitude of the trace. Calibration of the beat amplitude was therefore very difficult. Nevertheless, the frequency of limb movement recorded was a true representation of the animal's activity irrespective of changing amplitude calibration. The ease of analysis of these activity records should make the technique a valuable tool in the study of larval behaviour.

Television Video Tape Recording

Television is becoming increasingly used for the study of animal behaviour. Video-tape recording (V.T.R.) has considerable advantages over ciné film. The possibility of immediate recall of observed and recorded material is probably its greatest advantage. The television camera has also a greater potential than the ciné camera since lighting requirements are neither as great nor as critical, most T.V. cameras operating effectively in very dim light. The video system used in these studies comprised an Hitachi SV-612 time lapse V.T.R. displaying on a Sony monitor. The camera was an E.M.I. Surveyor II capable of taking pictures illuminated only by infra-red wavelengths. A special T.V. phototube was fitted to a Wild M5 stereomicroscope giving a trinocular viewing arrangement. Once the camera was mounted behind the microscope (as in Fig 1.2) a movable stage controlled the focussing. The microscope field of view could thus be checked directly (by eye) or via the T.V. monitor. The system produced sharp pictures, which lost little in recording or playback, except in slow motion mode which was interrupted by a "tracking band" traversing the screen and an inherent jerk as each band passed off the screen. This disturbance to the picture, though elimination should be possible with better video technology, represented only a minor nuisance to which the operator becomes accustomed during playback analysis. Fig 1.3 shows the major part of the experimental equipment in operation and a cypris larva can be seen on the monitor screen. This larva was alive and attached to a suction tip when the photograph was taken.

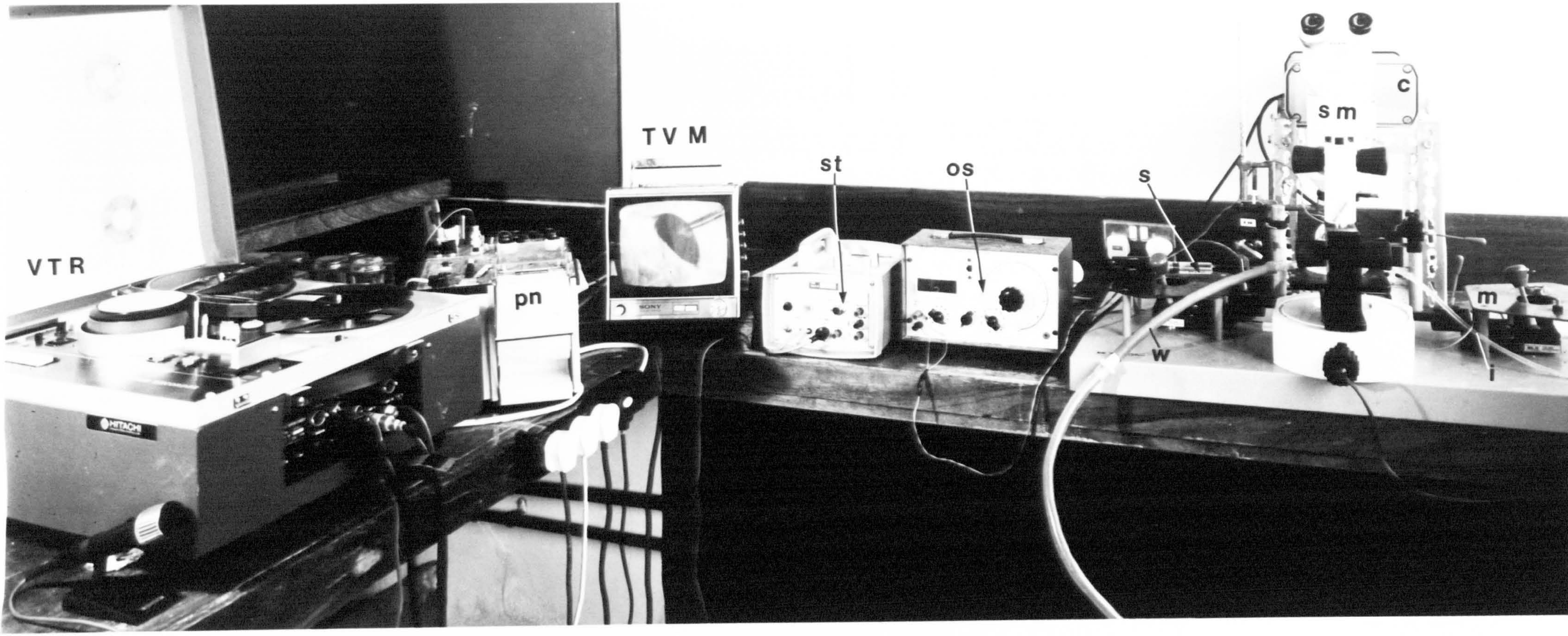


Fig 1.3 Apparatus for observing restrained larvae

c : t.v camera os : oscillator sm : stereomicroscope VTR : video-recorder

i : inlet pipe pn : pneumograph st : stroboscope w : waste pipe

m : micromanipulator s : syringe TVM : t.v. monitor

At present V.T.R. has been found to be a more useful tool than the pneumograph, although recent advances to develop a single wire micro-pneumograph electrode with a more reliable amplitude response are encouraging. Much of the work described below involves only V.T.R.. The frequency of limb beat was timed using a stopwatch and the slow motion playback mode, whilst beat amplitude was measured by marking the positions of the limbs at the beginning and end of a swimming stroke and this distance measured with a ruler. Accurate assessment of animal behaviour could be made with the opportunity to check the findings many times. The facility for voice recording on video tape enabled the operator to present a stimulus and mark the time of presentation accurately on the tape without having to observe the animal's response directly at the same time.

Like the pneumograph, however, V.T.R. has some disadvantages. Analysis of relatively short experiments recorded on video tape is very time consuming. Care must be exercised in the selection of information to be analysed and also the method of analysis. During the course of this dissertation I shall endeavour to offer suggestions on the efficient use of V.T.R. as it applies to the study of planktonic organisms.

Materials

Most of the cirripede larvae used were released and reared in the laboratory using well tried methods, similar to those of Gruffydd and Beaumont (1972) for scallop larvae and those of Tighe-Ford *et al.* (1970) for cirripede larvae. All the larvae were reared in 3 litre glass jars filled with filtered seawater and kept at constant temperature under normally prevailing light conditions. The jars were cleaned and sterilised

with hypochlorite solution every two days. At the same time the larvae were washed thoroughly in filtered seawater in appropriately sized sieves. Antibiotic solutions of penicillin and streptomycin at .03g and .05g per litre of culture respectively were initially added to the cultures to keep bacterial levels in check. However, this precaution was later deemed unnecessary as it was discovered, in common with Mullin and Brookes (1967), that there was no discernable difference in larval survival with or without antibiotics. Where possible, cultures of Skeletonema costatum were used as the food source following Moyse (1963). There were occasions when Skeletonema was unsuitable or unavailable and the flagellate algae Tetraselmis suecica, Rhodomonas baltica or Paylova lutheri were used. These flagellates appeared acceptable to the nauplii, especially when presented as mixtures. Normally between 250 - 500 ml of stock Skeletonema per jar were used giving average cell concentrations of 200 - 300 cells/µl. Occasionally this concentration had to be considerably reduced to prevent clogging of the limbs in the later stages (particularly with Balanus hameri nauplii). In many cases mortality at stage VI was very high unless algae were completely absent from the culture.

The following is a list of the barnacle species reared during the experimental period.

Elminius modestus (Darwin). Adults were collected mostly from the columns of the road bridge and pier at Menai Bridge and occasionally from the pier at Beaumaris (Anglesey). The most convenient method of release is to break up the adults and place them in a shallow tray with filtered seawater and attract the stage I nauplii to a light at the opposite end of the tray. The larvae were then maintained at 20°C with moderate aeration.

The stages were identified with reference to Knight-Jones and Waugh (1949).

Balanus balanoides (Linn.). These were collected solely from Menai Bridge and handled in the same way as E.modestus. The rearing temperature was 15°C. The stages were identified with reference to Crisp (1962a).

Balanus hameri (Ascanius). These were collected from 20 m depth near the Isle of Man, attached to the shells of the horse mussel, Modiolus modiolus, and the larvae were released as above. Some of the larvae used were in fact released from adults maintained for over a year in the laboratory at Menai Bridge. The rearing temperature was 10°C and the identification of the larval stages accomplished with reference to Crisp (1962b).

Balanus amphitrite communis (Darwin). Adults were obtained from Goa, India and I am indebted to Professor D.J.Crisp for providing them. These adults were maintained in a small tank at 25°C, and fed large quantities of mixed plankton, including many B.hameri and B.balanoides nauplii; both species of nauplii evoked a strong feeding response from adult B.amphitrite. The tank had a continuous seawater flow and the effluent from the tank was collected and treated with hypochlorite solution before disposal in order to prevent the introduction of exotic pests. The animals eventually produced many larvae which were collected from the seawater outlet in an 80 µm sieve. The larvae were washed every other day with seawater at 25°C, thereby eliminating any thermal shock during the cleaning process. Kalyanasundaram and Ganti (1974) found that there was a high mortality between stages III - IV when rearing B.amphitrite, which they related to the type of food offered to the larvae. A similar high

mortality between stages III - IV was found in the present study, but the problem was overcome by reducing the rearing temperature from 25 to 22°C once the larvae reached stage III . The majority of the larvae then reached the cyprid stage. The stages were identified with reference to Costlow and Bookhout (1958).

Chthamalus montagui (Southward). The adults were collected from Cable Bay (Anglesey) by chipping off pieces of rock to which they were attached. The rocks bearing the adults were maintained at 22°C in a small tank and the larvae collected as for B.amphitrite. The rearing temperature for the larvae was 22°C and the stages recognised with reference to Bassindale (1936).

Chthamalus malayensis (Pilsbry). These were also collected by Professor Crisp in Goa and handled as for B.amphitrite. Unfortunately they did not produce sufficient larvae for experimentation although a few were successfully reared to the cypris stage.

Cyprids

Cyprids of B.balanoides, used for experiments in the later chapters of this thesis, were taken from the wild populations at Port Dinorwic (Gwynedd) in a medium mesh plankton net. They were thoroughly washed in filtered seawater and kept in 3 litre glass jars at 15°C. Every two days they were washed and the jars cleaned and sterilised, but in general the larvae were seldom kept in the laboratory longer than a week before an experiment and most were used within one day of capture. Cyprid density in the jars was quite high and the jars were therefore vigorously aerated. I

could detect no behavioural difference in an isolated experimental situation between animals which had been kept at high densities and those kept at low densities.

Section 2

The Effect of Temperature on the Swimming Activity of Barnacle Nauplii

The Effect of Temperature on the Swimming Activity of Barnacle Nauplii.

Introduction

Southward (1955) has shown that the cirral beating activity of adult barnacles is affected by temperature. As would be expected from poikilotherms, the frequency of cirral beat by adult barnacles increases with increasing temperature until, at higher temperatures, the animals become adversely affected by temperature and the cirral beat frequency declines. Southward showed that some barnacles like Elminius modestus had fast beat rates and could continue to beat from temperatures as low as 2°C to above 30°C. Others such as Balanus balanoides, although capable of beating at low temperatures, ceased to beat at temperatures approaching 30°C. The response to temperature shown by the cirral beat activity appeared to reflect the geographic distribution of the barnacle species and the temperature range to which they were normally exposed.

Later, Southward (1958) qualified his findings by investigating the tolerance of adult barnacles to extremes of temperature and found that the lethal effects of temperature on the barnacles were not the prime species delimiters. Although temperature was considered to be an important factor governing the distribution of intertidal organisms (see for example Southward and Crisp, 1954) the ability of the organism to cope within a given temperature range would help to determine the distribution of that organism rather than the lethal extremes of the range.

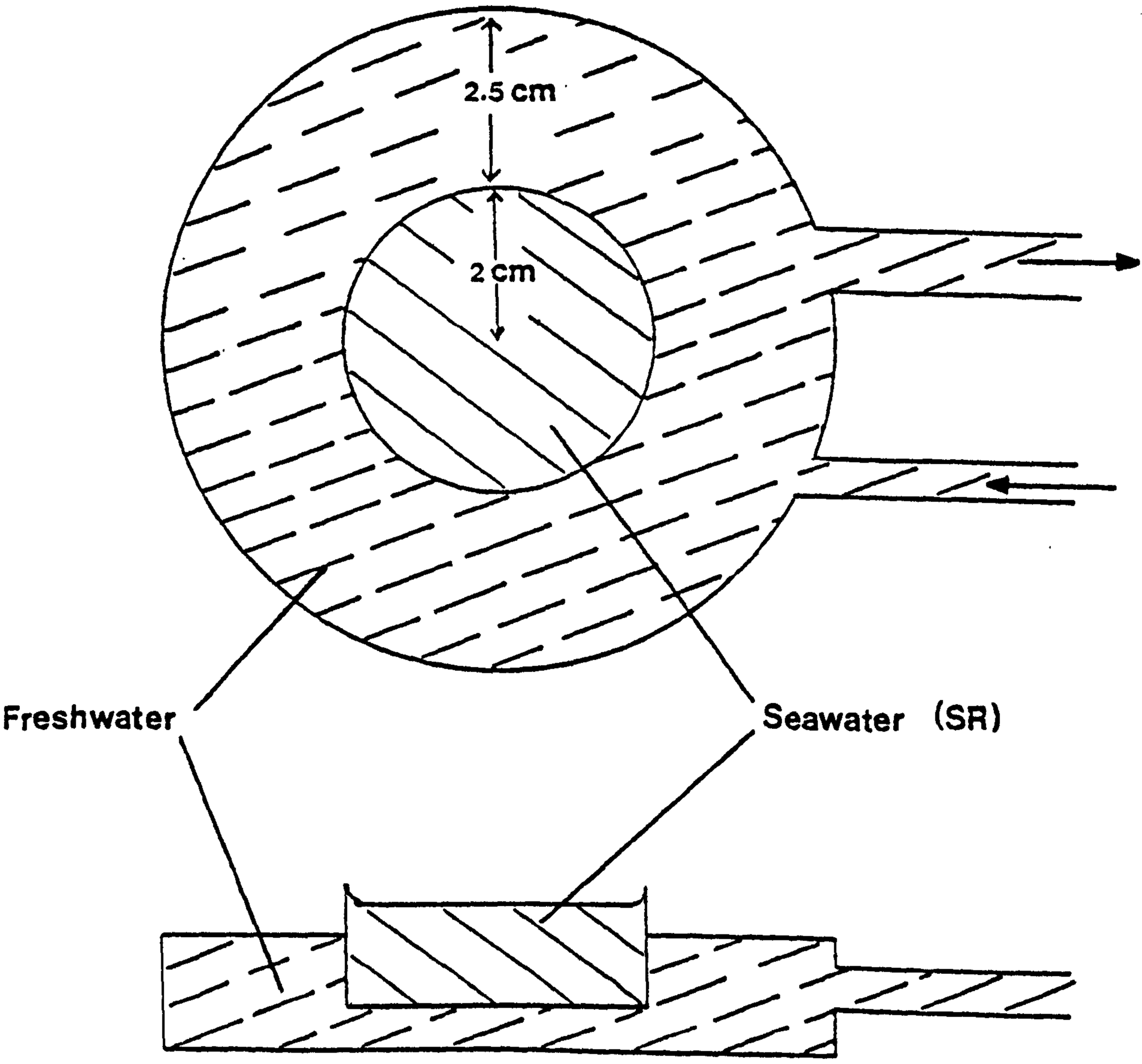
The nauplii of intertidal barnacles experience a more limited temperature range than the adults because they are always in seawater, at least until just before settlement, and the larval life lasts no more than a month or so at most. In some cases, however, the larvae may experience considerable variation in temperature throughout the larval life and succeeding broods of larvae, either in the same or following years, may experience entirely different temperature conditions since yearly variation in seasonal seawater temperature can be considerable (see Cooper, 1958). It was decided therefore to use a suction restraint technique to investigate the swimming activity of five species of barnacle larvae each with either a different life history, or a different geographic distribution from the others.

METHODS

Temperature control within narrow limits was necessary to investigate the response of cirripede larval limb beat to changes in temperature, hence the flowing seawater system described in 'Methodology' above was not used. Instead the larvae were restrained in between 20 - 30 ml of filtered seawater in a glass chamber enclosed in a water jacket with circulating water at the appropriate temperature. A diagram of the chamber is shown in Fig 2.1. The circulating water was maintained at constant temperature by a cooling coil which opposed a 1.5 Kw heater in a water bath. The seawater in the observation chamber (SR, Fig 2.1) quickly attained the temperature of the surrounding water and showed no measurable deviation from that temperature throughout the experiment.

FIG.2.1

Temperature Control Chamber



The larvae were restrained on a suction tube ('Methodology' section 1) in the centre of the observation chamber and viewed from above with a binocular microscope. Fig 2.2 shows a nauplius larva of Elminius modestus (stage VI) on a suction tube with the limbs visible and unobstructed by the restraining tube. The larvae were not acclimated at temperatures other than their rearing temperature (see section 1 'Materials') but merely allowed the normal 45 mins. settling time on the suction tube at each temperature before beginning each experiment. The frequency of limb beat was measured using a stroboscope (Strobe Automation 15K) for the higher frequencies and timing 20 limb beats with a stopwatch at frequencies below the useful limits of the strobe. The stroboscopic measurements were made by adjusting the flash frequency to that of the limb movement. When the limbs appeared stationary the strobe flash frequency was noted. The strobe was then set to twice the noted frequency and the attitude of the limbs observed. If the limbs appeared doubled and stationary, the noted flash frequency was taken as the larval limb beat frequency. If, however, the limbs still appeared single after doubling the flash frequency, which could happen had the noted flash frequency been half that of the actual limb beat frequency, then the procedure was repeated at higher frequencies until a doubling of the flash frequency gave two images of the limbs. This measurement was made twice for each animal throughout the temperature range and only during a period of prolonged swimming in order to obtain the best estimate of average limb beat rate. The same procedure was followed each time to ensure that the results for different larvae and between species were comparable.

In conjunction with the measurement of limb beat frequency observations were made on the amount of time spent swimming by each species of nauplius at various temperatures. The nauplii were again restrained in the

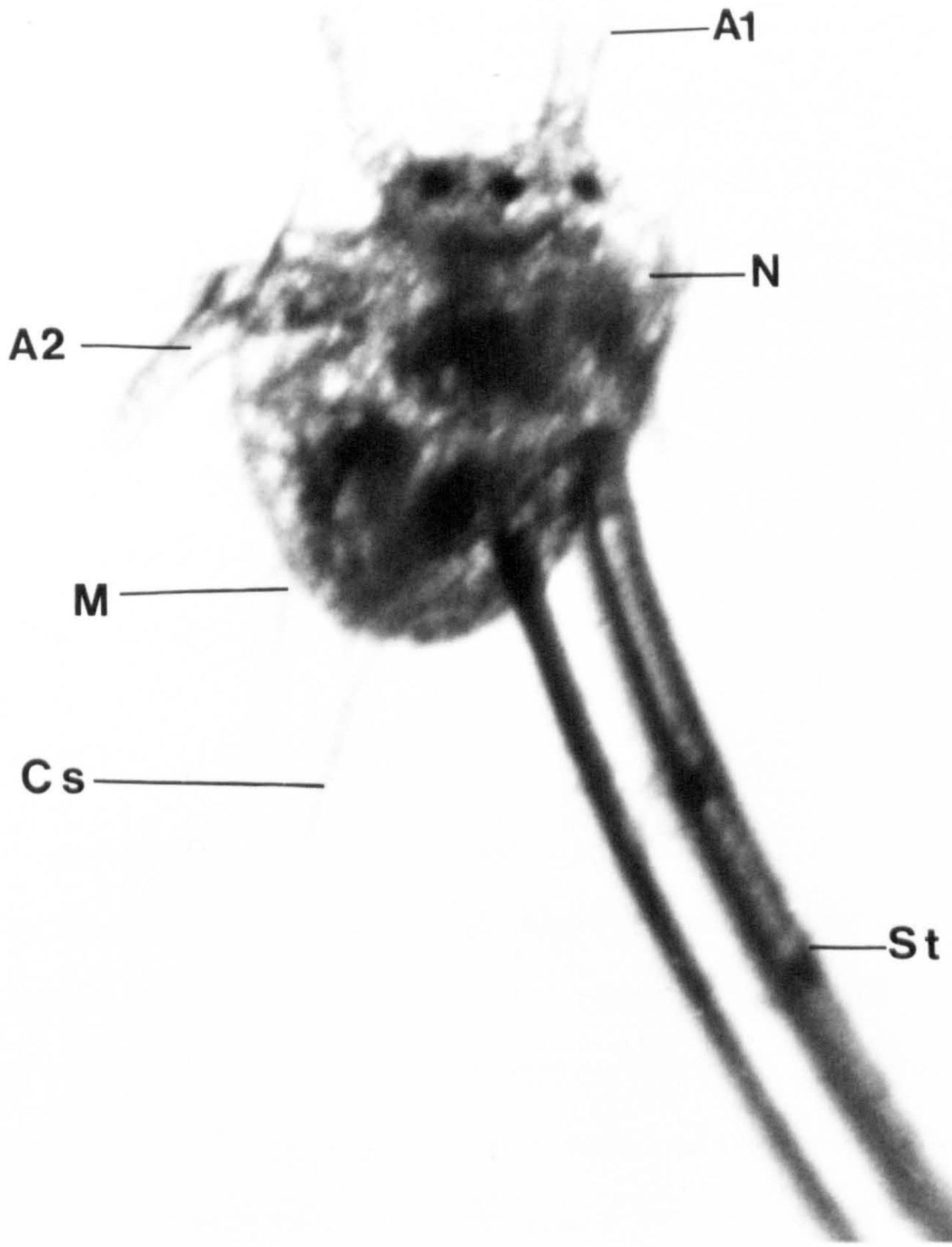


Fig 2.2 Restrained S VI E.modestus nauplius

A1 : antennule A2 : antenna

Cs : caudal spine m : mandible

N : nauplius St : suction tip

controlled temperature chamber (Fig 2.1). The total time spent swimming was measured by stopwatch over a five minute period. Approximately six, five minute, periods were measured for each animal at each temperature and up to six animals measured for each species. The time involved in this type of observation necessitates observations on each larva from its rearing temperature either to its highest or lowest swimming temperature. The result was not affected if the temperature was first increased or decreased. At least 45 mins. were allowed for each $5^{o}C$ change in temperature thus each series of observations occupied approximately seven hours to obtain representative values.

This particular experiment presented an opportunity to make a comparison between restrained and free swimming larvae, largely to establish that the suction restraint had no effect on them. Using low power magnification on the stereomicroscope the swimming times of free swimming stage IV E.modestus nauplii were measured in the same temperature control chamber. The whole of the observation chamber could be seen and individual larvae followed for the full five minute period on most occasions. It was sometimes difficult to decide when the nauplii had stopped swimming so that observations on free swimming nauplii introduced a greater error than those of restrained larvae.

As the observations of swimming times were made over long time periods a series of control experiments were conducted to determine whether or not the swimming times varied with time as well as temperature. In each case at least one animal from each species was maintained at a constant temperature for up to seven hours restrained within the observation chamber. Approximately every fifteen minutes the percentage time spent swimming in a

five minute period was determined.

Results

The results shown in Table 2.1 detail the mean limb beat frequency at various temperatures for five species of barnacle nauplii. Figures are given in Hz (beats per second) and accompanied by the standard error of the mean.

The results are shown graphically in Fig 2.3 (a and b). The shape of all five graphs is basically similar. Those for B.balanoides and B.hameri (Fig 2.2 b) show a lower beat rate over the temperature range than the others. B.balanoides and B.hameri are relatively large larvae released in the spring when the water temperature is cold, whereas E.modestus, C.montagui and B.amphitrite are smaller and mainly released in warmer waters. Figs 2.4 - 2.8 show the results graphed in the form of Arrhenius plots, the natural log of beat rate against the inverse of the absolute temperature. A thermal coefficient for limb beat movement can be obtained using the Van't Hoff equation, and expressed in the form of a Q_{10}, the coefficient of the change in rate per $10^{o}C$ change in temperature.

$$Q_{10} = \{K_1^{(10/t_1-t_2)}\}/ K_2$$

where K_1 and K_2 represent the limb beat rates at temperature t_1 and t_2 respectively. Table 2.2 shows the coefficients for each species between 5^{o} - $15^{o}C$ and also 15^{o} - $25^{o}C$.

Table 2.1

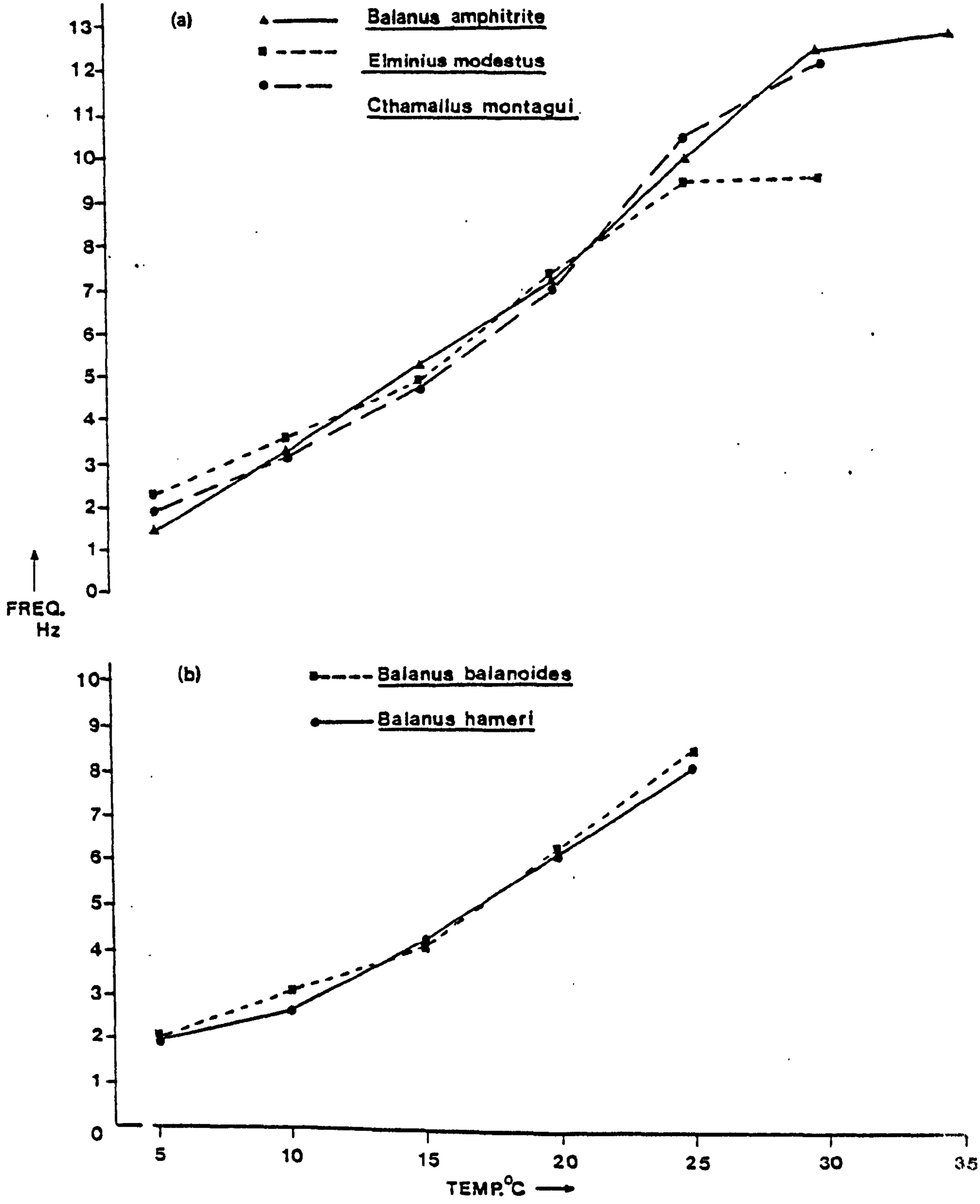
Mean beat frequency for 5 species of barnacle nauplius, ± the standard error of the mean, shown over the temperature range 5 - 35°C.

	Balanus balanoides	Balanus hameri	Balanus amphitrite	Elminius modestus	Chthamallus montagui
TEMP.	Stage IV	Stage II	Stage IV	Stage IV	Stage IV
5°C	2.074±0.03 (47)	1.997±0.02 (85)	1.491±0.03 (73)	2.386±0.07 (20)	1.914±0.03 (60)
10°C	3.099±0.02 (47)	2.654±0.02 (89)	3.241±0.06 (86)	3.631±0.07 (20)	3.185±0.05 (82)
15°C	4.017±0.05 (10)	4.356±0.11 (15)	5.259±0.11 (20)	4.898±0.20 (20)	4.771±0.18 (36)
20°C	6.228±0.11 (10)	6.078±0.06 (15)	7.146±0.21 (20)	7.219±0.21 (20)	6.788±0.17 (20)
25°C	8.445±0.15 (3)	8.083±0.05 (3)	9.929±0.37 (20)	9.406±0.20 (20)	10.404±0.18 (20)
30°C	---	---	12.475±0.35 (20)	9.424±0.23 (20)	12.113±0.27 (20)
35°C	---	---	12.717±0.07 (10)	---	---

Figures in brackets show the number of observations.

FIG. 2.3

Plot of Limb Beat Frequency against Temperature
for 5 species of Barnacle Nauplii.



Figs 2.4 to 2.8

Diagrams showing the Arrhenius plots of natural log of limb beat rate against the inverse of temperature in ^{o}K, for 5 species of barnacle nauplii.

Fig 2.4 B.balanoides.

Fig 2.5 B.hameri.

Fig 2.6 B.amphitrite.

Fig 2.7 E.modestus.

Fig 2.8 C.montagui.

FIG. 2.4

Arrhenius plot for B. balanoides S IV nauplii

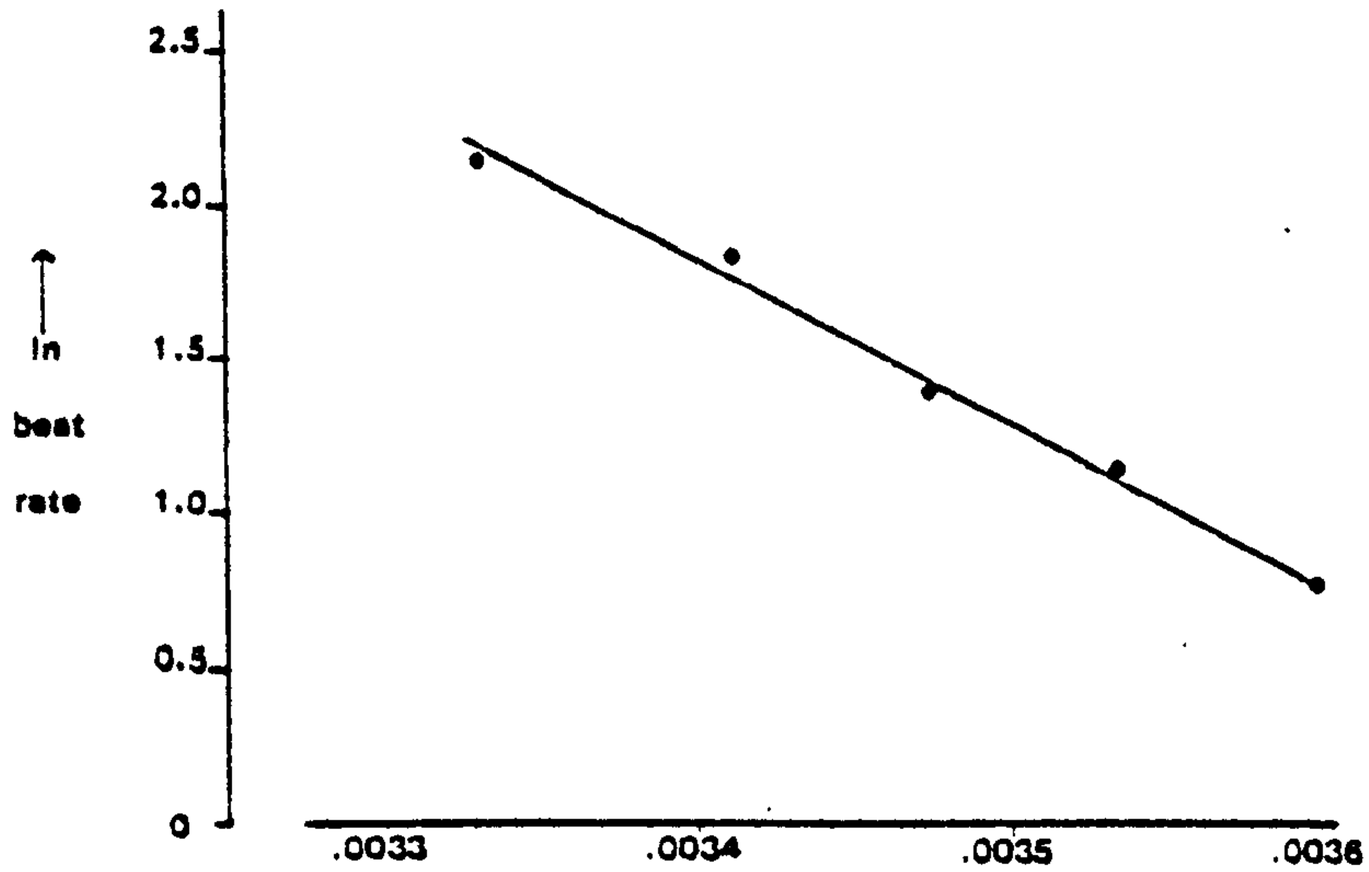


FIG. 2.5

Arrhenius plot for B. hameri S IV nauplii

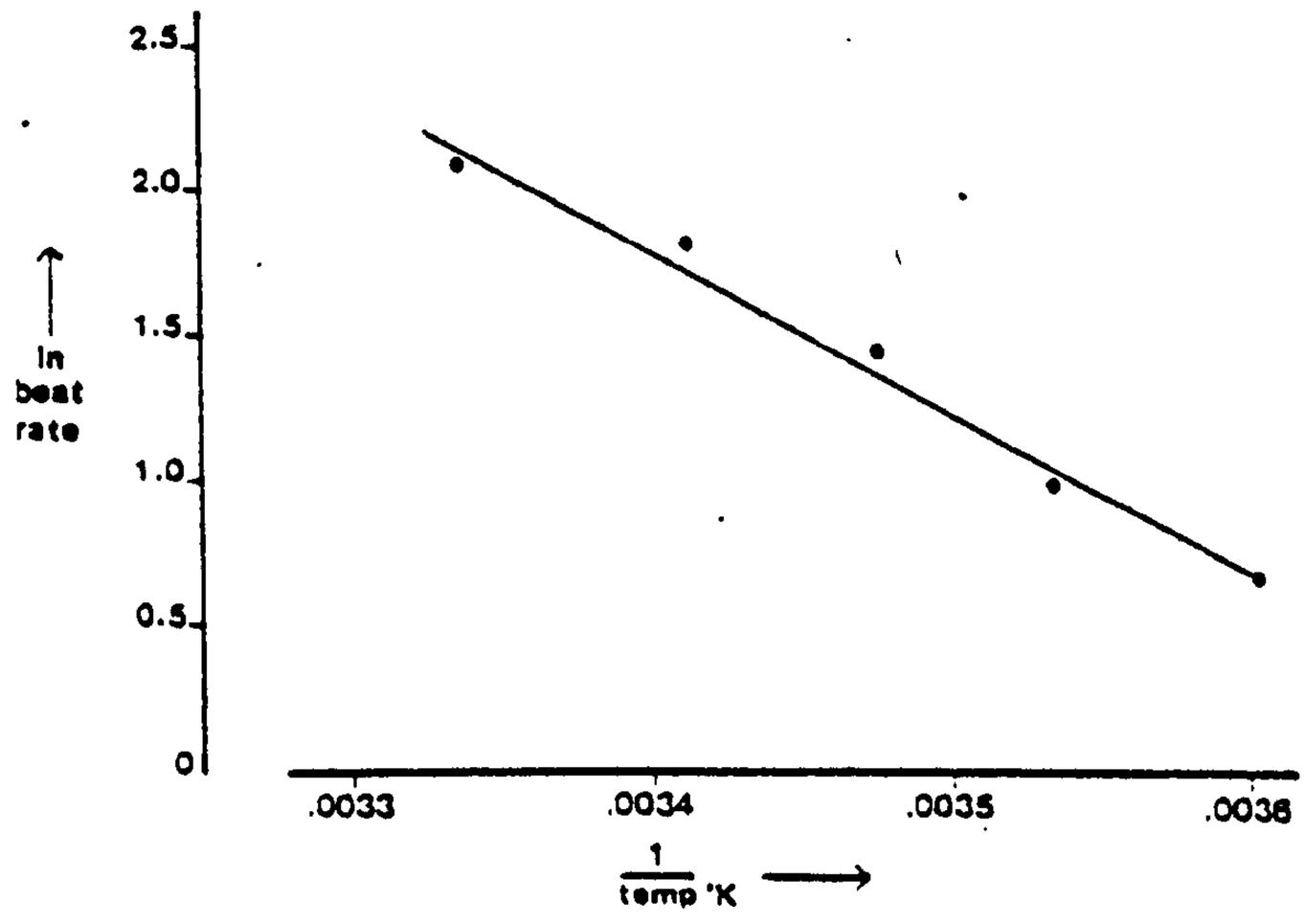


FIG. 2.6

Arrhenius plot for B. amphitrite S IV nauplius

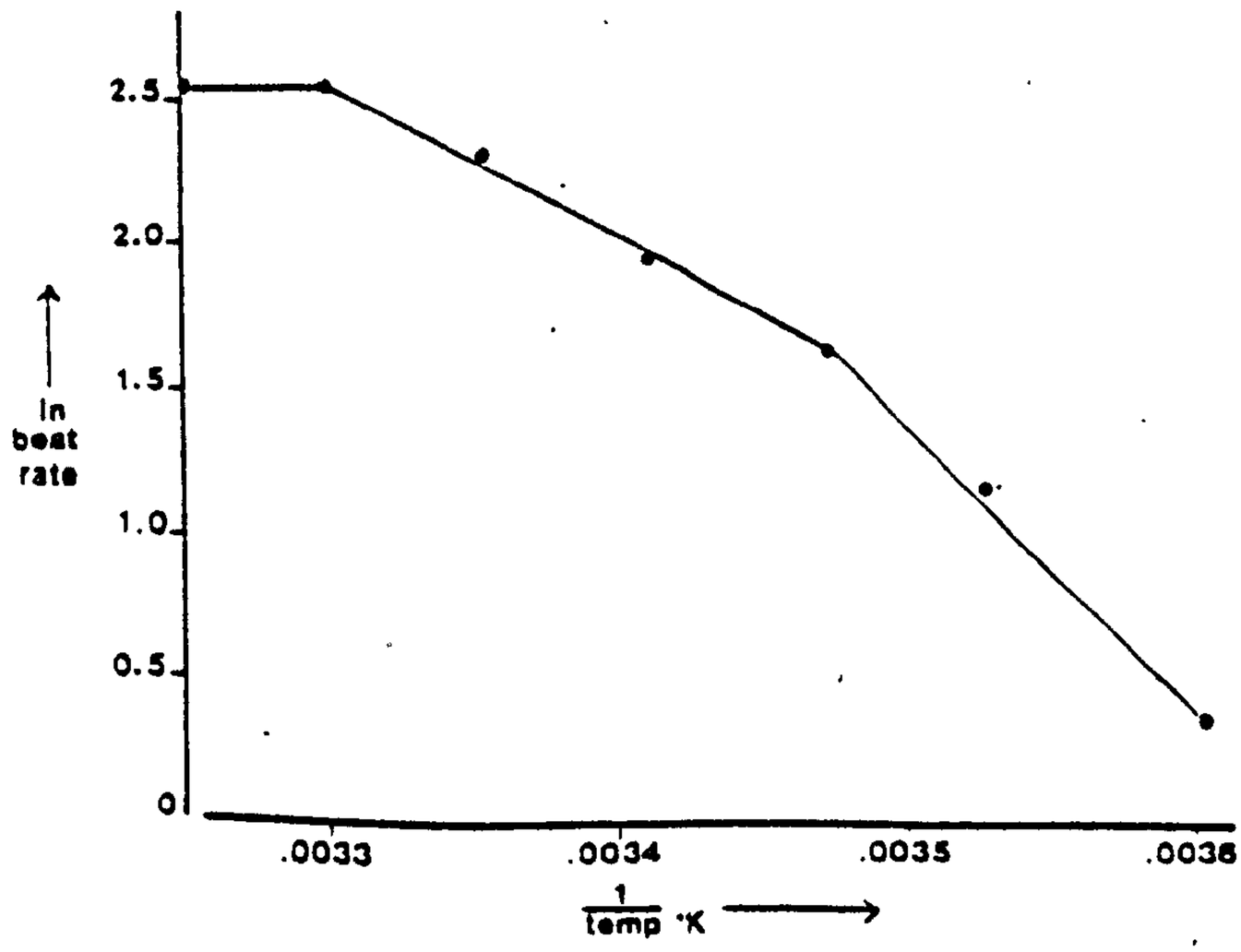


FIG. 2.7

Arrhenius plot for E. modestus S IV nauplii

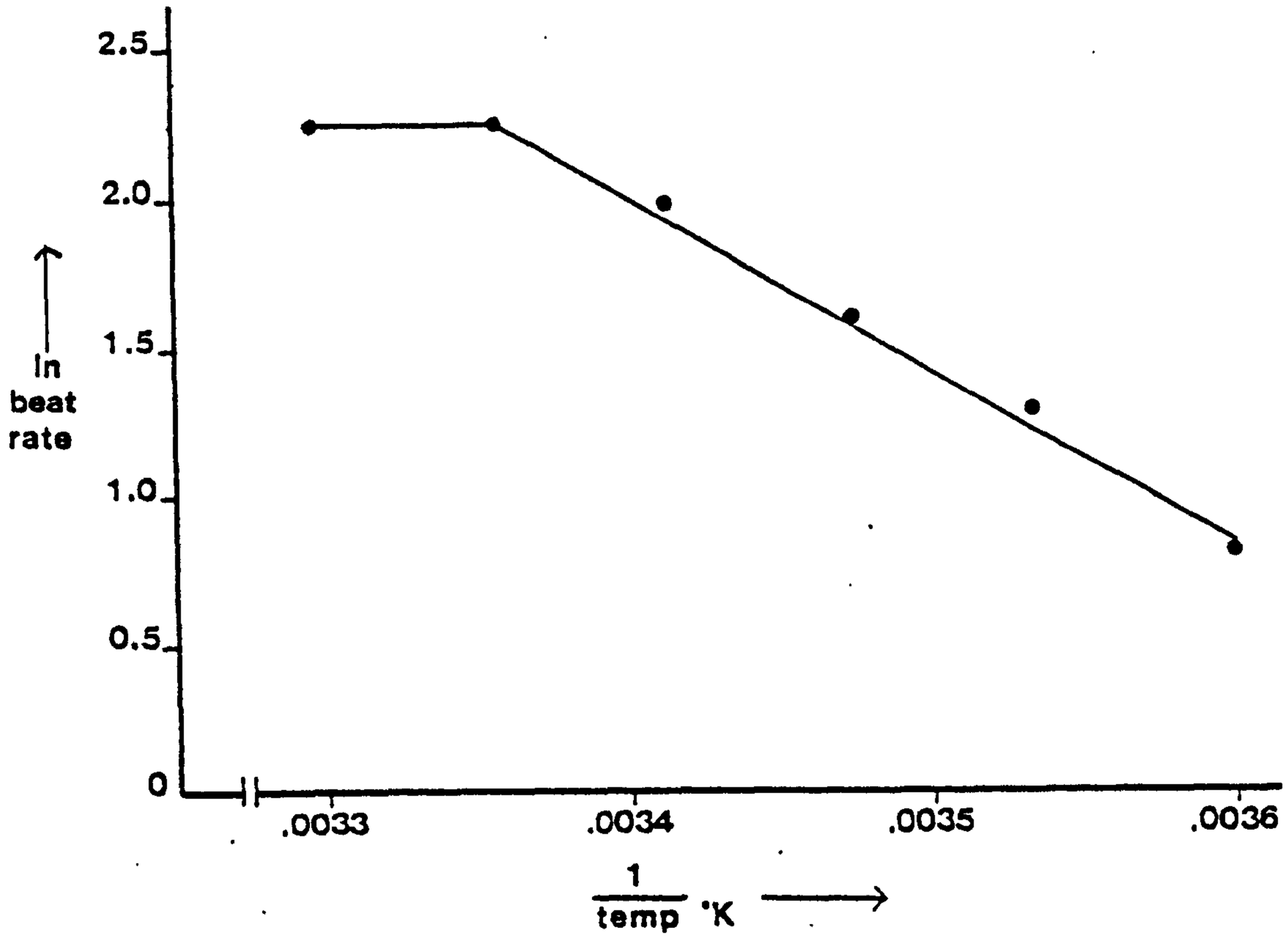


FIG. 2.8

Arrhenius plot for C. montagui S IV nauplii

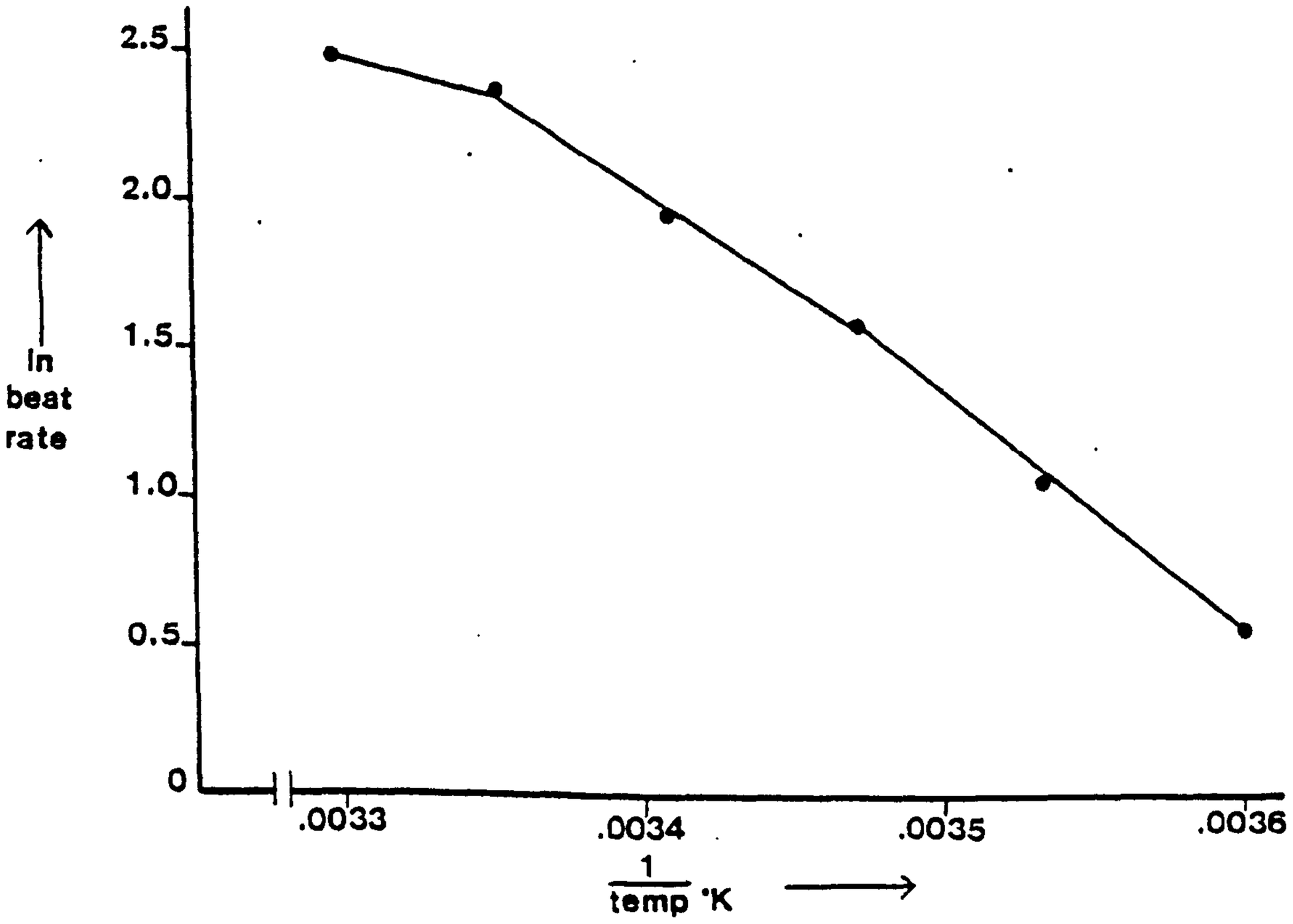


TABLE 2.2

Q_{10} for limb beat rates of 5 species of barnacle nauplii.

	Q_{10}(5°-15°C)	Q_{10}(15°-25°C)
B.balanoides	1.9368	2.0562
B.hameri	2.1820	1.8545
B.amphitrite	3.5270	1.8880
E.modestus	2.1240	1.9204
C.montagui	2.4932	2.1820

The Q_{10} for all species between 15° - 25°C is very similar and approximately 2.00. In other words a 10°C rise in temperature results in a two-fold increase in beat rate. A Q_{10} of around 2.00 is in fact very common in biological systems and it can be seen from the Arrhenius plots (Figs 2.4 - 2.8) how similar is the slope of each graph over that temperature range. At the lower temperature range (5° - 15°C) three species, B.balanoides, B.hameri and E.modestus (Figs 2.4, 2.5 and 2.7 respectively), also have a Q_{10} of approximately 2.00. However, over the lower temperature range, a 10°C rise in temperature causes a 2.5 times increase in rate for C.montagui (Fig 2.8) and a 3.5 times increase in limb beat rate for B.amphitrite (Fig 2.6).

Temperature, therefore, has a greater affect on the beat rate over the lower temperature range in the warm water barnacles which breed only in the summer than in the cooler water forms which predominate in the spring. At higher temperatures B.balanoides and B.hameri nauplii appear to be adversely affected by temperature and cease to swim at temperatures above 25°C. The other three species can swim at temperatures as high as 30°C although both Elminius and Chthamallus cease swimming above this temperature, the effect of temperature being reflected in the flattening of the Arrhenius plot (Figs 2.7 and 2.8). B.amphitrite swims well at 30°C but shows a flattening of the Arrhenius plot (Fig 2.6) between 30°C and 35°C signifying an adverse

effect of temperature on the larvae. A small proportion of B.amphitrite nauplii were able to swim intermittently at temperatures as high as 40°C.

Table 2.3 shows the percentage of time spent swimming by the five species of barnacle nauplii in a five minute period at various temperatures. The column for E.modestus has two figures; the first figure is that for restrained larvae and the second, in brackets, is that for free swimming larvae. The two results shown for Elminius provide an opportunity to directly compare the behaviour of free swimming larvae with that of larvae which had been anaesthetised and then restrained on the suction tube.

Fig 2.9 shows a plot of the percentage time spent swimming against temperature for restrained and free swimming nauplii of E.modestus. The points are means for up to thirty measurements and are accompanied by bars representing the standard error of the mean. The graphs are very similar except that the variation for the free swimming nauplii is greater than that for the restrained animals. This difference may well be due to the much closer examination possible with restrained larvae. The results for restrained and free swimming larvae were compared using the small sample t-Test as shown in Table 2.4. In each case there is no significant difference between the means for the percentage time spent swimming by free swimming or restrained larvae, the probability of obtaining the observed difference by chance being in all cases greater than 0.05. The percentage time spent swimming by restrained and free swimming larvae was clearly very similar over the temperature range investigated. It seems likely, therefore, that the suction restraint technique caused no major changes to the behaviour patterns of the larvae during these investigations.

TABLE 2.3

The mean percentage time spent swimming by the nauplii of five species of barnacle, ± standard error of the mean, over the temperature range 5 - 35°C. Figures in brackets = free swimming larvae.

TEMP°C	Balanus balanoides Stage IV	Balanus hameri Stage II	Balanus amphitrite Stage IV	Elminius modestus Stage IV	Chthamallus montagui Stage IV
5°C	86.13±0.63 [40]	87.64±0.78 [25]	30.25±3.17 [17]	99.11±0.38 [15] (98.59±0.45) [9]	68.44±1.11 [25]
10°C	74.67±0.92 [45]	80.68±1.38 [25]	67.65±3.11 [30]	98.56±0.37 [20] (97.44±0.70) [9]	89.25±1.19 [25]
15°C	67.80±2.14 [45]	65.60±0.96 [25]	91.78±0.61 [30]	95.87±0.92 [20] (94.26±1.56) [9]	88.61±0.97 [25]
20°C	47.08±2.13 [35]	35.68±1.59 [25]	90.55±0.31 [51]	81.45±2.02 [20] (79.27±3.88) [11]	86.67±0.97 [25]
25°C	16.47±2.13 [55]	13.29±0.46 [25]	89.22±0.76 [56]	52.00±4.07 [20] (60.51±4.68) [11]	87.34±0.34 [25]
30°C	----	----	85.09±0.78 [35]	----	83.07±1.08 [25]
35°C	----	----	60.17±3.21 [22]	----	----

Figures in square brackets, [], show the number of observations.

FIG. 2.9

Percentage of time spent swimming, in a five minute period, by restrained and free swimming *E. modestus* larvae.

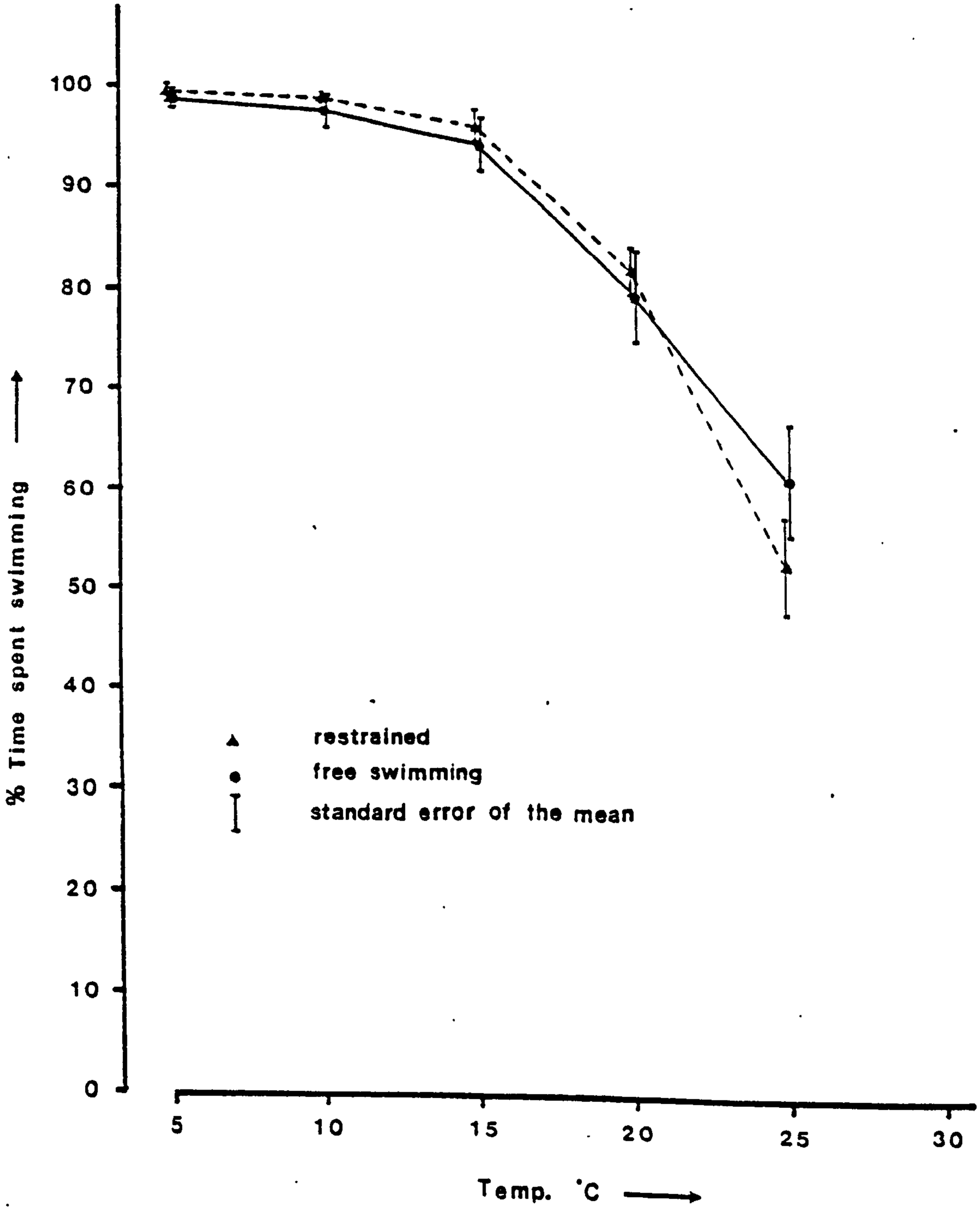


TABLE 2.4

A small sample t-Test comparison of the percentage time spent swimming by free swimming and restrained E.modestus nauplii (S IV), over the temperature range 5 - 25°C.

TEMP°C	t-Value	degrees of Freedom	Mean % of combined data
5°C	0.8655	22	98.85
10°C	1.5510	27	97.97
15°C	0.9330	27	95.07
20°C	0.5529	29	80.36
25°C	1.3087	29	56.26

d.f. are the degrees of freedom for the t-test and p is the probability of obtaining the observed differences by chance. Mean % of combined data shows the average percentage time spent swimming for E.modestus larvae by combining results for free swimming and restarined nauplii.

The results for the remaining species are plotted in Fig 2.10 (a & b). The graphs for E.modestus (Fig 2.9), B.balanoides and B.hameri (Fig 2.10 a) are very similar showing a decrease in swimming activity as the temperature increases. This result is clearly different from the response represented by the limb beat frequencies (Fig 2.3 a & b). Throughout the temperature range, 5 - 25°C, E.modestus spends a greater percentage of time swimming than either B.balanoides or B.hameri. In fact it was very rare to find either of the latter species swimming continuously for the whole five minute period, whereas, at the lower temperatures, several of the E.modestus nauplii swam throughout. This difference between the nauplii is perhaps attributable to size, as the larvae of E.modestus are smaller than those of B.balanoides and B.hameri. The larger limb span of the larvae of the latter two species perhaps enables them to move proportionally more water for their size than E.modestus larvae can and thus they need spend less time swimming to maintain their position in the water column.

Fig 2.10 b shows the plot of percentage time spent swimming against temperature for B.amphitrite and C.montagui. Over the middle temperature range there was only a very slight decrease in swimming time with increased temperature. Only at the extremes of the range (both high and low) was a large decrease in activity demonstrated. The graphs for the British species C.montagui, which predominates in the summer to autumn months, and the Indian species B.amphitrite, a tropical warm water barnacle, are very similar. C.montagui, however, swims very little at temperatures above 30°C and exhibits a marked reduction in activity below 10°C. B.amphitrite will continue to swim above 30°C but less often and shows a marked reduction in activity below 15°C. The higher latitude form, C.montagui, is less affected at the lower temperatures whereas the lower latitude form, B.amphitrite, can

Fig 2.10 (a)+(b)

Mean percentage time spent swimming in a 5 minute period for 4 species of S IV nauplii at various temperatures

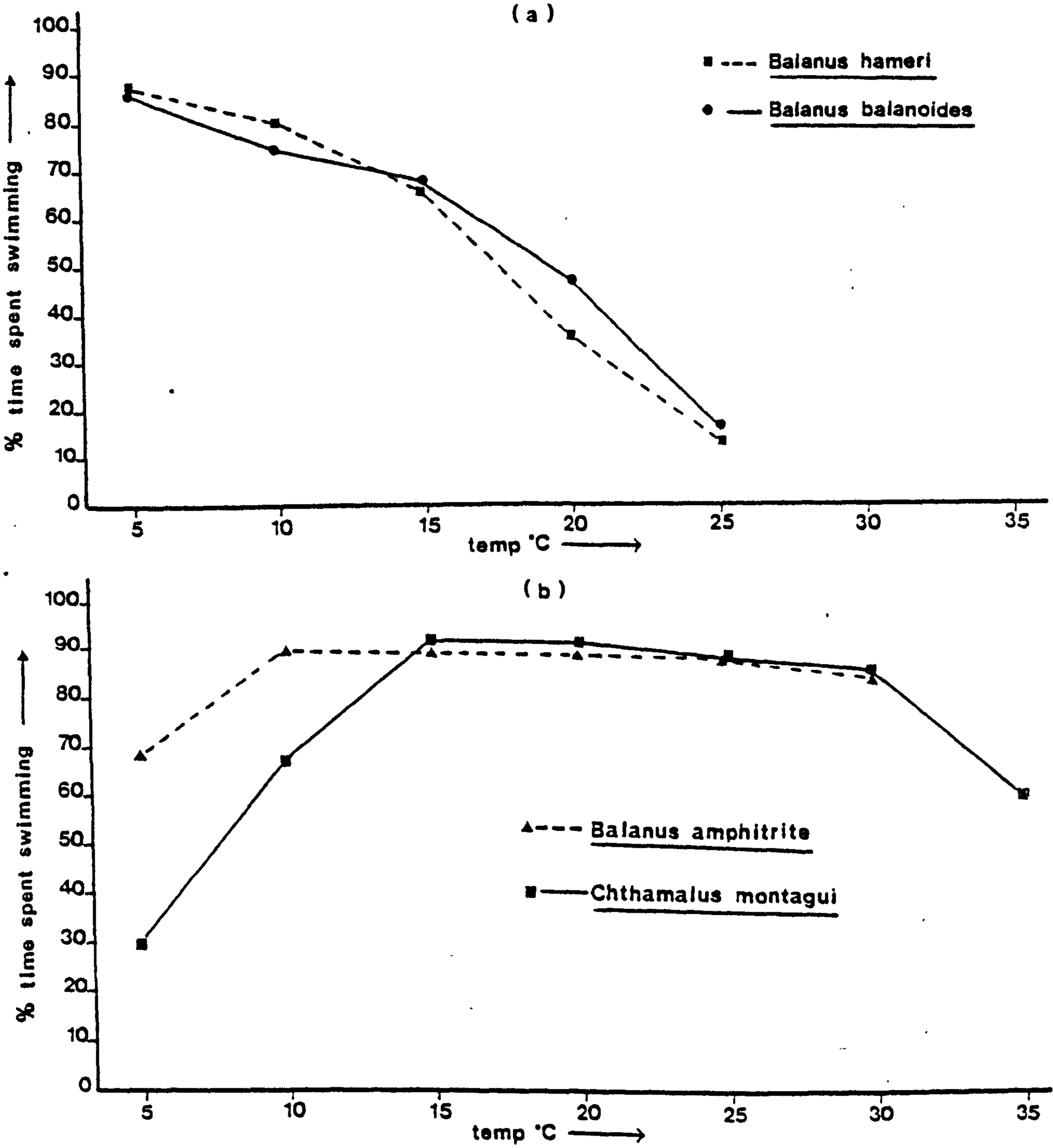
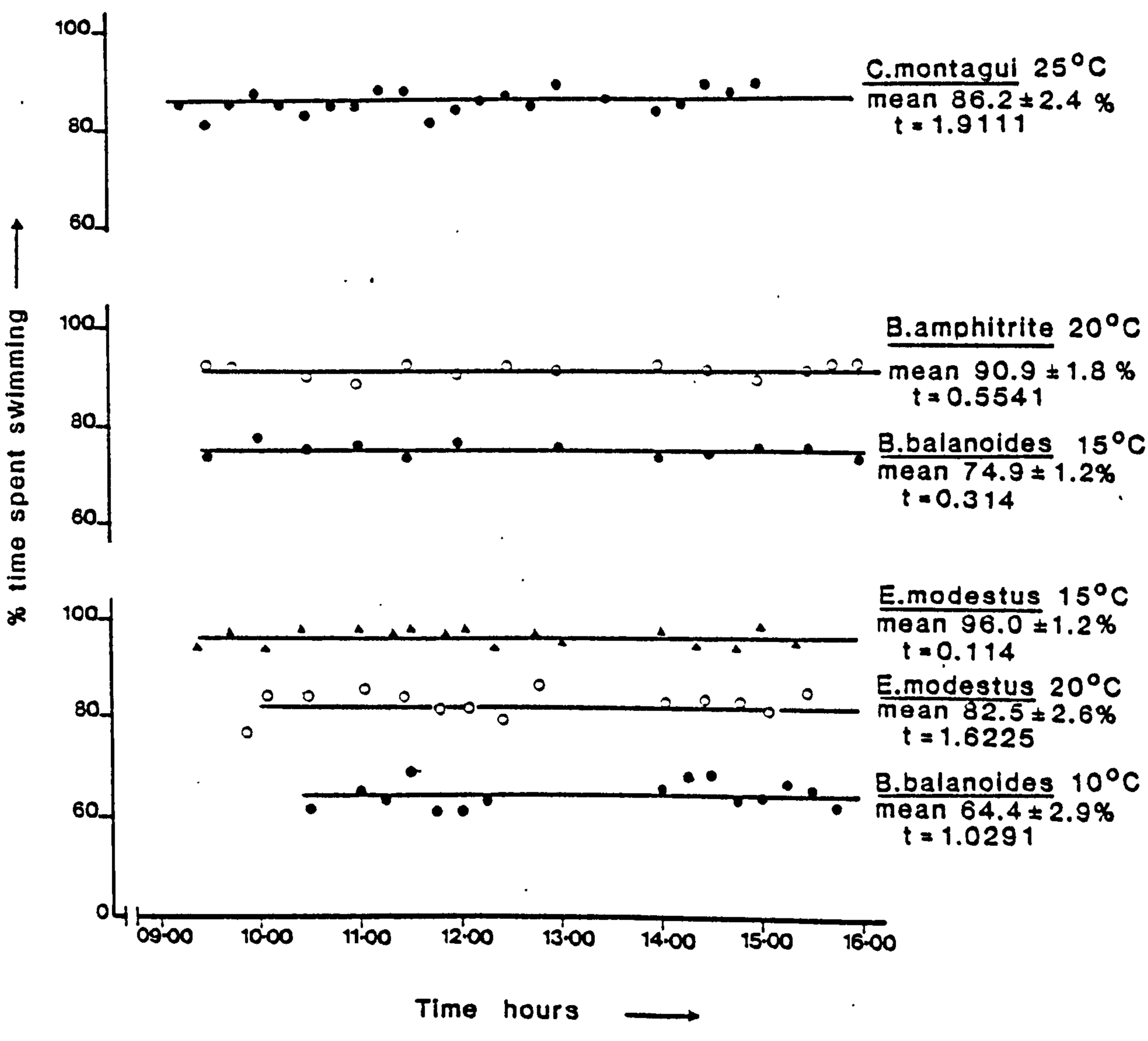


Fig 2.11

Observations of the percentage time spent swimming in a five minute period for 5 species of S IV barnacle nauplii measured regularly over a 6 - 7 hour period. The mean value $\pm$ standard error for the period is shown with each graph and the t-value for a comparison with the mean obtained from shorter term observations, at the same temperature, detailed in Table 2.3.

Fig. 2.11 Long term controls, % time spent swimming against time



continue to swim at higher temperaures.

Fig 2.11 shows that there was little change over long intervals of time in the percentage of time spent swimming by the five species of barnacle nauplii. In each case a small sample t-Test was performed to compare the difference between the mean time spent swimming over the six to seven hour period of the observation, with that obtained for the equivalent temperature shown in Table 2.3. There was no significant difference shown for any species, the probability of obtaining the observed differences by chance being greater than 0.05 in all cases. The mean and t-values for each species are shown in Fig 2.11. The changes in the time spent swimming at different temperatures by the nauplii, detailed in Table 2.3, were therefore longterm phenomena.

Conclusions and Discussion

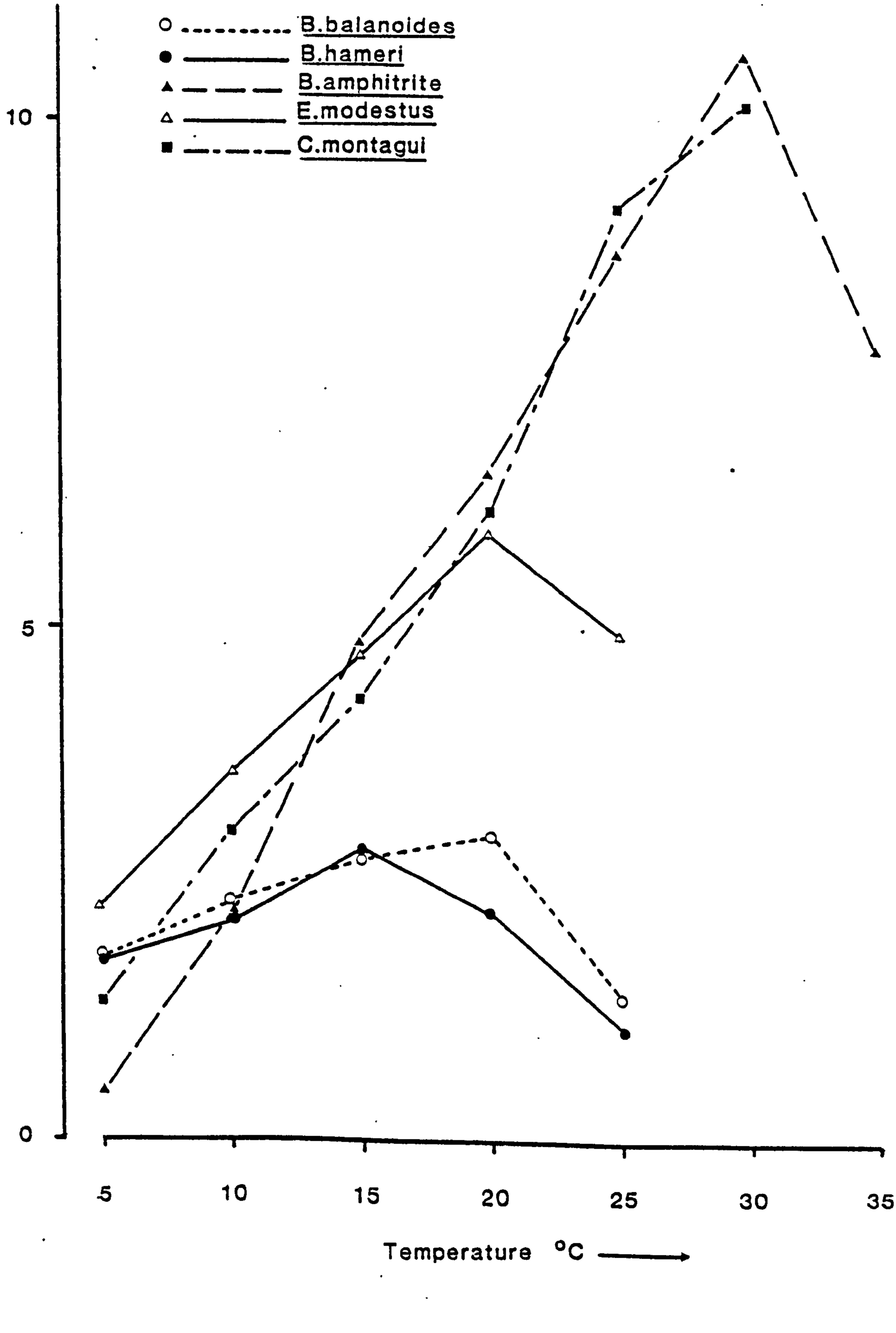
The above results show that the limb beat frequency and the amount of time spent swimming by barnacle nauplii are altered by the environmental temperature. It is possible to analyse the swimming activity of the larvae at various temperatures in terms of the average number of strokes made by each species, calculated from the beat rate and the percentage of time spent swimming. Thus the average number of strokes in a second will equal the beat frequency in Hz times the proportion of time spent swimming. Table 2.5 shows the results obtained for the average number of strokes made in a second by the five species of barnacle nauplii. The result of Table 2.5 is plotted in Fig 2.12. The shape of the plot suggests that each species could be placed in one of three groups. One group would contain B.balanoides and

Table 2.5

The average stroke rate per second of five species of barnacle nauplii.

	Balanus balanoides	Balanus hameri	Balanus amphitrite	Elminius modestus	Chthamallus montagui
TEMP.	Stage IV	Stage II	Stage IV	Stage IV	Stage IV
5°C	1.7863	1.7502	0.4510	2.3648	1.3099
10°C	2.3140	2.1412	2.1925	3.5787	2.8426
15°C	2.7235	2.8575	4.8267	4.6957	4.2276
20°C	2.9321	2.1686	6.4707	5.8799	5.8832
25°C	1.3909	1.0742	8.8587	4.9005	9.0869
30°C	------	------	10.6150	------	10.0623
35°C	------	------	7.6518	------	------

Fig 2.12 Plot of average number of strokes per second at various temperatures for 5 species of S IV barnacle nauplii



B.hameri which are again very similar showing only a small rise in average stroke rate with increasing temperature. B.amphitrite and C.montagui could form a second grouping where a large change in stroke rate was observed with increasing temperature. E.modestus would fall into a group on its own being somewhat intermediate between the other two groups.

Throughout this investigation a grouping of this nature has been observed which could be explained by the similarity of larval life between the species within the groups. The question arises as to why the stroke rate in B.balanoides and B.hameri varies little over the temperature range whilst that for B.amphitrite, C.montagui and to a lesser extent E.modestus varies considerably over the range.

All the barnacle nauplii studied are more dense than sea water and inactive larvae will sink in the water column. Swimming enables the larvae to remain in the upper layers of the sea where food organisms are relatively abundant. If the larvae cannot consistently move sufficient water to overcome the tendency to sink they will fall out of the trophic zone and be unable to feed. Alternatively, if the larvae swim faster because of an increase in the temperature a greater percentage of available energy may go into swimming and the ability of the larvae to replace that energy becomes a limiting factor for continued larval development. The successful larvae will be those which balance the necessity of remaining in the trophic zone to the energy expenditure in doing so.

B.balanoides and B.hameri retain a fairly constant stroke rate over a wide temperature range by reducing the amount of time spent swimming as the temperature and limb beat rate increase. B.amphitrite and C.montagui do not

maintain a constant stroke rate, the increase in limb beat rate with increased temperature was not offset by a response which reduces the percentage of time spent swimming by the larvae. E.modestus larvae show an intermediate situation where there is a slight response reducing the time spent swimming as the stroke rate increases with temperature.

The larvae of both B.balanoides and B.hameri abound in the plankton of British waters in the spring (Pyefinch, 1948, Crisp, 1962 b) when conditions are unstable and temperatures may vary quite considerably (e.g. Cooper, 1958). The maintenance of a constant swimming stroke rate with changing temperature would be advantageous allowing the larvae to keep station in the water column without undue energy expenditure during unstable conditions.

C.montagui larvae have a much shorter planktonic life than either B.balanoides or B.hameri (Bassindale, 1936). Chthamallus larvae are released into the plankton during the summer months in Britain when conditions in the sea are relatively stable. The same is true for the tropical barnacle B.amphitrite, the larvae of which also have a short planktonic life, normally in very stable conditions (Costlow and Bookhout, 1958). Neither B.amphitrite nor C.montagui larvae would have an obvious need to maintain a constant stroke rate over a wide temperature range.

E.modestus is eurythermal (Southward, 1955) and in Britain can release larvae all year round (Crisp and Davies, 1955). It might have been expected therefore, to have also shown a reasonably constant stroke rate over a wide temperture range. However, Elminius originated in the sub-tropical waters of New Zealand and is thus a warmer water species (Moore, 1944) than B.balanoides and B.hameri. The recent successful colonisation of British,

and other north-temperate, shores by Elminius is in part due to its eurythermal characteristics (Southward 1955) , aided perhaps, by its slight but possibly significant response of decreased swimming activity as the temperature and limb beat frequency increase.

Section 2a

The Limb Beat Rate of Barnacle Nauplii

Using the Impedance Pneumograph

Limb beat rate of barnacle nauplii using the impedance pneumograph

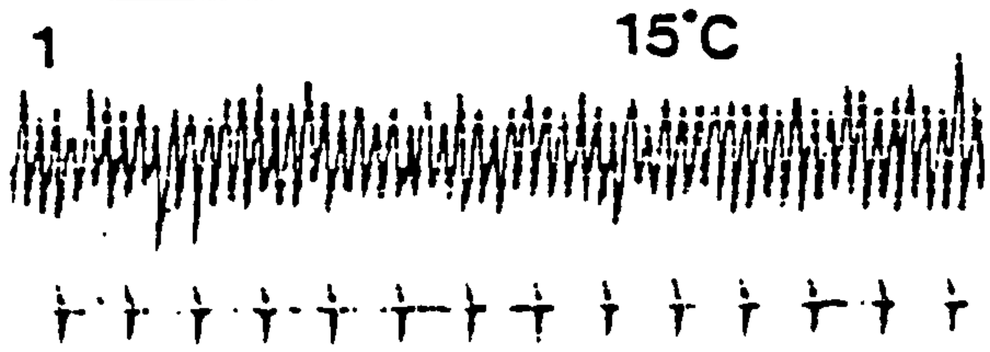
Figs 2a.1 and 2a.2 have been included as an appendix to the previous section on the limb beat frequencies of barnacle nauplii. These figures represent pneumograph traces of the antennal movements of all five species of barnacle nauplii. A sample trace is shown followed by the seawater temperature and the limb beat frequency shown on the trace. The strobe-measured frequency at an equivalent temperature is also given. The traces (Figs 2a.1 and 2a.2) are direct measurements from the antennae of the nauplii, peak to peak representing one complete beat. The time marks at the bottom of the trace represent one second. For every species the limb beat frequency measured from the traces correspond to those of the earlier, more thorough investigation, measured by stroboscope or stopwatch.

The traces for B.hameri (Fig 2a.1 b) show the limitations of the present pneumograph technique for the accurate recording of limb beat amplitude. The difference in limb beat amplitude between the two animals was negligible yet the signal strength shows a considerable difference on the trace. This resulted from the difficulty of positioning the electrodes in exactly the same way each time, and from the tendency for the larvae or the current to move the wires out of position.

The beat frequency is readily discernible from the traces, as are the movements of the antennae during the 'folding behaviour'. Fig 2a.1 (a 2&3) show instances of the periodic folding of the antennae by B.balanoides. The nauplii show a break in the rythmical swimming beat and a much slower movement takes place often with a greater amplitude than normal swimming. Fig 2a.3 shows a graphical reconstruction of the movements shown in Fig 2a.1

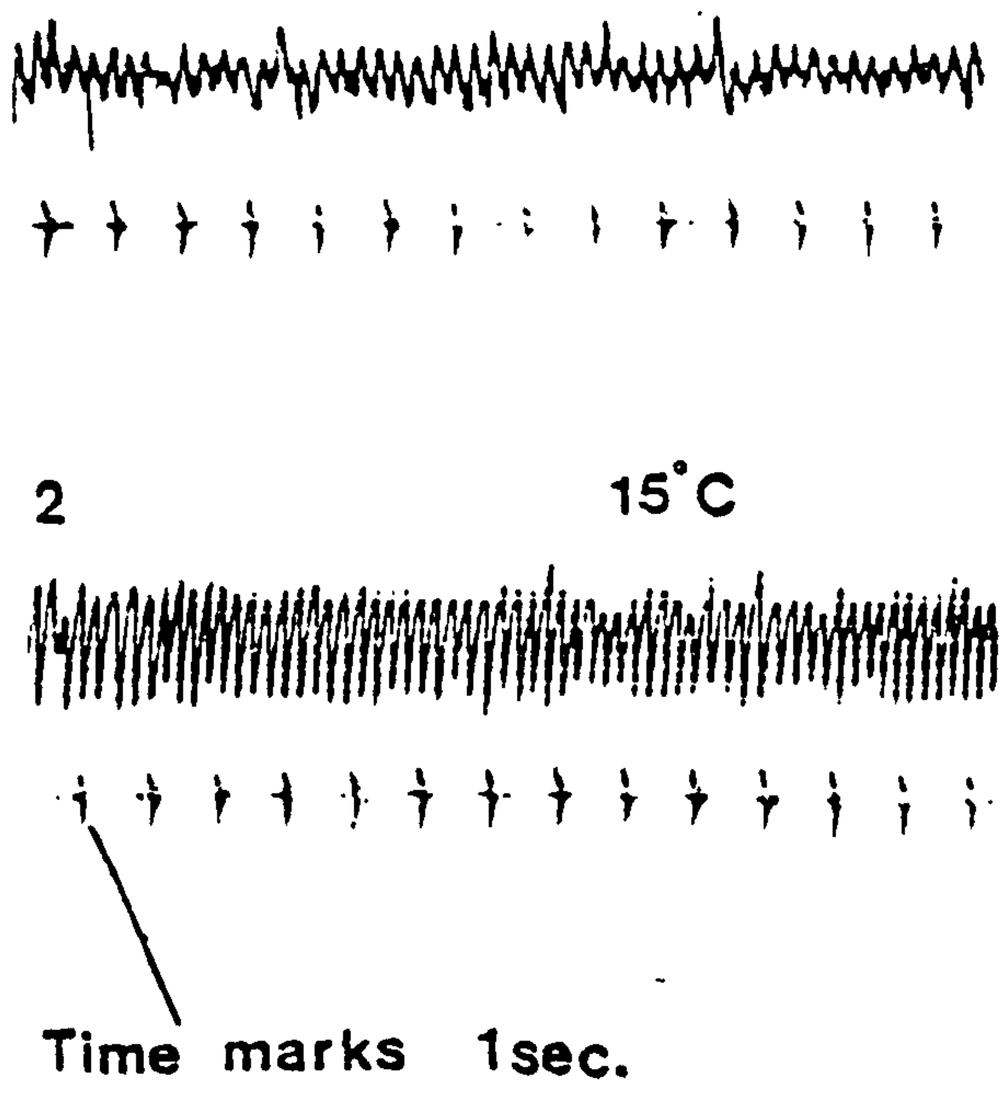
Fig 2a.1 : Sample Pneumograph Traces

(a) B.balanoides



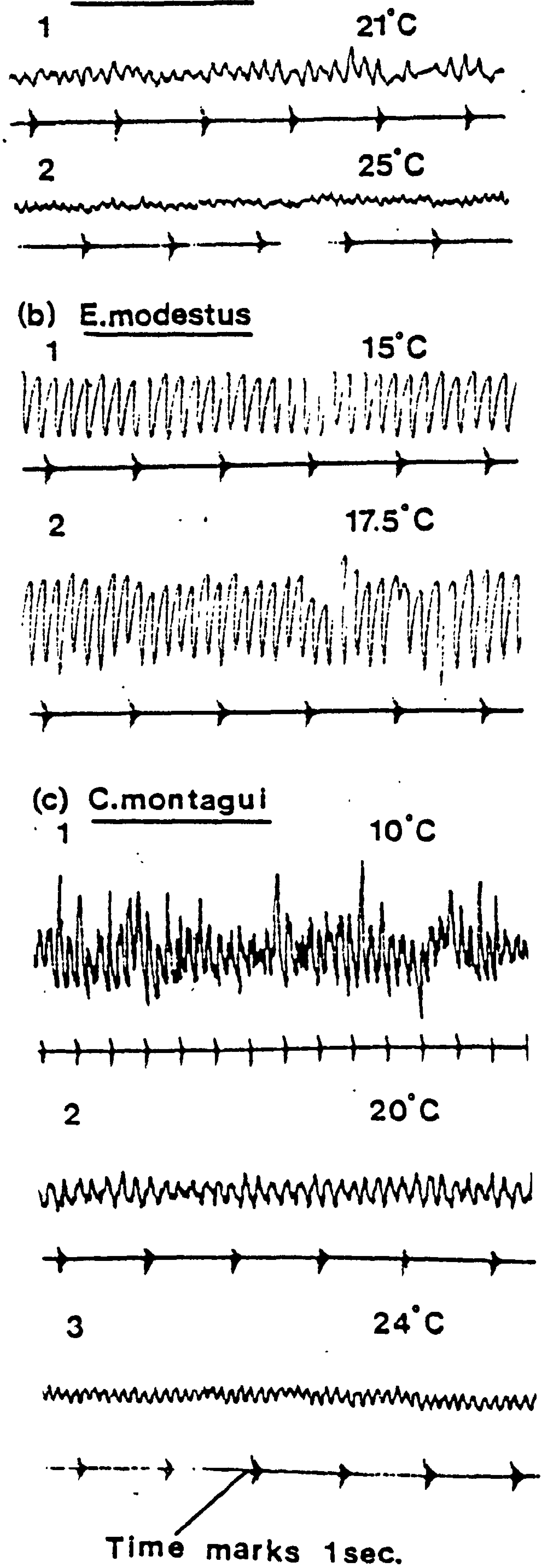
	FREQUENCY Hz.	
	Trace	Strobe ±95% limits
1	4.00	4.017±0.11 (15°C)
2	4.625	—
3	6.00	6.228±0.24 (20°C)

(b) B.hameri



1	3.313	2.654±0.05 (10°C)
2	4.429	4.158±0.22 (15°C)

Fig 2a.2 : Sample Pneumograph Traces



FREQUENCY Hz

	Trace	Strobe
(a) B.amphitrite 1 (21°C)	7.00	7.142±0.42 (20°C)
(a) B.amphitrite 2 (25°C)	10.00	9.929±0.75 (25°C)
(b) E.modestus 1 (15°C)	5.42	4.898±0.42 (15°C)
(b) E.modestus 2 (17.5°C)	6.25	7.219±0.42 (20°C)
(c) C.montagui 1 (10°C)	3.5	3.185±0.1 (10°C)
(c) C.montagui 2 (20°C)	6.445	6.938±0.34 (20°C)
(c) C.montagui 3 (24°C)	9.75	10.404±0.36 (25°C)

Fig 2a.3

A graphical reconstruction of a portion of the pneumograph trace for a stage IV B.balanoides nauplius at 17°C (from Fig 2a.1 [a 2]) showing the sequence of events during folding behaviour.

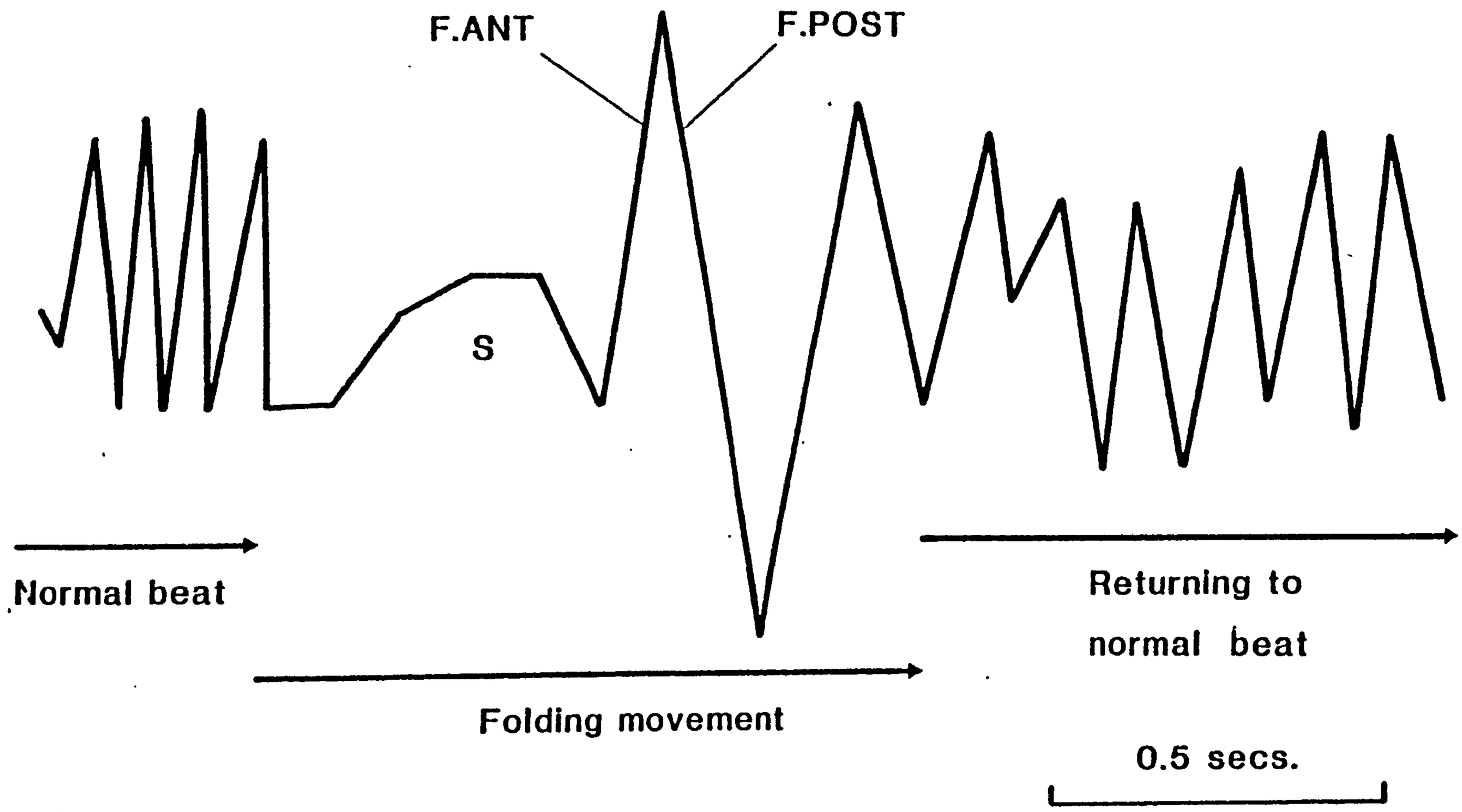
s = slow, small amplitude antennal movement.

F.ant = long anterior antennal swing.

F.post = long posterior antennal swing.

Fig 2a.3 Reconstruction of pneumograph trace

for a S IV B.balanoides nauplius at 17°C



(a 2). As can be seen from the time scale, the folding and unfolding of the antennae lasts between 1.5 and 2 seconds. In many cases the antennae initially moved slowly back and forth with a small amplitude of movement (S Fig 2a.3). The antennae then folded anteriorly, much further than on a normal swimming beat (F.ANT Fig 2a.3), followed by a posterior fold again of greater amplitude than the normal beat (F.POST Fig 2a.3). During the posterior fold of the antennae the setae of the antennae and mandibles were brushed across each other and the caudal region of the nauplius (see Lockhead 1936, Norris and Crisp, 1953). The normal swimming beat was then slowly re-established. The use of such reconstructions of the traces obtained from this small scale pneumograph technique could prove useful in ascertaining many of the limb movements involved in locomotion or feeding of very small organisms, from crustacean larvae to adult polyzoa.

Section 3

The Capture of Microalgae by the Nauplii of E.modestus and B.balanoides

The capture of microalgae by the nauplii of E.modestus and B.balanoides.

Introduction

Lockhead (1936) discussed the feeding mechanism of the nauplius larvae of Balanus perforatus. He watched the nauplii in small volumes of water and pieced together the movements of the three pairs of limbs. The swimming stroke was found to be relatively inefficient as the limbs were not fully feathered on the recovery stroke. This inefficiency of limb beat was offset to some degree by the fact that the limbs did not beat in phase with one another such that the mandibles could still be pushing water behind the larvae while the antennae were moving forwards on the recovery stroke. The recovery stroke of the limbs thus brought water from the side and behind the larvae towards the ventral surface of the body. Lockhead considered that the current produced by the recovery phase of the swimming stroke directed food particles towards the mouth, where they were trapped under the labrum by the setae on the mandibular and antennular endopodites and pushed into the mouth by the limb gnathobases.

Norris and Crisp (1953), while considering the distribution and planktonic stages of B.perforatus, suggested another mode of feeding which barnacle nauplii could employ. They proposed that barnacle nauplii could filter particulate matter from the seawater on the meshes of the net formed by the overlapping setules of the limb setae, particularly those of the antennae. During the characteristic folding behaviour, shown by all barnacle nauplii, particulate matter could be transferred from the antennae to the mandibles and thence onto the ventral surface of the body as the spiny ventral thoracic process bent forwards onto the folded limbs. Food

particles thus collected could then be pushed towards the mouth by the action of the limb gnathobases.

Gauld (1959) summarised both versions of the feeding mechanism of barnacle nauplii but preferred that of Norris and Crisp as being closer to his own observations. Rainbow and Walker (1976) used a scanning electron microscope to show that the ventral thoracic process and the ventral surface of the nauplius were covered in spines which could assist in cleaning particles from the limbs and possibly to retain such filtered particles on the ventral surface of the body prior to being moved towards the mouth by the limb gnathobases.

As has been shown in Section 2 above, the limb movements of many barnacle nauplii are quite rapid and the animals' progress through the water is also relatively fast. It was therefore decided to look more closely at the limb movements of restrained barnacle nauplii, and in particular the way in which the limbs were used to capture microalgae.

METHODS

Nauplii of the barnacles E.modestus and B.balanoides were released and reared in the laboratory as described in 'Methodology' above. Active stage IV larvae were restrained in flowing seawater at the temperature at which they had been reared (E.modestus 20°C, B.balanoides 15°C), using a suction tip as described above. Observations were made using a Wild M5 stereomicroscope and the larvae held at an appropriate angle to view the limb movements and the labrum. Methyl Blue dye, Carmine granules and

mixtures of algae and carmine granules were introduced to the seawater, in front of the nauplius, through a small bore plastic tube similar in construction to that of the suction tube used to restrain the larvae. Methyl Blue dye in seawater solution was used to make the water currents caused by the normal swimming of the nauplii visible. Suspensions of Carmine granules in seawater were used to ascertain the fate of particles attaching to the limbs of the nauplii. Mixtures of Skeletonema costatum or Rhodomonas baltica and Carmine granules in seawater were used to investigate the movements of particles in the vicinity of the nauplii at a time when the larvae could be expected to feed.

A qualitative observation of the 'stickiness' of the nauplius' limbs was made by brushing the side of a very fine, clean needle along the setae of the antennae. Both sides of the needle were then tested for efficiency in picking up Carmine granules in seawater. The needle was thoroughly cleaned with detergent, distilled water and seawater before use.

Results

General Observations

Throughout the investigation no qualitative differences were demonstrated between the behaviour and limb movements of E.modestus and B.balanoides. The results described below will therefore apply to both species.

The normal swimming beat of the nauplius was frequently punctuated by one of three behaviour patterns. First, the larvae could stop swimming with limbs extended. The length of these periods of inactivity was variable, lasting from two to three seconds up to as much as thirty seconds. Secondly, distinct changes in rhythm could be observed during swimming. This change in rhythm lasted less than a second. Thirdly, the larvae could stop swimming and fold their limbs across the ventral surface of the body while bending the caudal region of the body anteriorly towards the labrum. The third change in normal swimming beat will be referred to as folding behaviour. The antennae and mandibles performed a longer slower beat than normal as the caudal region bent forward to meet them. The setae of opposing limbs were brushed through each other and against the spinous caudal region (see Section 2a, Fig 2a.3).

Folding behaviour lasted 1.5 - 2.0 seconds in B.balanoides and slightly less in E.modestus. Often two to three folds directly followed one another. On rare occasions a modification to the normal folding behaviour was observed. The limbs and caudal region of the body moved in the manner described above, but initially the antennae were bent forward to such an extent that the splayed setae brushed across the frontal horns. When the antennae bent backwards to fold across the mandibles and caudal region the antennules also bent backwards at either side of the labrum. Modifications to the normal folding behaviour were most often seen in larvae with clean limbs, however the frequency of normal folding behaviour was greatest for animals with limb setae clogged with particles.

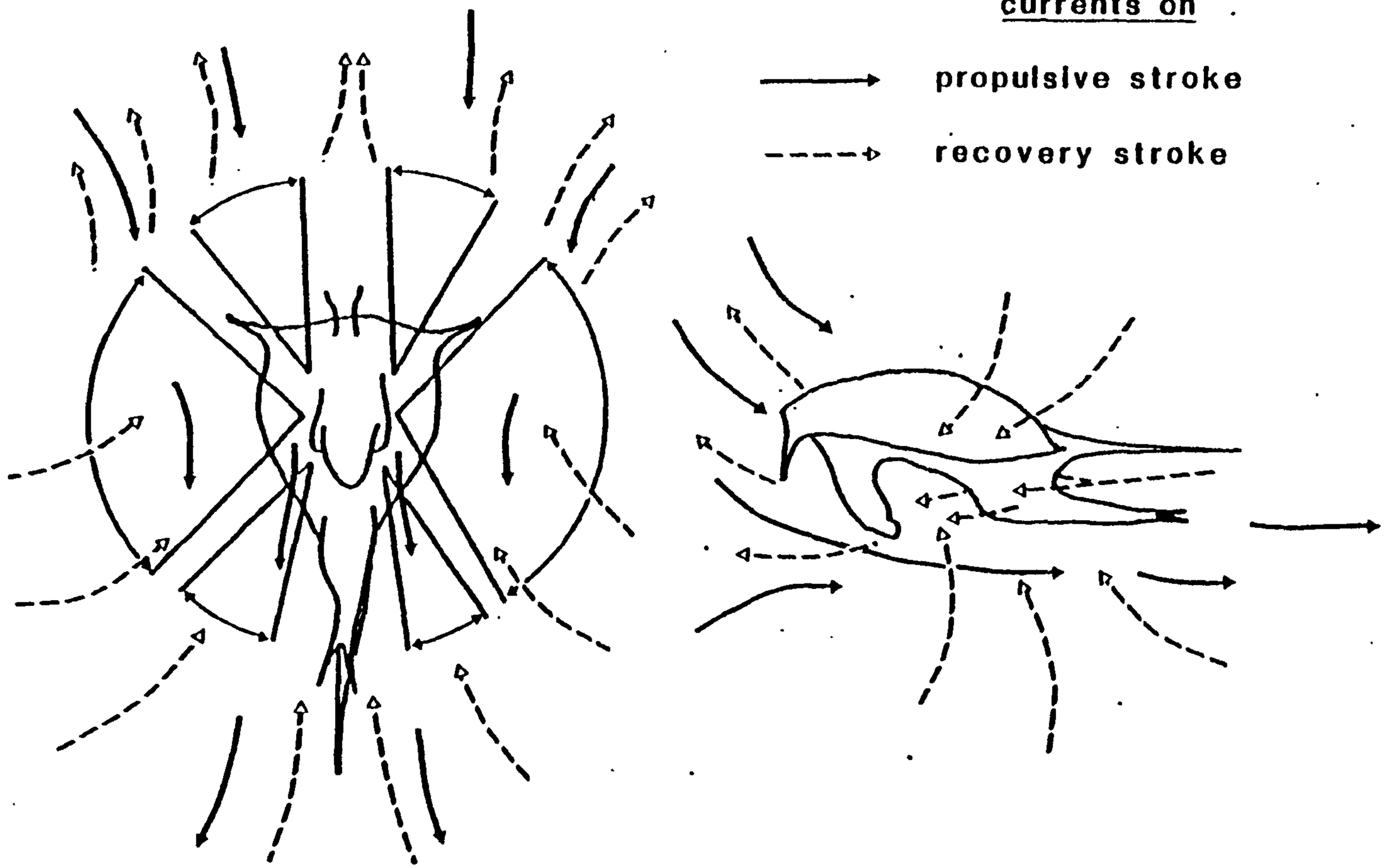
Water currents

The introduction of Methyl Blue solution in front of the restrained nauplii showed how the water was moved by the larvae during the swimming strokes. Figure 3.1 shows the movements of water around the nauplius during normal swimming. The solid arrows show water movements during the propulsive stroke and the dashed arrows indicate the movements of water during the recovery stroke of the limbs. The dye was moved posteriorly by the larvae in small parcels representing the quantity of water moved on the propulsive stroke (Fig 3.1 solid arrows). The dye parcels were separated by colourless seawater which had been brought towards the nauplii from the side and behind during the recovery stroke (Fig 3.1 dashed arrows). No strong water current was seen to be directly aimed at the mouth under the labrum, although water was brought in towards the ventral surface of the nauplii from the posterior and lateral regions of the larvae during the recovery stroke.

The fate of clogging particles

When suspensions of Carmine granules were introduced into the vicinity of the nauplii the granules tended to get caught up on the setae of the limbs. The setae of the antennae were tested for stickiness as described above and the side of the needle which had been brushed against the setae picked up considerably more carmine granules in seawater than the clean side. Therefore, it is likely that the setae are coated with some sticky substance. Carmine granules on the limbs were probably trapped by the sticky coating on the limb setae as well as in the net formed by the setae

Fig 3.1 The movements of water currents around a swimming barnacle nauplius



(a) Ventral view
limb movements
shown diagrammaticaly

(b) Side view
for clarity
limbs not shown

and setules.

Over a period of one or two hours, in clean fine-filtered seawater, most of the adherent granules on the limbs were removed, except when the setae were exceptionally heavily smothered in particles. During the folding behaviour, granules stuck to the limbs were aggregated and passed from the antennae to the mandibles. Particles on the antennules were passed to the antennae or knocked clear into the surrounding water during normal swimming as the antennules and antennae often brushed against each other. The aggregates of granules eventually passed to the caudal region from the mandibles during the folding behaviour, and often ended up on the caudal spine. At every transfer stage some granules were brushed off into the surrounding water but the majority were brushed off from the caudal region during subsequent folding behaviour patterns. Granules which were trapped on the limbs were not seen to be brushed onto the ventral surface of the body and thence worked forward towards the labrum and the mouth by the gnathobases of the limbs.

The ingestion of particles

When carmine granules alone were presented to the nauplii very few were ever ingested. The nauplii would, however, ingest more granules when these were presented in a mixture with algal cells. The capture of the granules and the algal cells by the nauplii of *E.modestus* and *B.balanoides* was extremely fast and many observations of the act of capture and ingestion were necessary to form a picture of the mechanism. Particles captured by the nauplii were always brought to the ventral surface of the body in the

recovery stroke currents shown in Fig 3.1 (dashed arrows). The movements of the mandibles tend to be towards each other on the propulsive stroke, as shown in Fig 3.1, which tends to trap particles brought in by the recovery stroke, particularly that of the antennae. The particles become trapped in the space between the labrum and the ventral thoracic process enclosed by the mandibles on either side (see Fig 3.2). Captured particles were worked along the spinous ventral surface of the nauplii (see Rainbow and Walker, 1976) by the setae of the mandibular endopodite (termed feeding setae by Lockhead 1936) towards the dense array of spines surrounding the mouth (Rainbow and Walker 1976). The granules were then forced into the oesophagus by the action of the limb gnathobases (termed antennal jaw processes by Lockhead 1936). In many cases where particles were seen to be ingested it appeared as though the recovery stroke of the limbs, particularly the antennae, seemed to be exaggerated such that more seawater than normal was brought towards the nauplii . It is possible that the brief change in the normal swimming rhythm mentioned above may be caused by such an exaggeration of the recovery stroke .

Not all the particles captured in the way described were ingested. Many of the carmine granules appeared to be actively rejected by the nauplii. Granules which were ultimately rejected were often stroked towards the labrum by the setae of the mandibles as if to be ingested. However, during a folding behaviour pattern such particles were knocked off the ventral surface of the larvae by a combination of the ventral thoracic process and the limb gnathobases whilst the limbs were folded. When the limbs bent forwards the rejected particles were moved slightly forward in the water and then swept behind the larvae during the backward thrust of the limbs as normal swimming beat was established.

Fig 3.2

A side and ventral view diagram of a stage IV E.modestus nauplius showing the position of the mandible in relation to the labrum and vetral thoracic process.

c = carapce.

cs = caudal spine.

l = labrum.

m = mandible.

m.end = mandibular endopodite.

m.exo = mandibular exopodite.

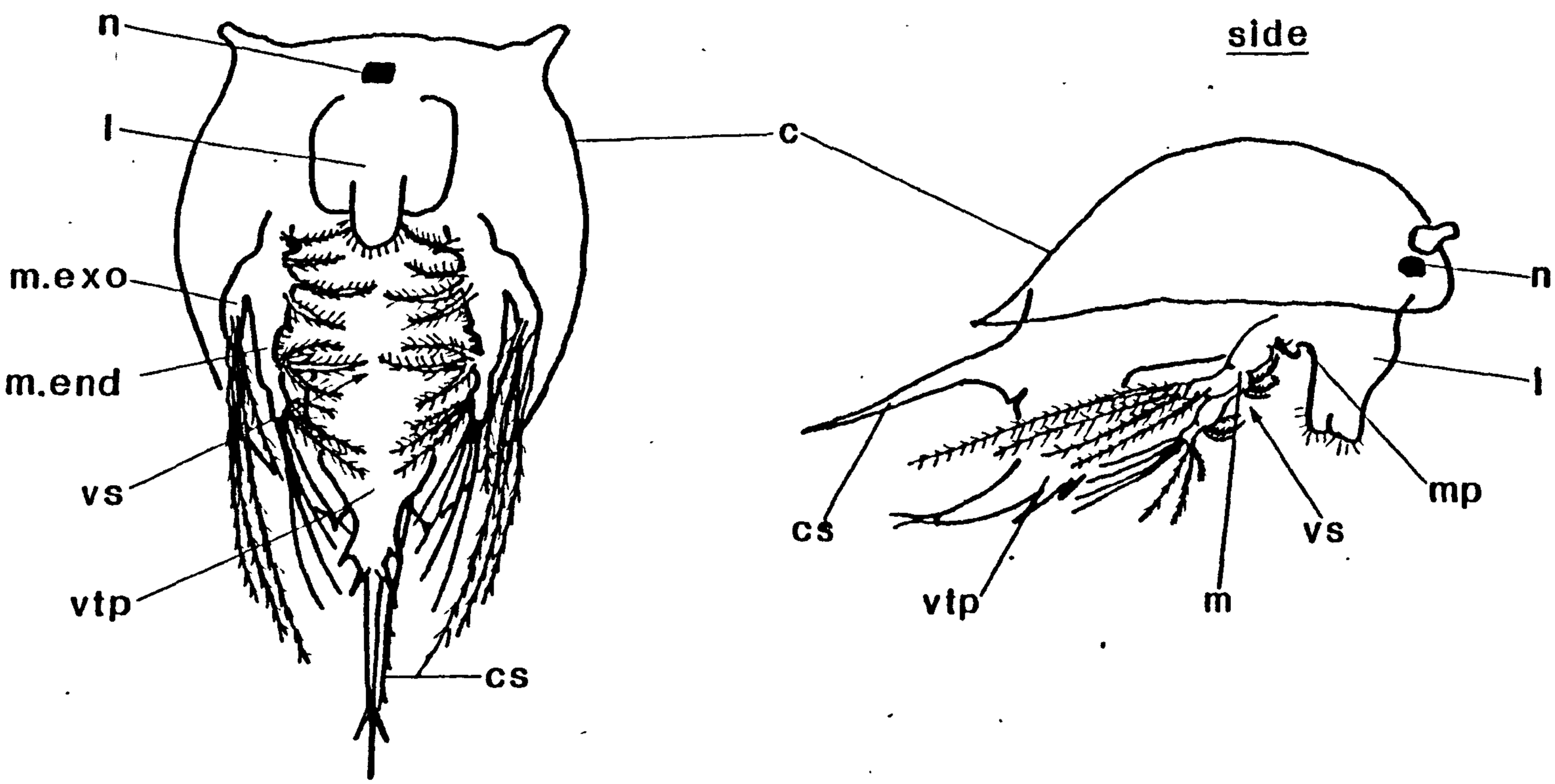
mp = position of mouth.

n = nauplius eye.

vs = ventral space.

vtp = ventral thoracic process.

Fig 3.2 Diagram of a S IV E.modestus nauplius showing the position of the mandibles (other limbs omitted)



200 µm

CONCLUSIONS

Norris and Crisp (1953) have suggested that folding behaviour was an important part of the feeding process of the nauplii of B.perforatus. The limb setae bear setules which cross each other forming a net like appearance. The suggestion of Norris and Crisp (1953) was that the nauplii filtered food organisms from the water column in the network of the limb setae, and in particular on the setae of the antennae. Particulate matter thus filtered would be transferred from the limbs during folding behaviour and thence worked towards the mouth along the ventral surface of the body. The observations of the present study show that particles which become trapped on the limbs of E.modestus and B.balanoides nauplii were cleaned off during folding behaviour and never directed towards the mouth as an immediate consequence of folding behaviour. The folding behaviour may well be simply a limb cleaning operation as suggested by Lockhead (1936). It is possible that a limb covered in particles does not propel the animal as efficiently as a clean limb. Although a clogged limb may be more solid and hence more effective on the propulsive swimming stroke it will be more difficult to close the setae and feather the limb on the recovery stroke, thus making the whole swimming stroke less efficient. Furthermore, limbs with adherent organic particles become a focus for microbial growth and possible infection of the larvae. It has been noticed by several workers who rear barnacle larvae in Menai Bridge that, if algal food is too dense in the culture, the limbs of the larvae tend to become clogged and the nauplii start to die. This is particularly true for the larger and later stage nauplii where it has sometimes been found necessary to maintain them with only very small amounts of microalgae to prevent limb clogging. Perhaps under some conditions the larvae become covered in particles so quickly that

they cannot keep the limbs clean, causing excessive energy expenditure, exhaustion, and failure to be able to keep off the bottom of the culture vessels.

The setae of the limbs are sticky which could assist in the method of feeding postulated for the larvae by Norris and Crisp (1953). This stickiness, however, may have been evolved because in clean water it increases the efficiency of the limb as a paddle. A coating of a viscose fluid on the setae and over the inter-setule gap would be easily compressible on the recovery stroke, yet increase the efficiency of the power stroke without a great increase in limb weight. Walker (1973) suggested that the frontal horns produced a 'sticky' secretion which was transferred to the limbs and assisted with algal capture. He was uncertain exactly how the secretion reached the limbs. The observation of the modification to folding behaviour where the antennae were bent forwards to brush across the frontal horns shows how the secretion could be transferred to the antennae and thence during folding behaviour to the mandibles. It is unlikely, however, that such a secretion would play a direct role in the capture of algal cells on the limbs.

The suggestion of Lockhead (1936) that the water currents produced by the nauplius during the recovery phase of the swimming stroke carry food particles to the mouth is probably more accurate. Water was certainly moved towards the ventral surface of the barnacle nauplius by the recovery stroke of the limbs yet there appeared to be no direct current carrying particles up under the labrum. Particles behind and to the side of the nauplii which were brought towards the ventral surface of the larvae by the recovery stroke were in fact trapped by the backward stroke of the mandibles. Once

trapped in the ventral space (see Fig 3.2) the particles were manipulated towards the labrum by the stout setae on the mandible. Finally, the food particles were forced into the mouth by the limb gnathobases as described by Rainbow and Walker (1976). The observations of the present study further suggest that the larvae may be able to occasionally exaggerate the recovery stroke to bring more water and, if present, more food particles within the scope of the mandibular trap. Such a behaviour pattern would undoubtedly mean that the larvae had a measure of control over their feeding rate.

Section 4

Changes in the Limb Beat Movements of Barnacle Nauplii in the Presence of Food Organisms

Changes in the Limb Beat Movements of Barnacle Nauplii in the Presence of Food Organisms

Introduction

The swimming of barnacle nauplii maintains their position in the water column. The early nauplius stages are strongly photopositive (Barnes and Klepal 1972), although there is some evidence to suggest that the larvae become photonegative after they feed (Singarajah, Moyse and Knight-Jones 1967). It seems clear that swimming and feeding in the barnacle nauplius are inextricably linked (Lockhead 1936, Gauld 1959). The common view of the feeding mechanisms of many planktonic larvae has been that feeding occurs as an automatic consequence of swimming. The larvae would, therefore, feed at the same speed in low algal concentrations as in high ones.

The observations described in Section 3 above would suggest that the barnacle nauplius could exercise some control over it's feeding rate, even if only by rejecting some particles, or ignoring them, when they were brought close to the mandibular feeding apparatus in the course of normal swimming. Quantitative studies of copepod feeding by Adams and Steele (1966) led them to postulate that the copepods altered their feeding speed to suit the algal availability. Frost (1972) expanded this hypothesis with a wealth of quantitative data on the feeding of Calanus and produced a careful mathematical model (Lam and Frost 1976) reflecting the feeding strategies used by the copepod. Frost's investigations concluded that calanoid copepods fed more slowly in low algal concentrations, but at a optimal rate above a certain algal concentration, thus balancing the energy

expended on feeding to that gained by the food intake.

Algal food for planktonic organisms often occurs in patches (Cushing 1962, Cassie 1962,1963) and the areas between patches may have quite low algal concentrations. It is likely that planktonic organisms may move into and out of areas of high algal density fairly regularly. The opportunist, herbivorous plankter would be able to utilise patches of high algal density fully, yet not expend energy on feeding when the food return from a barren area between algal patches was low. The mechanisms by which planktonic herbivores may adjust their feeding speed have not been fully investigated. The following series of observations were performed to investigate the swimming response of tethered barnacle nauplii to patchy changes in the algal concentration around them.

Methods

Nauplii of either B.balanoides or E.modestus which had been reared in the laboratory (see 'Methodology' above) were restrained on a suction tip such that they faced into a seawater current in the apparatus described in Methodology, Fig 1.2, Fig 1.3. The observation chamber had a continuous flow of fine-filtered, ultra-violet irradiated seawater at 0.25 - 0.5 cm s^{-1} and the temperature was monitored continuously with a thermistor. The larvae were drawn at random from the laboratory culture, the first successful attachment being used for observation.

Records of the limb beat movements of the nauplii were made in three ways which are described fully in 'Methodology' above. 1). Pneumograph records of the antennal limb beat were made. 2). Pneumograph records were

made simultaeneously with video-tape recordings. Limb beat frequency and amplitude could be obtained from either record. Frequency was obtained more quickly from the pneumograph records than from the video-recordings. Beat amplitude could be measured directly from the video-recordings and the values obtained used to calibrate the pneumograph amplitude scale. 3) Video-tape recordings only were used and analysed as described in 'Methodology'. The results obtained from the above techniques are given below in an attempt to establish the usefulness of the techniques in pursuit of the study of planktonic organisms.

After 45 minutes the larvae had become habituated to the restraint and the recording was initiated. A control beat rate and amplitude record was obtained from the larvae, normally over the succeeding 20 - 30 minutes. 1 ml. samples of algae, which had been concentrated by centrifugation and resuspended from a weaker stock culture, were injected into the seawater flow through a rubber connection at point I ('Methodology' Fig 1.2). The algae passed into the observation dish and received thorough mixing before leaving the observation dish at the outlet.

In a series of control trials suspensions of Skeletonema costatum or Tetraselmis suecica were introduced into the seawater flow and the change in algal concentration of the effluent was monitored. The effluent from the chamber was collected in separate 100 ml. beakers for each minute following the injection. The concentration of algal cells in each sample was estimated either by haemocytometer counting or by coulter counting. The total number of algal cells injected was calculated as the sum of the total number of cells in each of the timed samples. The percentage of cells from the initial injection still remaining in the observation chamber after time

t can be expressed as follows:

$$\% \text{ cells remaining} = \left(\sum \text{cells in effluent} - {}_{o}^{t}\sum \text{ cells in effluent}\right) \times 100$$
$$\text{cells in the effluent}$$

Where $\sum$cells in effluent = the total number of cells in the initial injection and ${}_{o}^{t}\sum$ cells in effluent = the sum between time 0 and t of the cells collected in the effluent. Table 4.1 gives the figures obtained for trials with two species of algae, the numbers of cells in each of the samples, the total numbers of cells in the initial injection, the algal concentration in the observation chamber of 50 ml. capacity immediately after the injection and the percentage of cells remaining in the observation dish at time t. Fig 4.1 shows a plot of the percentage of cells remaining in the observation chamber against time after injection for three trials with T.suecica and three trials with S.costatum. In all the trials shown in Fig 4.1 no algae was found in the effluent after 5 minutes.

For both algal species the shape of the graph in Fig 4.1 approximates to that of exponential decay, equations of which take the form :-

$$W_t - A = (W_o - A)e^{-kt} \quad \ldots\ldots\ldots(1)$$

In this case A = 0, since the final concentration will be zero, thus eq.(1) will reduce to :-

$$W_t = W_o\, e^{-kt} \quad \ldots\ldots\ldots\ldots\ldots(2)$$

where W_t = concentration (% of cells remaining in the observation chamber) at time t and W_o = the initial concentration (i.e. 100%). The least squares fit of such an equation to the data of Table 4.1 can be

TABLE 4.1

SKELETONEMA

Sample time	Cells per Sample	Initial algal Concentration	% of Total Remaining	at Time
0 - 1 minute	13.045×10^6		14.45	1 minute
1 - 2 minutes	1.555×10^6		4.25	2 minutes
2 - 3 minutes	0.433×10^6		1.42	3 minutes
3 - 4 minutes	0.216×10^6		-	4 minutes
4 - 5 minutes	-			
Total	15.249×10^6	305.0		
0 - 1 minute	14.133×10^6		22.23	1 minute
1 - 2 minutes	2.507×10^6		8.44	2 minutes
2 - 3 minutes	0.909×10^6		3.44	3 minutes
3 - 4 minutes	0.516×10^6		0.60	4 minutes
4 - 5 minutes	0.108×10^6		-	5 minutes
5 - 6 minutes	-			
Total	18.173×10^6	363.5		
0 - 1 minute	11.036×10^6		10.23	1 minute
1 - 2 minutes	0.538×10^6		5.85	2 minutes
2 - 3 minutes	0.445×10^6		2.23	3 minutes
3 - 4 minutes	0.274×10^6		-	4 minutes
4 - 5 minutes	-			
Total	12.294×10^6	245.9		

TETRASELMIS

Sample time	Cells per Sample	Initial Algal Concentration	% of Total Remaining	at Time
0 - 1 minute	4.800×10^6		23.76	1 minute
1 - 2 minutes	0.936×10^6		8.89	2 minutes
2 - 3 minutes	0.400×10^6		2.54	3 minutes
3 - 4 minutes	0.160×10^6		-	4 minutes
4 - 5 minutes	-			
Total	6.296×10^6	125.9		
0 - 1 minute	5.254×10^6		24.23	1 minute
1 - 2 minutes	1.254×10^6		6.15	2 minutes
2 - 3 minutes	0.320×10^6		1.53	3 minutes
3 - 4 minutes	0.106×10^6		-	4 minutes
4 - 5 minutes	-			
Total	6.934×10^6	138.7		
0 - 1 minute	12.400×10^6		19.48	1 minute
1 - 2 minutes	2.200×10^6		5.19	2 minutes
2 - 3 minutes	0.600×10^6		1.30	3 minutes
3 - 4 minutes	0.200×10^6		-	4 minutes
4 - 5 minutes	-			
Total	15.400×10^6	308.0		

Table 4.1 : The measurement of the rate of algal clearance from the observation chamber. The number of cells in the effluent and the percentage of cells still remaining is shown with the algal concentration (cells/µl) immediately after the injection.

Figs 4.1 and 4.2

The disappearance of algal cells from the observation dish with time. The percentage of Skeletonema and Tetraselmis cells remaining in the dish is plotted against time from the injection.

Fig 4.1 curve fitted to

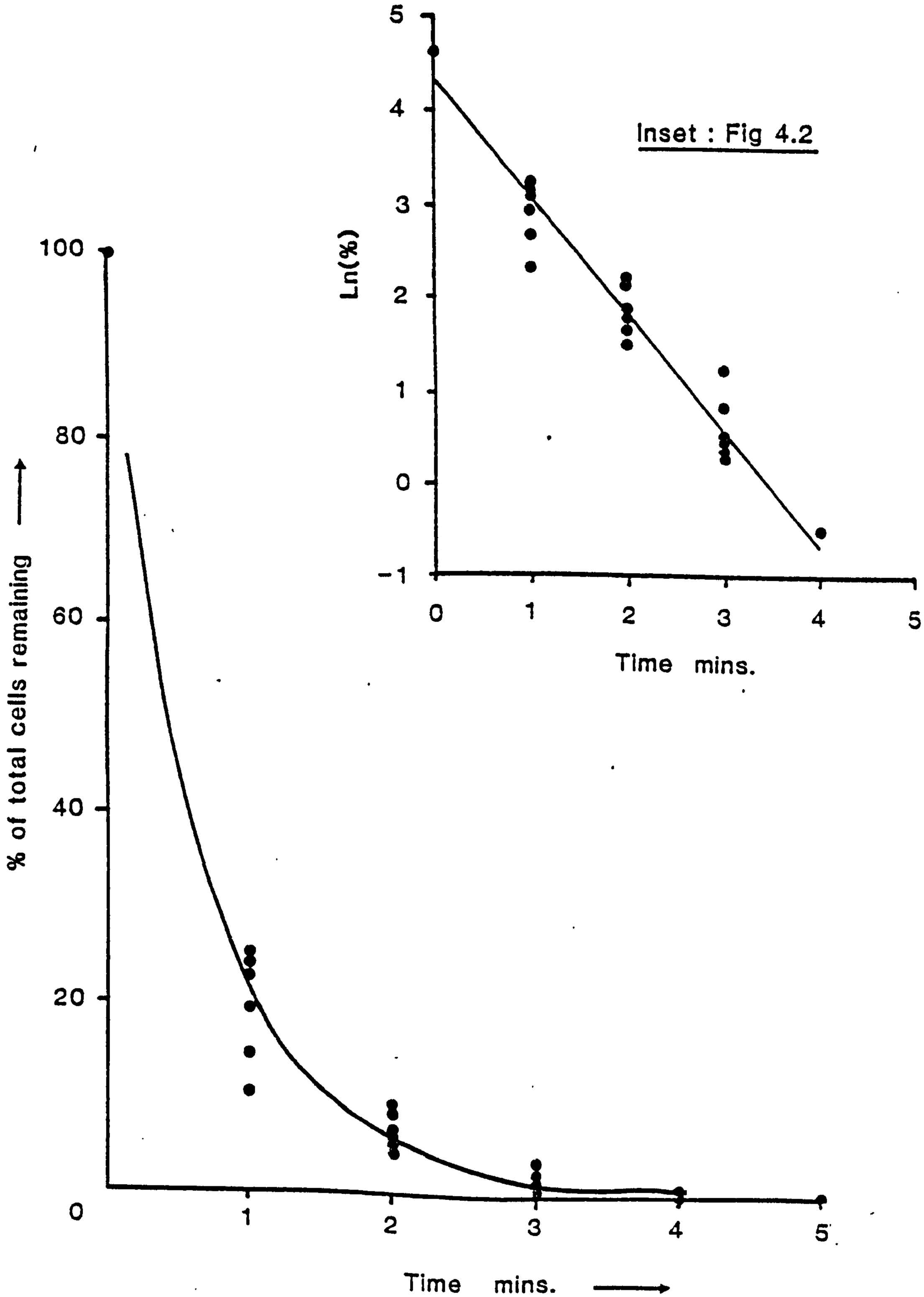
$$\%\text{cells remaining} = 83e^{-1.28\ \text{time}}$$

Fig 4.2 (inset). Natural log of %cells remaining against time after injection. The regression was fitted by least squares to

$$\ln\% = 4.42 - 1.28\ \text{time}$$

correlation coefficient = -0.981 for 23 degrees of freedom.

Fig 4.1 Time of algal clearance from the observation dish



obtained by linear regression analysis of the natural Log of the percentage of cells remaining against time. Fig 4.2 (inset with Fig 4.1) shows the plot of the natural Log of % of cells remaining in the observation chamber against time. The regression line fitted to the data takes the form :-

$$\text{Ln(\%of cells remaining)} = 4.42092 - 1.2745\ \text{time} \quad \ldots\ldots\ldots (3)$$

The correlation coefficient for (3) above is equal to -0.9805 for 23 degrees of freedom corresponding to a probability < 0.001 that the slope of the regression line equals zero. The intercept was tested for deviation from the true intercept (which must be very close to 100% just after the algal injection) in the same manner as a test of the intercept to zero (see for example Snedecor and Cochran 1967). The Students t-Value for the comparison = 0.3955 for 23 degrees of freedom corresponding to a probability of 0.6961 that the difference between Ln(100) and the intercept of (3) could have been obtained by chance.

In the experiments reported below, where algal cell concentrations are shown, they have been derived from the measurement of the algal concentration in the effluent sample taken for one minute after the initial injection. The percentage of cells remaining in the observation chamber at time one minute was derived from (3) and thus the number of cells in the sample represented 100% - percentage of cells remaining in the observation chamber . The total number of cells in the initial injection was calculated by extrapolation.

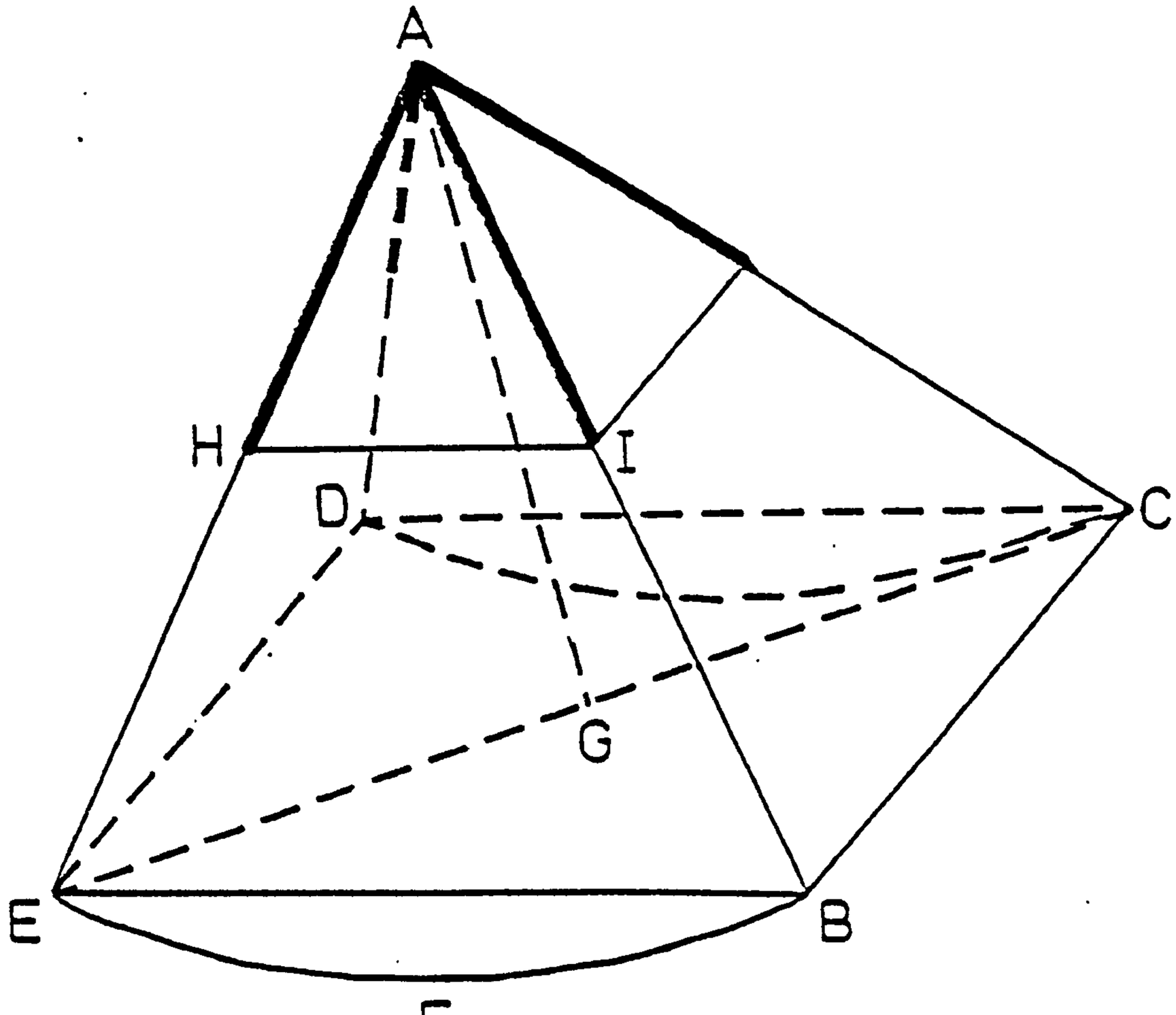
Presentation of the results.

Where appropriate the results of the observation described below are expressed in three ways. The frequency of the nauplius antennal beat is given measured in Hz. Amplitude of the antennal beat is given in μm. and the volume through which the antennae move, the swept volume, is given in mm^3 / minute. Each measure was calculated as the average for recorded values over a minute. The swept volume was calculated from the limb beat frequency, amplitude and the amount of time spent swimming by the larvae during the minute in which frequency and amplitude were averaged. This swept volume was calculated for the propulsive phase of the swimming stroke and does not include water moved on the recovery stroke because the geometry of the limb on the recovery stroke was less readily approximated. It is most likely, however, that the amount of water moved on the recovery stroke will be proportional to that moved on the power stroke. For the purposes of the calculation, the antennae were considered as triangular paddles moving through part of a sphere which is determined by the dimensions of the antennae and the amplitude of stroke on the propulsive phase of the limb beat. Fig 4.3 shows a schematic representation of the antennal limb beat used in the calculation of the swept volume. For ease of calculation the swept volume was determined as that of the pyramid A B C D E plus the volume of a rod, of segmental cross section, B C D E F.

The following three measurements were assumed as standard for stage IV nauplii. The average antennal and exopodite length and the antennnal width was measured from several dissected limbs for B.balanoides and E.modestus. The exopodite length was measured from near the basal antennal segment to the tip of the exopodite and the antennal length from the basal antennal

Fig 4.3

Diagram of the figure used to represent the swept volume of the nauplius antenna.



A = Base of the limb.

ΔABC = Antenna at the start of a stroke.

ΔADE = Antenna at the end of a stroke.

AH = Exopodite length.

HE = Length of setae.

BC = Antennal width.

IH = Exopodite amplitude of movement.

segment to the ends of the setae. The antennal width was measured as the greatest width of the antennae towards the tips of the setae. The dimensions thus measured were as follows :-

Elminius modestus

Exopodite length (AH Fig 4.3)..... 0.18 mm.

Antennal length (AB Fig 4.3)..... 0.44 mm.

Antennal width (BC Fig 4.3)..... 0.33 mm.

Balanus balanoides

Exopodite length ..... 0.35 mm.

Antennal length ..... 0.62 mm.

Antennal width ..... 0.54 mm.

The distance moved by the tips of the setae (BE Fig 4.3) was estimated from the limb measurements given above and the recorded limb beat amplitude (IH Fig 4.3).

$$BE = (IH \times AB)/AH$$

The ratio of the sides of two isosceles triangles having the same apex are equal.

The height of the pyramid (AG Fig 4.3) was found by first calculating GE (Fig 4.3).

$$GE = BC/[2Sin(C\hat{E}B)]$$

where $$C\hat{E}B = Tan^{-1}(BC/BE)$$

By Pythagoros theorem

$$AG = (AB^2 - GE^2)^{0.5}$$

The volume of the pyramid then becomes

$$V_P = 0.33 \times AG \times BC \times BE$$

The cross sectional area of the remaining shape can be obtained as

$$\text{Sector area ABFE} = 0.5 \times B\hat{A}E \times AB^2$$

where $B\hat{A}E$ is in radians and

$$0.5 \times B\hat{A}E = \text{Sin}^{-1}(IH/[2 \times AH])$$

$$\text{Triangle area ABE} = 0.5 \times AB^2 \times \text{Sin}(B\hat{A}E)$$

$$\text{Segment area BEF} = \text{Area ABFE} - \text{Area ABE}$$

$$\text{Volume segment cylinder Vs} = \text{Area BEF} \times BC$$

$$\text{Swept Volume by both antennae Vm} = 2 \times (Vp + Vs)$$

Thus from the simplified representation of an antennal beat depicted in Fig 4.3, it is possible to obtain a theoretical estimate of the swept volume during a propulsive stroke. If Vm is multiplied by the beat frequency in Hz and the number of seconds in which the larva was actively swimming during that particular minute, then an estimate can be obtained for the average swept volume per minute.

The estimate of the swept volume will be proportional to the volume of water moved by the antennae during a propulsive stroke. The difficulties involved in describing the effective surface area of the limb, or in assessing entrainment of water from beyond the scope of the limbs, make it impractical to obtain a reasonable estimate of net water transport by the limbs. For the purposes of this investigation the swept volume will be adequate because it will be proportional to net water transport by the limbs and thus will vary directly with changes in net water transport.

Results

(1) Uncalibrated pneumograph record from an E.modestus S IV Nauplius

Fig 4.4 shows the plot obtained from the average beat frequency and pneumograph scale deflection for a stage IV E.modestus nauplius restrained in clean flowing seawater at 10°C for 3 hours 46 minutes. The record was intended for use as a control observation for further pneumograph records, however, the pneumograph technique was not extensively employed in this work for the reasons discussed above (see 'Methodology') and exemplified below. The graph is composed of groups of consecutive averages 5 - 7 minutes long (G1 - G4 Fig 4.4). The groups of observations (G1 - G4) were separated by further observations taken at approximately 10 minute intervals. Fig 4.4(a) represents the limb beat amplitude of the nauplius, which varies from 4 - 2.75 Hz throughout the period of observation with no apparent sudden or large changes in beat frequency.

Each of the groups of results were analysed using the single factor analysis of variance technique developed by Fisher (1935). Table 4.2 gives the mean frequency and standard error for each minute in each group and the analysis of variance table for each group. The F-value for groups G1, G2 and G4 show that there was no significant difference in mean beat frequency within each group. For group G2, however, the probability that the mean beat frequencies were drawn from the same population of means was 0.0018. The differences between means of limb beat frequency in group G2 were analysed by the sequential variant of the Studentised Q test for the least significant range (e.g. Snedecor and Cochran 1967). It can be seen from Table 4.2 that only the extreme difference between the mean for the 50th

Fig 4.4 Antennal movements of a stage IV E.modestus nauplius analysed from a pneumograph trace 14°c

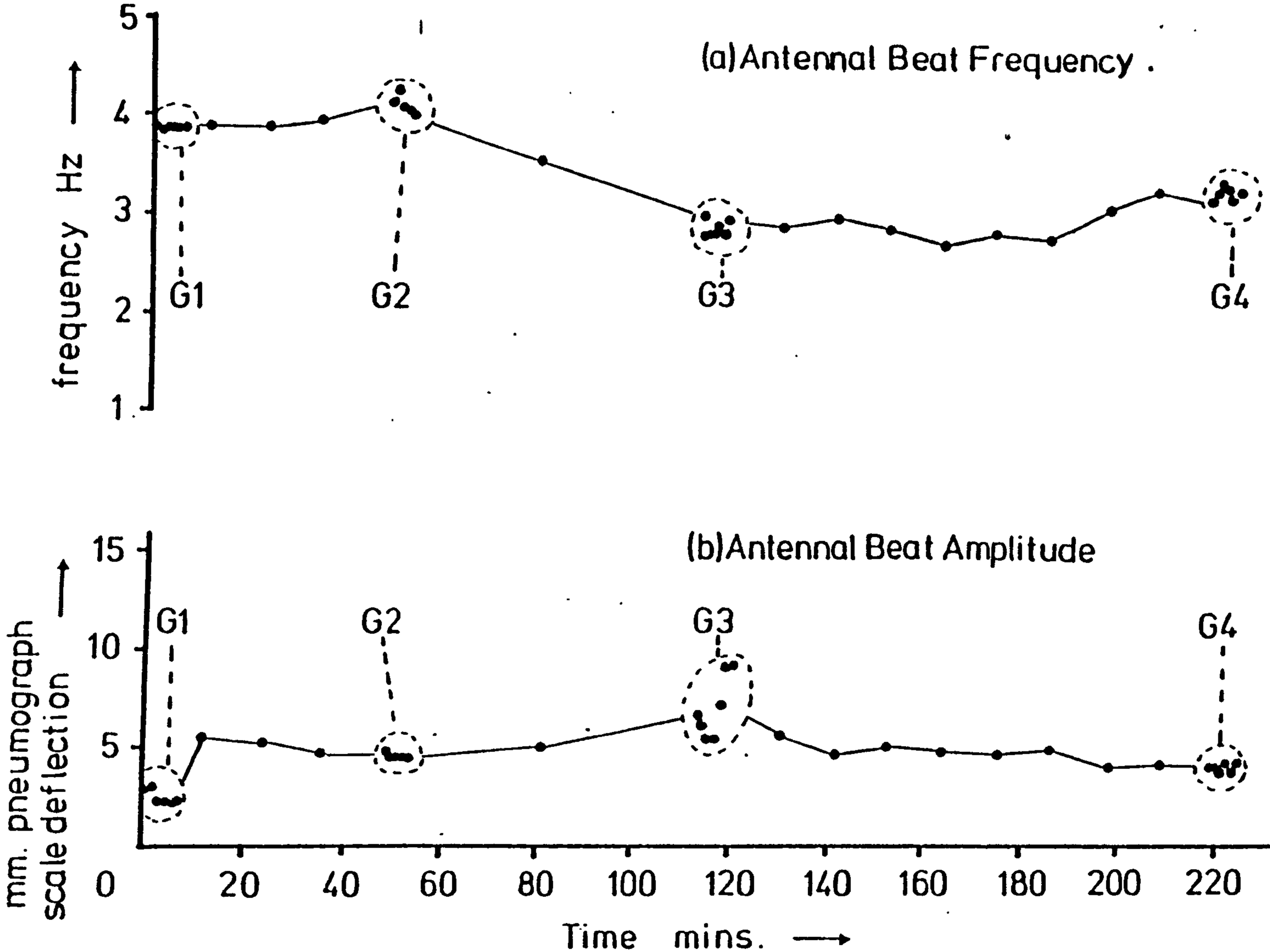


TABLE 4.2

GROUP 1 1-7 minutes

Minute	1	2	3	4	5	6	7
Mean Frequency (Hz)	3.80	3.78	3.84	3.80	3.80	3.80	3.86
Standard Error	0.042	0.041	0.042	0.042	0.042	0.042	0.042

Analysis of Variance Table

Source	Sum of Squares	D.F.	Mean Square	F	p
Between	0.240	6	0.0400	1.473	0.1932
Within	3.232	119	0.0272		
Total	3.472	125			

GROUP 2 49-53 Minutes

Minute	49	50	51	52	53
Mean Frequency (Hz)	4.12	4.23	4.12	4.04	3.99
Standard Error	0.039	0.055	0.039	0.039	0.045

Analysis of Variance Table

Source	Sum of Squares	D.F.	Mean Square	F	p
Between	0.6232	4	0.1558	4.721	0.002
Within	2.6400	80	0.0330		
Total	3.2631	84			

Comparison between Means. Sequential Neuman Keuls.

Group 2 Standard error of any mean = 0.0454

Test	$Q_{0.05}$	(a)	(f)	Difference Required For 5% Significance	Observed Difference
minute 50 - 53	3.74	5	80	0.17	0.24
minute 50 - 52	3.4	4	80	0.15	0.19
minute 49/51 - 53	3.4	4	80	0.15	0.13

Table 4.2 (cont.)

Group 3 115-121 minutes

Minute	115	116	117	118	119	120	121
Mean Frequency (Hz)	2.998	2.797	2.862	2.831	3.197	2.896	2.962
Standard Error	0.071	0.100	0.110	0.075	0.160	0.087	0.140

Analysis of Variance Table

Source	Sum of Squares	D.F.	Mean Square	F	p
Between	1.1005	6	0.1834	1.460	0.2064
Within	7.9136	63	0.1256		
Total	9.0141	69			

Group 4 219-223 minutes

Minutes	219	220	221	222	223
Mean Frequency (Hz)	3.189	3.236	3.307	3.330	3.378
Standard Error	0.057	0.054	0.074	0.060	0.098

Analysis of Variance Table

Source	Sum of Squares	D.F.	Mean Square	F	p
Between	0.3214	4	0.08034	1.162	0.3360
Within	4.4956	65	0.06916		
Total	4.817	69			

Table 4.2 : An analysis of variance for the mean antennal beat frequency of a B.balanoides stage IV nauplius as recorded by impedance pneumograph. (a)= the number of means in the Neuman Keuls test and (f)= the degrees of freedom for the standard error.

and the 53rd minutes was significant. No other differences between means were shown to be significant for group G2.

The analysis for limb beat amplitude in Table 4.3(a) shows that only for group G1 can all the means be considered to be drawn from the same population of means. The differences between means for groups G2 and G4 were again tested by the sequential variant of the Studentised Q test. As with the limb beat frequency data for G2, only the largest difference between means was found to be significant (viz. mins 48 and 51 for G2, mins 221 and 222 for G4). Group G3, however, as can be seen from Fig 4.4(b), showed considerable within group variation. Analysis of the differences between means using the sequential Studentised Q test (Table 4.3(b)) showed that the mean amplitude record for mins. 115 - 118 were significantly different from that in min. 119 which was in turn significantly different from those in mins. 120 - 121. No changes in environmental conditions could be detected which may have led to such a large variation in the limb beat amplitudes measured for group G3. In view of the less variable amplitude shown for the record of the remaining 210 minutes of observation, it is likely that the result for G3 was a consequence of electrode movement around the limb.

(2) Calibration of the amplitude records from pneumograph traces

In a series of experiments, pneumograph recordings of the antennal limb beat of nauplii were made and Video tape recordings were obtained simultaeneously. The limb beat frequency was measured directly from the pneumograph trace as described in 'Methodology' above. Antennal beat amplitude was measured from the Video tape recordings and used to calibrate

TABLE 4.3(a)

Group 1 1-6 minutes

Minute	1	2	3	4	5	6
Mean Amplitude	3.163	3.236	3.082	3.009	3.009	3.000
Standard Error	0.077	0.082	0.084	0.078	0.078	0.076

Analysis of Variance Table

Source	Sum of Squares	D.F.	Mean Square	F	p
Between	2.6311	5	0.5262	1.525	0.1816
Within	111.8273	324	0.3451		
Total	114.4583	329			

Group 2 49-53 minutes

Minute	49	50	51	52	53
Mean Amplitude	4.918	4.600	4.573	4.455	4.518
Standard Error	0.098	0.130	0.120	0.117	0.108

Analysis of Variance Table

Source	Sum of Squares	D.F.	Mean Square	F	p
Between	7.0964	4	1.774	2.464	0.0455
Within	194.4091	270	0.720		
Total	201.5055	274			

TABLE 4.3(a) cont.

GROUP 3 115-121 minutes

Minute	115	116	117	118	119	120	121
Mean Amplitude	6.75	6.35	5.367	5.367	7.117	9.183	9.383
Standard Error	0.381	0.470	0.354	0.298	0.355	0.558	0.540

Analysis of Variance Table

Source	Sum of Squares	D.F.	Mean Square	F	p
Between	487.2976	6	81.22	14.46	<0.001
Within	1139.808	203	5.615		
Total	1627.106	209			

GROUP 4 219-224 minutes

Minute	219	220	221	222	223	224
Mean Amplitude	4.267	4.326	4.000	4.558	4.140	4.430
Standard Error	0.119	0.135	0.097	0.131	0.113	0.112

Analysis of Variance Table

Source	Sum of Squares	D.F.	Mean Square	F	p
Between	8.6008	5	1.7200	2.839	0.0163
Within	152.6740	252	0.6059		
Total	161.2753	257			

Table 4.3(a) : The analysis of variance for the mean antennal beat amplitude of B.balanoides stage IV nauplius recorded by impedance pneumograph.

TABLE 4.3(b)

Sequential Neuman Keuls Analysis of Mean Beat Amplitude

Test	$Q_{0.05}$	(a)	(f)	Difference Required For 5% Significance	Observed Difference
Group 2	Standard error of any mean = 0.1144				
minute 51 - 48	3.86	5	270	0.442	0.463 *
minute 51 - 49	3.63	4	270	0.415	0.145
minute 52 - 48	3.63	4	270	0.415	0.400
Group 3	Standard error of any mean = 0.4326				
minute 117 - 121	4.17	7	203	1.804	4.016 *
minute 117 - 120	4.03	6	203	1.743	3.816 *
minute 118 - 121	4.03	6	203	1.743	4.016 *
minute 117 - 119	3.86	5	203	1.670	1.750 *
minute 116 - 121	3.86	5	203	1.670	3.033 *
minute 117 - 115	3.63	4	203	1.571	1.383
minute 115 - 121	3.63	4	203	1.571	2.633 *
minute 119 - 121	3.32	3	203	1.436	2.266 *
minute 120 - 121	2.77	2	203	1.198	0.200
Group 4	Standard error of any mean = 0.1187				
minute 221 - 222	4.03	6	252	0.478	0.558 *
minute 221 - 224	3.86	5	252	0.458	0.430
minute 223 - 222	3.86	5	252	0.458	0.418

Table 4.3(b) : A sequential Neuman Keuls analysis of the mean antennal beat amplitudes for B.balanoides shown in Table 4.3(a).
The analysis was performed only for groups of means showing a significant F-value from the analysis of variance. (a) = the number of means per test and (f) = the number of degrees of freedom for the standard error.
A * alongside an observed difference denotes a value in excess of that required for significance at the 5% level.
The absence of a star denotes that the probability that all the means, covered by the test, come from the same population of means is greater than 0.05.

the peak to trough distance of the pneumograph trace in μm. of beat amplitude. The tips of the antennal setae were difficult to see on the Video recordings, hence the amplitude of movement of the end of the antennal exopodite was measured. The mean limb beat amplitude, obtained from a sample of 30 - 50 beats in one minute and measured from the Video recordings, was equated to the mean of a similar number of peak to trough measurements from the same minutes recording on the pneumograph. The pneumograph record was calibrated to represent limb beat amplitude in mm. regularly, over long periods of observation, especially when the trace showed large changes in amplitude.

Fig 4.5 shows the result of a series of records of the antennal beat of an E.modestus stage IV nauplius obtained by using the impedance pneumograph calibrated for beat amplitude as described above. The record was made over a 90 minute period with 10 minute intervals between groups of observations. Figs 4.5 (a) and (b) represent antennal beat amplitude and frequency respectively. The plots suggest that after the introduction of S.costatum, R.baltica and possibly P.lutheri the frequency of limb beat, the amplitude, or both was increased. Fig 4.5 (c) shows the plot of the antennal swept volume (see above). In all cases, except after the introduction of T.suecica, there was a definite increase in the swept volume after the addition of algae, followed by a fall to a level close to that before the injection.

The initial concentrations of the algae in all the injections were low, particularly that for T.suecica. The experiment was performed at a time when algal blooms of Phaeocystis persisted in the Menai Strait. The quality of the seawater at the laboratory was well below normal due to contamination

Fig 4.5

The response of a stage IV E.modestus nauplius at 15°C to the presence of suspensions of Skeletonema, Pavlova, Tetraselmis, Isochrysis, and Rhodomonas, in the observation dish.

(a)...antennal beat amplitude (mm).

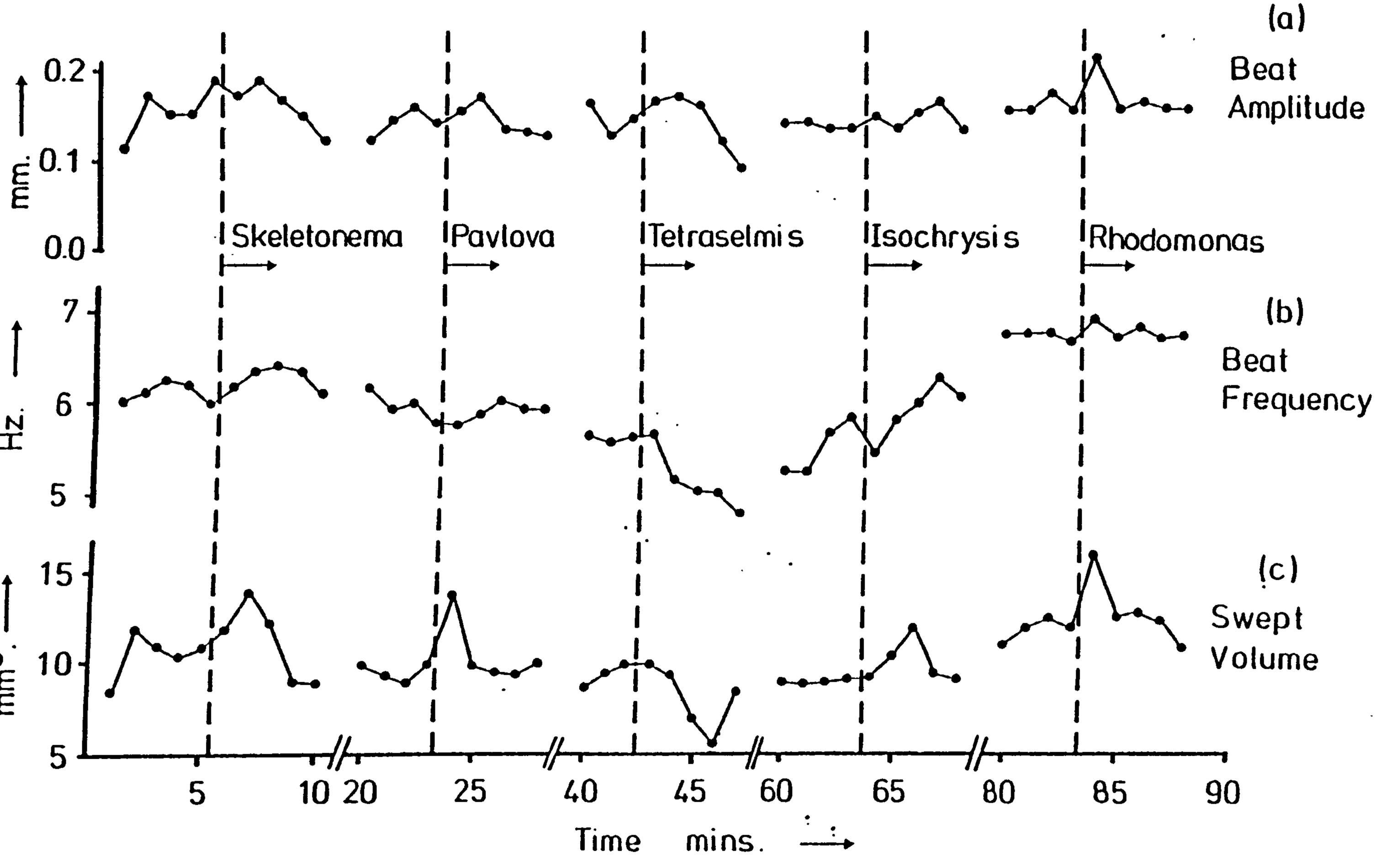
(b)...antennal beat frequency (Hz).

(c)...antennal swept volume (mm^3).

%time spent active by the nauplius in each minute (-)

(1)	98%	(2)	98%	(3)	96%	(4)	92%	(5)	85%	(6)	100%	(7)	100%
(8)	98%	(9)	95%	(10)	100%	(20)	96%	(21)	92%	(22)	95%	(23)	98%
(24)	100%	(25)	100%	(26)	100%	(27)	96%	(28)	100%	(40)	100%	(41)	98%
(42)	95%	(43)	100%	(44)	100%	(45)	100%	(46)	100%	(47)	96%	(60)	96%
(61)	98%	(62)	96%	(63)	96%	(64)	100%	(65)	98%	(66)	100%	(67)	99%
(68)	95%	(80)	98%	(81)	96%	(82)	100%	(83)	100%	(84)	98%	(85)	98%
(86)	100%	(87)	100%	(88)	95%								

Fig 4.5 The response of a stage IV E.modestus nauplius to the presence of algal suspensions. 15°C



with the breakdown products of the algal bloom in the Strait. Consequently many of the algal cultures maintained at the laboratory were weak and of poor quality.

(3) The antennal movements of Nauplii recorded on Video tape

Although the method described above can be used to calibrate a pneumograph trace in terms of actual antennal beat amplitude the process was found to be unreliable unless a check on the amplitude calibration was made very frequently. For this reason it was decided to use Video tape recording alone and to calculate the beat frequency and amplitude as described in 'Methodology'. The analysis of Video recordings in this manner was very time consuming yet its greater accuracy for the analysis of beat amplitude made the technique preferable to the pneumograph techniques described above.

Figs 4.6 - 4.11 are plots showing the analysis from Video recordings of the antennal movements of two species of barnacle nauplii. Four quantities are expressed on each graph, (a) limb beat frequency (b) limb beat amplitude (c) the swept volume and also the algal concentration in the observation chamber.

Fig 4.6 gives the recorded movements for a stage IV E.modestus nauplius at 15°C. This Fig represents a control observation as the algal concentration throughout the observation was zero. The swept volume (Fig 4.6 (c)) remained fairly constant over the whole period of observation, although the beat frequency (Fig 4.6 (a)) and the beat amplitude (Fig 4.6 (b)) become more variable after the first 35 minutes. The mean swept volume for a sample period between minutes 26 and 36 was

Figs 4.6 to 4.11

The response of E.modestus and B.balanoides stage IV nauplii to the presence of algal suspensions.

Measures of (a) antennal beat frequency (Hz), (b) antennal beat amplitude (μm) and (c) antennal swept volume (mm^3) have been plotted against time from the start of the observation. The concentration of algae in the dish (calculated from eq.(3)) has been shown at the bottom of the graphs.

Fig 4.6 Control, E.modestus, 15°C, no algae.

Fig 4.7 E.modestus, 20°C, Skeletonema.

Fig 4.8 E.modestus, 15°C, Skeletonema.

Fig 4.9 E.modestus, 15°C, Seawater, Skeletonema.

Fig 4.10 B.balanoides, 15°C, Skeletonema.

Fig 4.11 B.balanoides, 10°C, Tetraselmis.

Fig 4.6

E.modestus stage IV at 15°C :- control.

Z time spent active in each minute (-)

(1)	78%	(6)	77%	(11)	77%	(16)	87%	(21)	88%	(26)	72%	(28)	83%
(29)	83%	(30)	83%	(31)	82%	(32)	93%	(33)	83%	(34)	82%	(35)	90%
(36)	93%	(37)	92%	(41)	93%	(46)	87%	(51)	92%	(56)	78%	(61)	95%
(66)	100%	(70)	100%										

Fig 4.6 Control observation from an *E.modestus* stage IV nauplius. 15°C

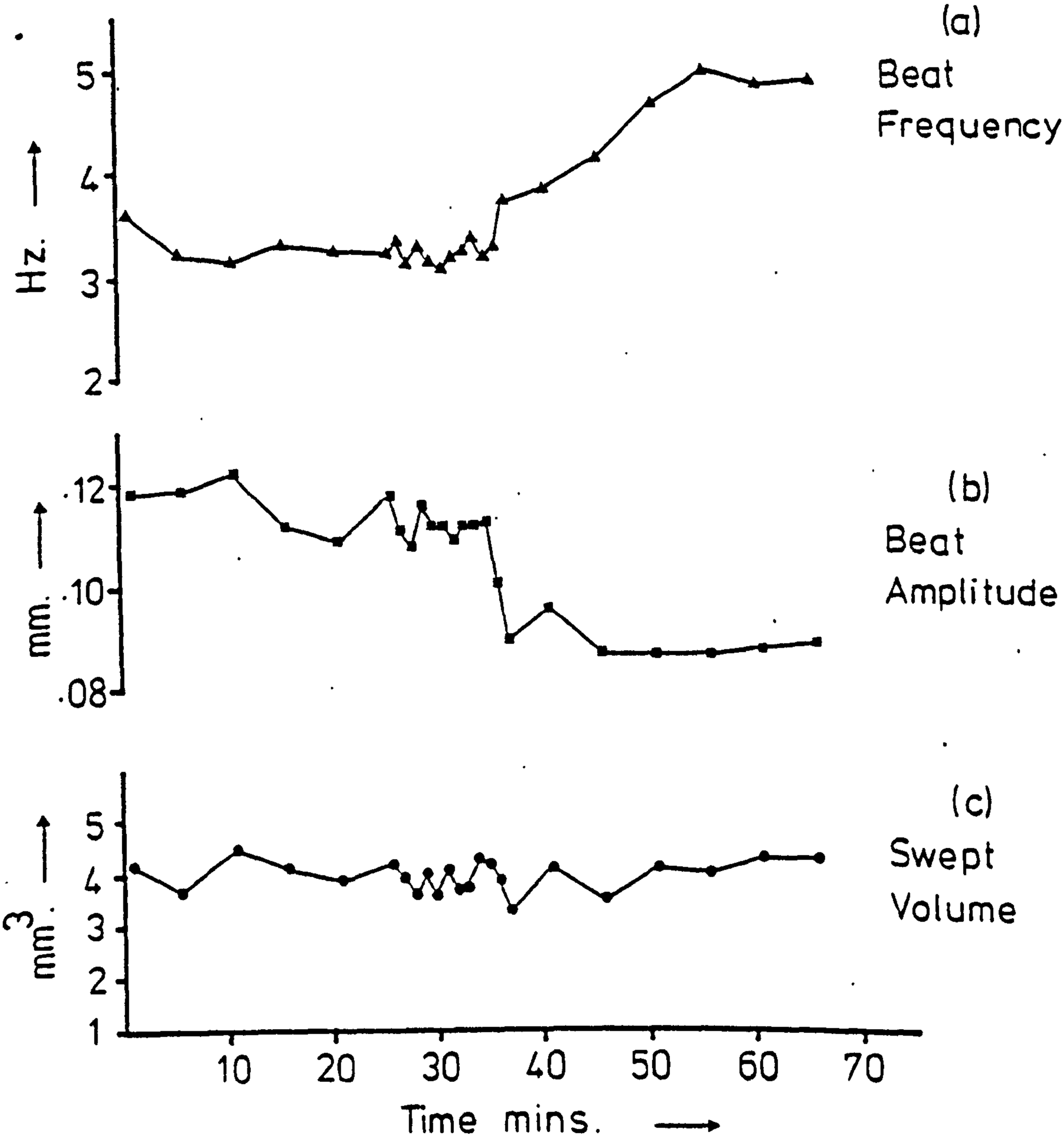


Fig 4.7

E.modestus stage IV at 20°C with Skeletonema injection to the seawater flow at minutes 23 and 53.

% time spent active in each minute (-)

(1) 77% (6) 80% (11) 63% (16) 80% (21) 78% (22) 80% (23) 77%

(24) 85% (25) 92% (26) 98% (27) 85% (28) 60% (29) 93% (30) 100%

(31) 93% (36) 68% (41) 58% (46) 90% (51) 93% (52) 77% (53) 97%

(54) 97% (55) 97% (56) 93% (57) 97% (58) 93% (59) 93% (60) 85%

(61) 90%

Fig 4.7 Antennal movement of an E.modestus S IV nauplius at 20°C in response to S.costatum

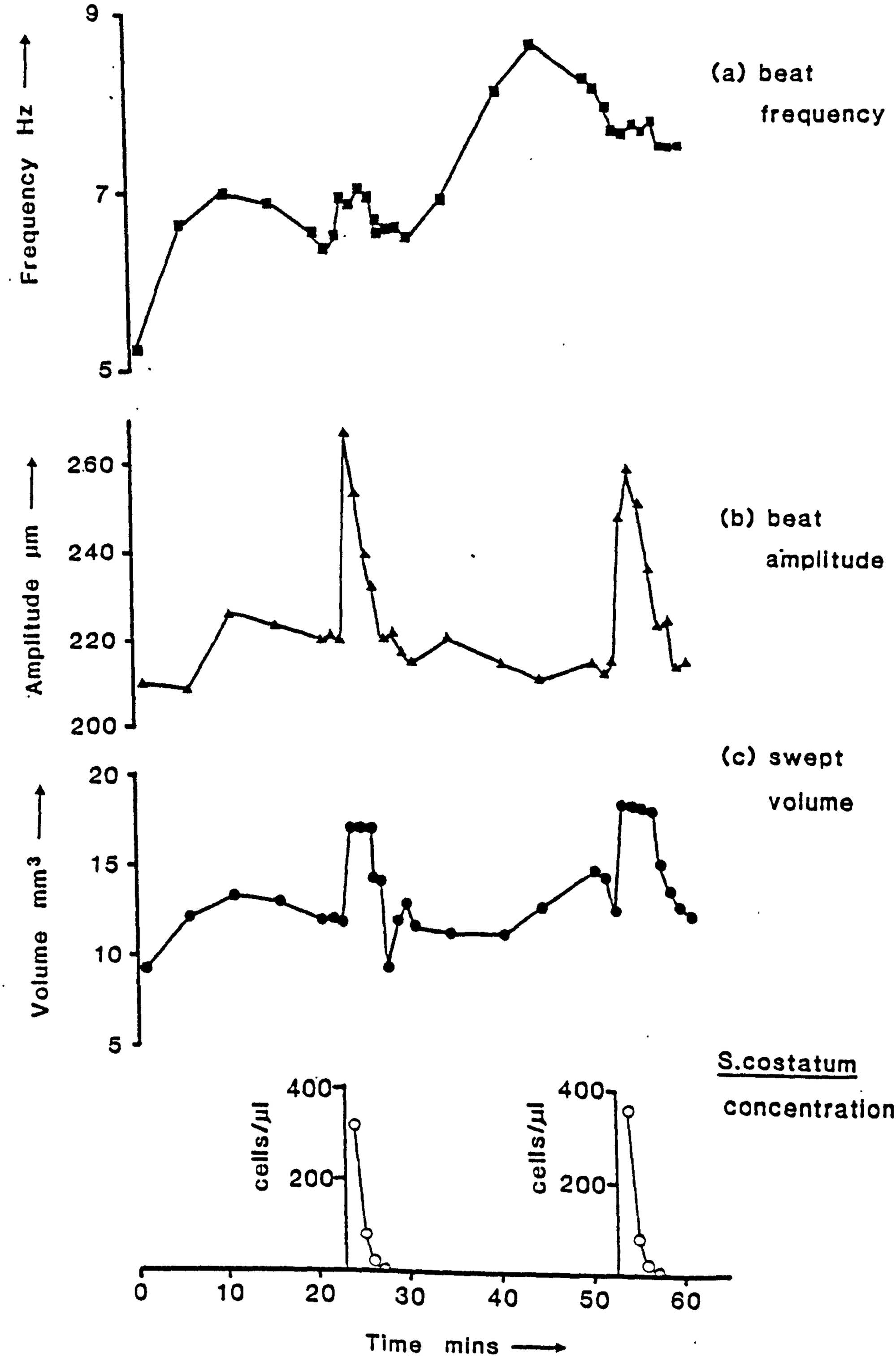


Fig 4.8

E.modestus stage IV at 15°C Skeletonema injection to seawater flow at minutes 26 and 60.

100% swimming activity except minute (6) = 90% active.

Fig 4.8 Antennal movement of an E.modestus S IV nauplius at 15°C, in response to S.costatum

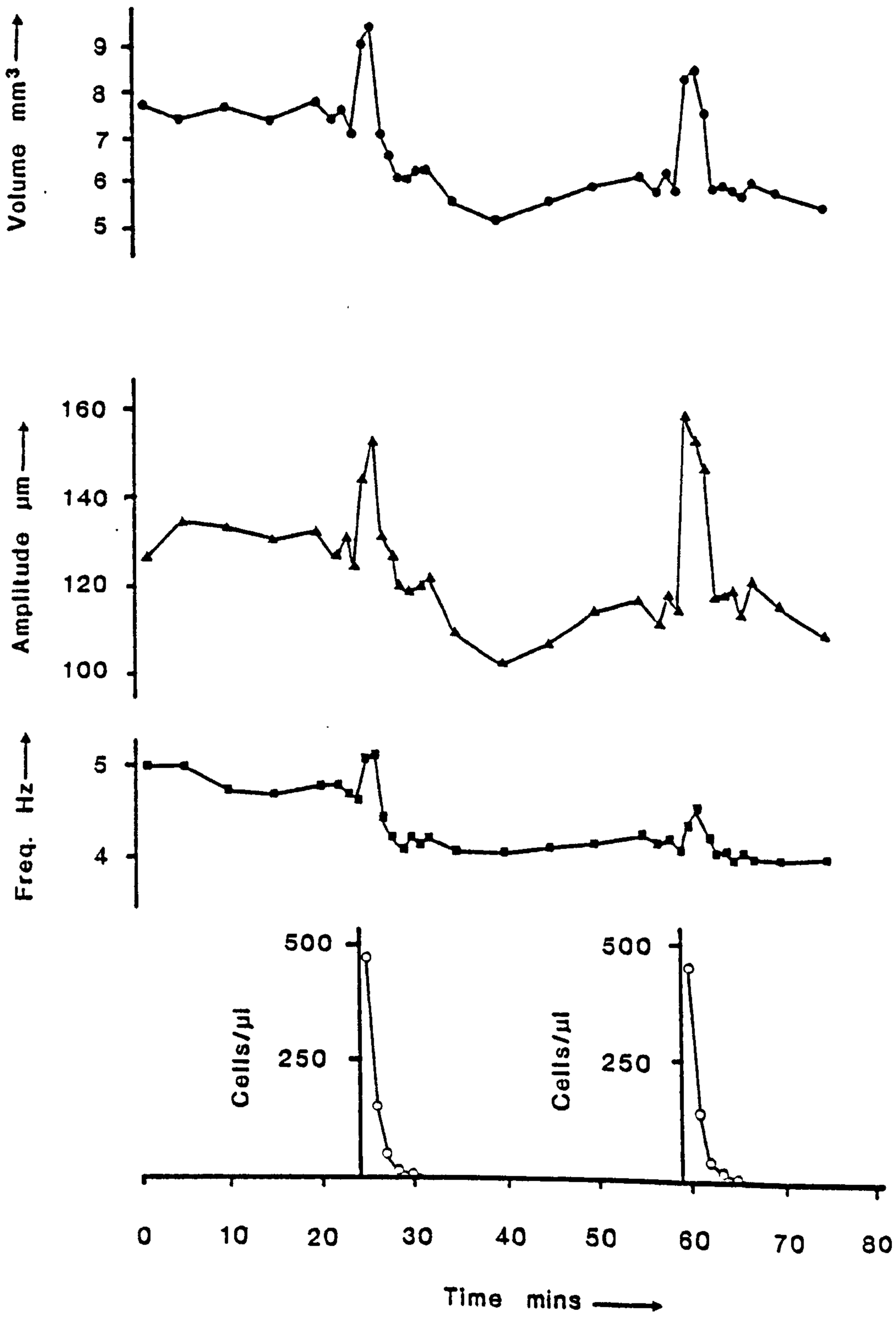


Fig 4.9

E.modestus stage IV at 15°C seawater control injection at minute 26, Skeletonema injection to seawater flow at minute 60.

No break in activity, 100% swimming by the nauplius throughout.

Fig 4:9 Antennal movement of an E.modestus S IV nauplius at 15°C in response to seawater and S.costatum

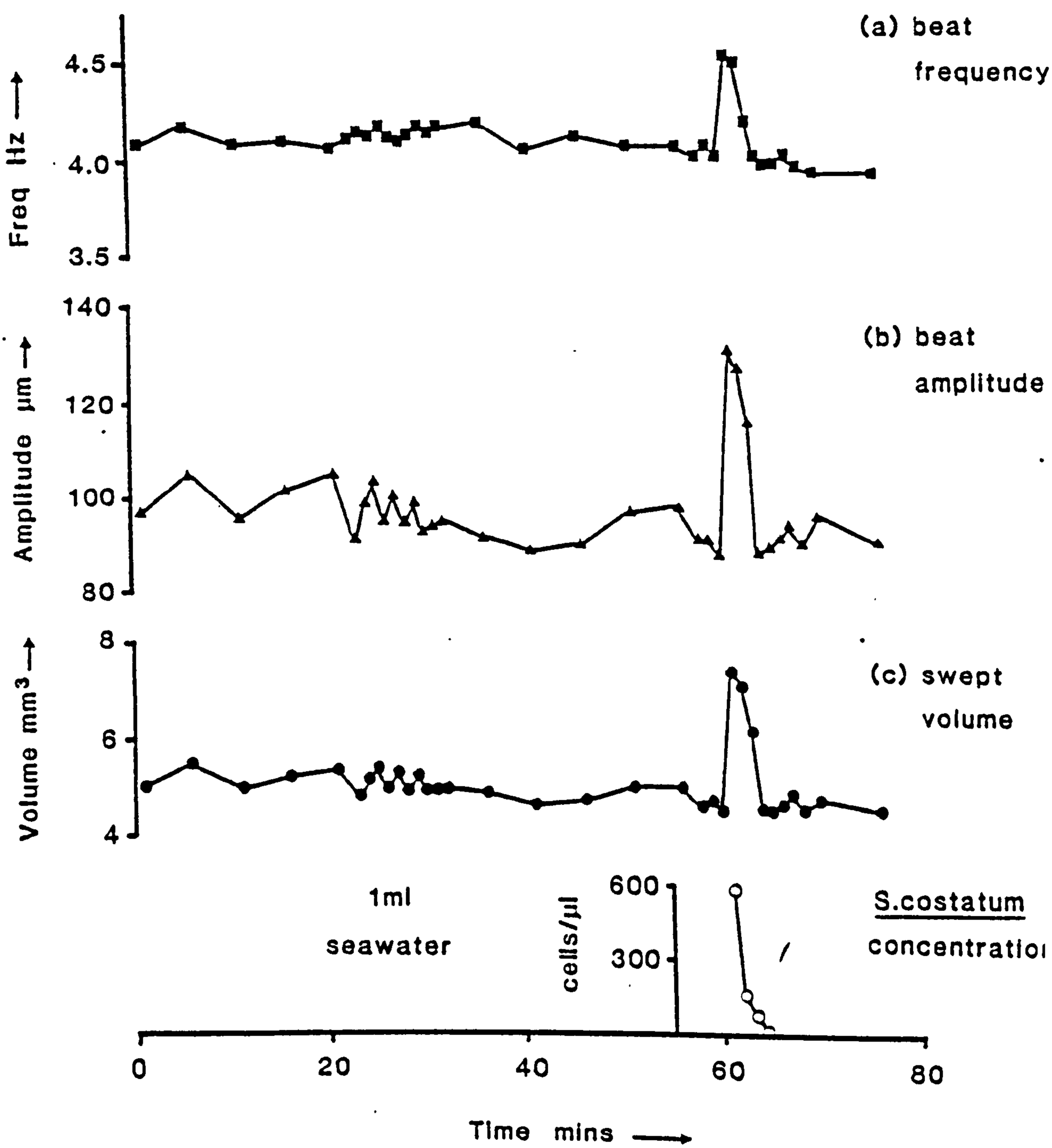


Fig 4.10

B.balanoides stage IV at 15°C with injections of Skeletonema to the seawater flow at minutes 25 and 60.

% time spent active in each minute (-)

(1) 50% (6) 88% (11) 95% (16) 60% (21) 95% (23) 95% (24) 88%

(25) 90% (26) 100% (27) 100% (28) 100% (29) 95% (30) 100% (31) 90%

(32) 95% (33) 73% (36) 40% (41) 95% (46) 95% (51) 68% (56) 50%

(58) 50% (59) 55% (60) 68% (61) 95% (62) 100% (63) 95% (64) 95%

(65) 95% (66) 95% (67) 78% (68) 80% (71) 95% (74) 100%

Fig 4.10 Antennal movement of a B.balanoides S IV nauplius at 15°C in response to S.costatum

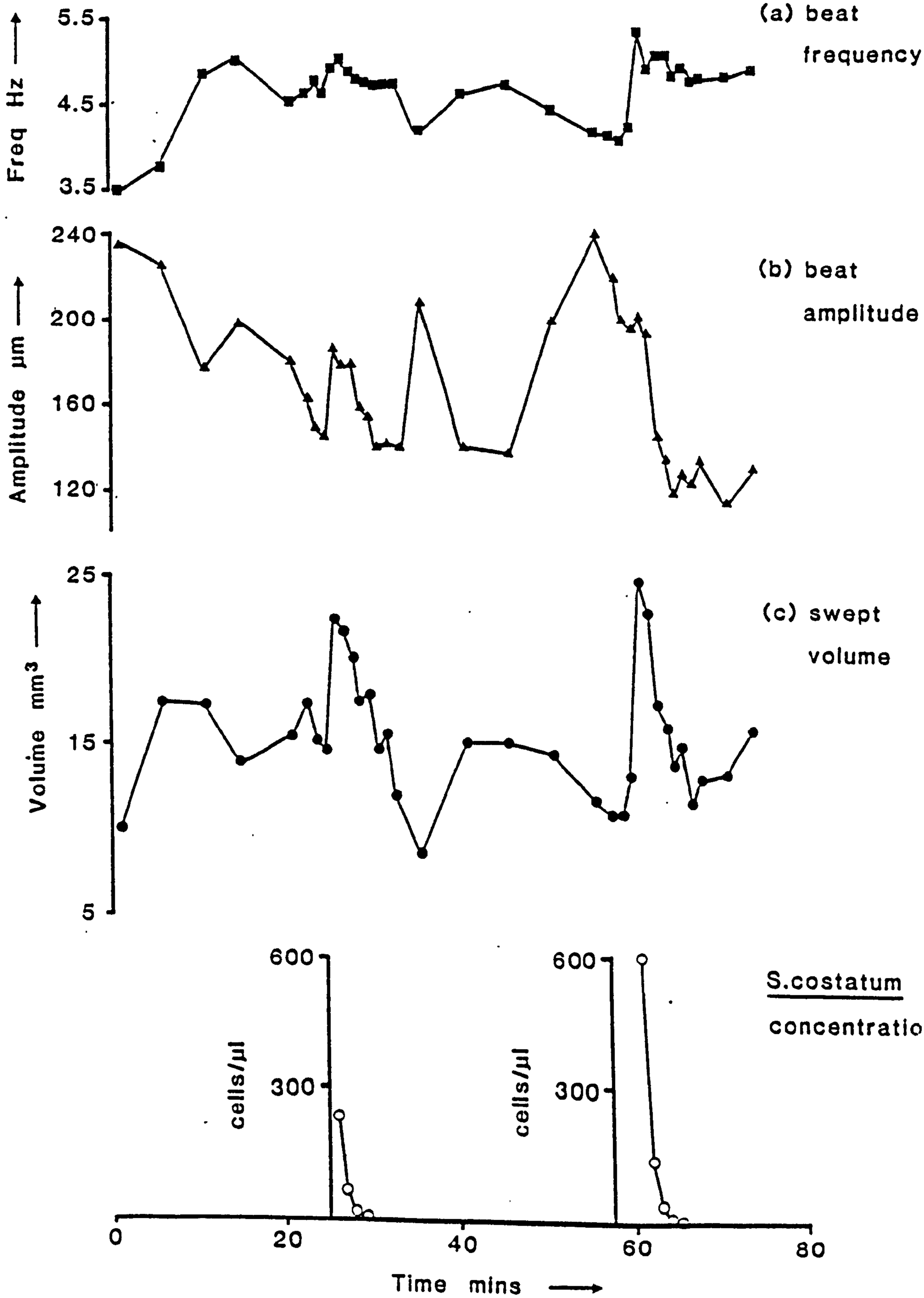


Fig 4.11

B.balanoides stage IV at 10°C with a Tetraselmis injection to the seawater flow at minute 21.

% time spent active in each minute (-)

(1) 70% (2) 70% (3) 70% (4) 63% (5) 55% (6) 70% (7) 63%

(8) 63% (9) 70% (10) 70% (11) 70% (12) 55% (13) 63% (14) 63%

(15) 70% (16) 55% (17) 65% (18) 70% (19) 63% (20) 63% (21) 75%

(22) 73% (23) 75% (24) 70% (25) 40% (26) 58% (27) 63% (28) 60%

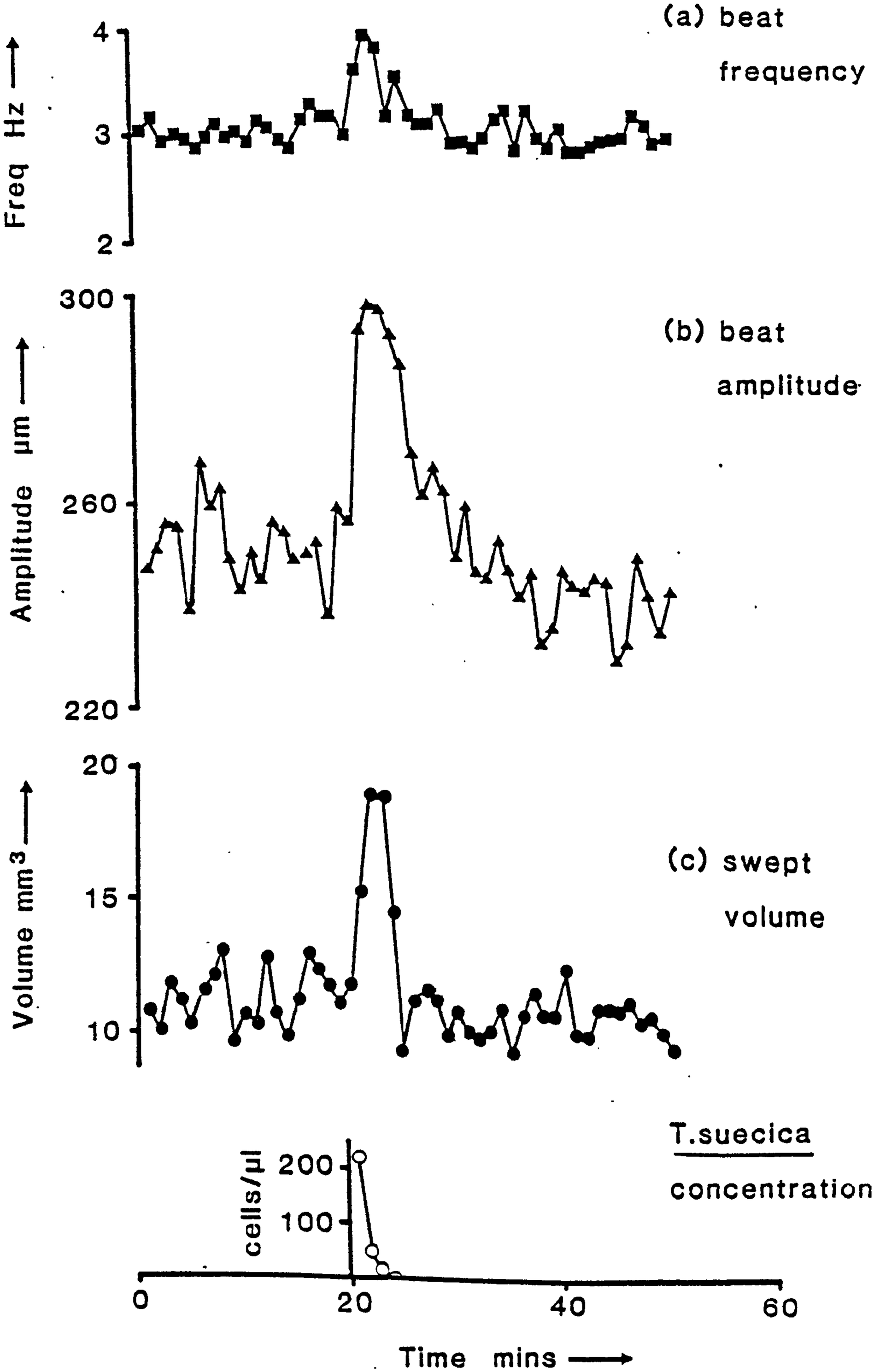
(29) 50% (30) 65% (31) 58% (32) 60% (33) 60% (34) 60% (35) 50%

(36) 68% (37) 63% (38) 68% (39) 68% (40) 73% (41) 63% (42) 63%

(43) 68% (44) 68% (45) 70% (46) 70% (47) 58% (48) 63% (49) 65%

(50) 58%

Fig 4.11 Antennal movements of a B.balanoides S IV at 10°C in response to T.suecica



found to be 3.94±0.436 mm^3 (± standard error). For the remaining period of observation the mean swept volume was found to be 4.00±0.22 mm^3 (± standard error). Using the small sample t-Test the calculated value of t for the difference between the means was 0.3464 for 21 degrees of freedom. The means did not, therefore, differ significantly, the probability of obtaining the observed difference by chance being 0.7325.

Table 4.4 shows a similar type of analysis for the remaining five Figs (4.7 -4.11). The table shows the mean and standard error of the mean of the swept volume for the periods when algae were present in the observation dish, and for the remainder of the observation period. Comparisons of the respective mean values (with and without algae present) were calculated by small sample t-Test and the resultant t-values and the probability associated with that value are shown in Table 4.4.

In all cases, except for that depicted in Fig 4.8, there was a highly significant increase in the swept volume when algae were present in the seawater. It can be assumed, therefore, that the volume of water handled by the nauplii also increases in the presence of food algae. The result for Fig 4.8 shows no significant difference between the swept volume in the presence of algae and the mean swept volume in the absence of algae. Fig 4.8 does, however, show that beat frequency, amplitude and swept volume do increase just after the injection of algae. Unlike the other records the response of this animal did not appear to be as prolonged, and the swept volume returned to a level similar to that before the injection while algae were still present in the observation chamber. If, instead of using the full time period of algal presence in the chamber to calculate the mean swept volume as has been done for Table 4.4, the swept volume for the first

TABLE 4.4

FIG	TEMP. °C	INJECTION	MEAN SWEPT VOLUME	STANDARD ERROR	t-VALUE	D.F.	p
4.7	20	None	11.70	± 1.60 mm^3	4.3180	27	0.001
		Skeletonema	17.20	± 1.10 mm^3			
4.8	15	None	6.30	+ 0.90 mm^3 −	1.6195	32	0.115
		Skeletonema	7.70	+ 1.03 mm^3 −			
4.9	15	Seawater	5.10	+ 0.31 mm^3 −	0.2994	27	0.767
		None	4.90	± 0.35 mm^3	4.8338	27	0.001
		Skeletonema	6.25	± 1.50 mm^3			
4.10	15	None	13.84	± 1.00 mm^3	4.0222	32	0.001
		Skeletonema	20.15	+ 2.48 mm^3 −			
4.11	10	None	10.80	± 0.30 mm^3	9.9990	48	0.001
		Tetraselmis	16.70	± 3.28 mm^3			

Table 4.4 : Comparison of the mean swept volume for E.modestus (Figs 4.7 - 4.9) and B.balanoides (Fig 4.10 - 4.11) stage IV nauplii in the presence of algae or after an injection of seawater (Fig 4.10) with the control mean swept volume. The comparison was made by small sample t-Test. D.F. = degrees of freedom for the t-Test and p = the probability of obtaining the observed difference by chance.

two minutes (25 - 26) after the first injection and the first three minutes (60 - 62) after the second injection, were used to calculate the mean swept volume in the presence of algae, then a different result was obtained. The mean swept volume for such a truncated period of algal presence was 8.5±0.83 mm^3. The comparison by small sample t-Test of this figure for swept volume in the presence of algae with that for the remaining period without algae (6.3 ± 0.9 mm^3, Table 4.4) gives a t-value of 2.1067 for 29 degrees of freedom. The probability of obtaining the observed difference between the means by chance was therefore 0.044. Thus, even for the animal represented by the plots in Fig 4.8 there was a significant increase in the swept volume just after the introduction of algae.

Fig 4.9 shows an analysis of the limb beat movements of an E.modestus stage IV nauplius at 15°C. It was in part a control observation as the first injection to the system was of filtered seawater whilst the second was of the diatom Skeletonema. The increase in swept volume in the presence of algae is clearly shown in Fig 4.9 and no increase is shown after the injection of seawater. Table 4.4 shows that there was no significant difference between the mean swept volume following the injection of seawater and of that in the remainder of the observation period, excluding the period following the algal injection. A highly significant increase in the swept volume was demonstrated in the period following the algal injection.

Table 4.5 shows the algal concentration, swept volume, and the percentage increase in swept volume, in the presence of algae, over the mean swept volume when no alga was present in the seawater, for each minute after an injection of Skeletonema. The data were plotted in Fig 4.12 as percentage increase in swept volume against algal concentration in cells per

TABLE 4.5

Species	Algal Conc^{n}. (cells / µl)	Swept Volume Without Algae (mm^3)	Swept Volume With Algae (mm^3)	% increase in Swept Volume
E.modestus				
	586.32	4.9	7.4	51.0
	453.42	6.3	8.9	41.3
	437.79	6.3	8.3	31.7
	359.61	11.7	18.0	53.8
	323.12	11.7	17.0	45.3
	136.34	4.9	7.1	44.9
	105.43	6.3	9.4	49.2
	101.80	6.3	8.5	34.9
	83.62	11.7	17.8	52.1
	75.14	11.7	16.6	41.9
	38.12	4.9	6.1	24.5
	29.48	6.3	7.1	12.7
	28.46	6.3	7.5	19.1
	23.38	11.7	17.7	51.3
	21.01	11.7	16.4	40.2
	10.66	4.9	4.5	--
	8.24	6.3	6.6	5.0
	7.69	6.3	5.8	--
	6.54	11.7	17.7	51.3
	5.87	11.7	14.3	22.2
B.balanoides				
	594.14	13.8	24.2	75.4
	234.53	13.8	22.2	61.0
	138.15	13.8	22.5	63.0
	54.53	13.8	21.1	52.9
	38.62	13.8	20.0	44.4
	15.25	13.8	17.0	23.2
	10.80	13.8	17.5	26.8
	4.26	13.8	16.1	16.7

Table 4.5 : The percentage increase in swept volume over control for E.modestus and B.balanoides stage IV nauplii in the presence of the diatom Skeletonema costatum.

percentage of time spent swimming by the larvae, could be increased to increase the swept volume. The results suggest that all three strategies were used by the nauplius. In all cases, the presence of algae in the water caused an increase in beat amplitude. An increase in beat frequency tended to be small and was not shown in every case. The percentage time spent swimming by the nauplii was increased more often by B.balanoides than by E.modestus as a means of increasing the swept volume. Section 2 above showed that over the temperature range of these observations E.modestus spent a greater percentage of time swimming in clean seawater than did B.balanoides. B.balanoides may, therefore, have had more scope to increase the time it spent swimming than did E.modestus.

concentration as high as 600 cells per µl.

As has been shown above (Section 3) the movements of the antennae of barnacle nauplii play an important role in the capture of algal food. The antennae are also the principal organs of locomotion by which the larvae maintain their position in the water column. The need to retain position in the water column is still apparent when the nauplii feed, hence it could be expected that the amount of water handled by the larvae would increase if both tasks were performed at the same time. As the nauplii appear to less than maximally increase their swept volume at algal concentrations below 100 cells per µl, it follows that less energy is expended in feeding at low concentrations than at higher ones. It could be suggested that the energy return from a maximal increase in water movement at low algal concentrations would not be sufficient to offset the increased energy expenditure by the larvae in producing a maximal increase in water handled. The feeding of the barnacle nauplius may thus be linked to the algal availability in such a way as to maximise the net energy gain to the larvae in a similar manner to that postulated for the feeding strategies of some copepods by Frost (1972) and Lam and Frost (1976).

Adams and Steele (1966) suggested that the feeding strategy of Calanus, demonstrated during shipboard experiments, could best be explained if the copepods were able to adjust their feeding speed, although they offered no suggestion as to how the animals might do so. The barnacle nauplius has been shown to alter the amount of water handled when algae are present in the water and, therefore, at a time when the larvae could be expected to be feeding. The increase in swept volume demonstrated could be achieved by the larvae in several ways. The limb beat amplitude, frequency, or the

µl. Squares represent the results for stage IV E.modestus larvae whilst the dots represent those for stage IV B.balanoides larvae. The results for both species show that the amount of water moved by the larvae increases with increasing concentration of algae up to about 100 cells per µl. The swept volume reaches a plateau beyond 100 cells per µl. and does not appear to alter with increasing algal concentration, at least up to 600 cells per µl. Although there are fewer data points for B.balanoides the general form of the curve is the same for both species. It would appear, however, that the maximum percentage increase in swept volume for B.balanoides may be slightly higher than that shown by E.modestus.

Discussion

The above results suggest that when the nauplius larvae of B.balanoides and E.modestus encounter patches of algae the average amplitude, and often the frequency of antennal beat, increases. This increase results in an increase in the volume through which the antennae move and hence a proportional increase in the volume of water moved by the nauplii. The larvae of the two barnacle species were shown to move more water in the presence of a range of algal species than they do in clean seawater.

Furthermore, the concentration of the algae appears to affect the extent to which either species will increase the amount of water handled. The percentage increase in swept volume over control (Fig 4.12) shows that at low algal concentrations the nauplii do not maximally increase their swept volume. Only when the algal concentration approaches 100 cells per µl is the maximal increase shown. The maximal increase in the swept volume was approximately 50% of the control level and was maintained at algal

Fig 4.12 Increase in the volume swept by the antennae of barnacle nauplii in response to S.costatum.

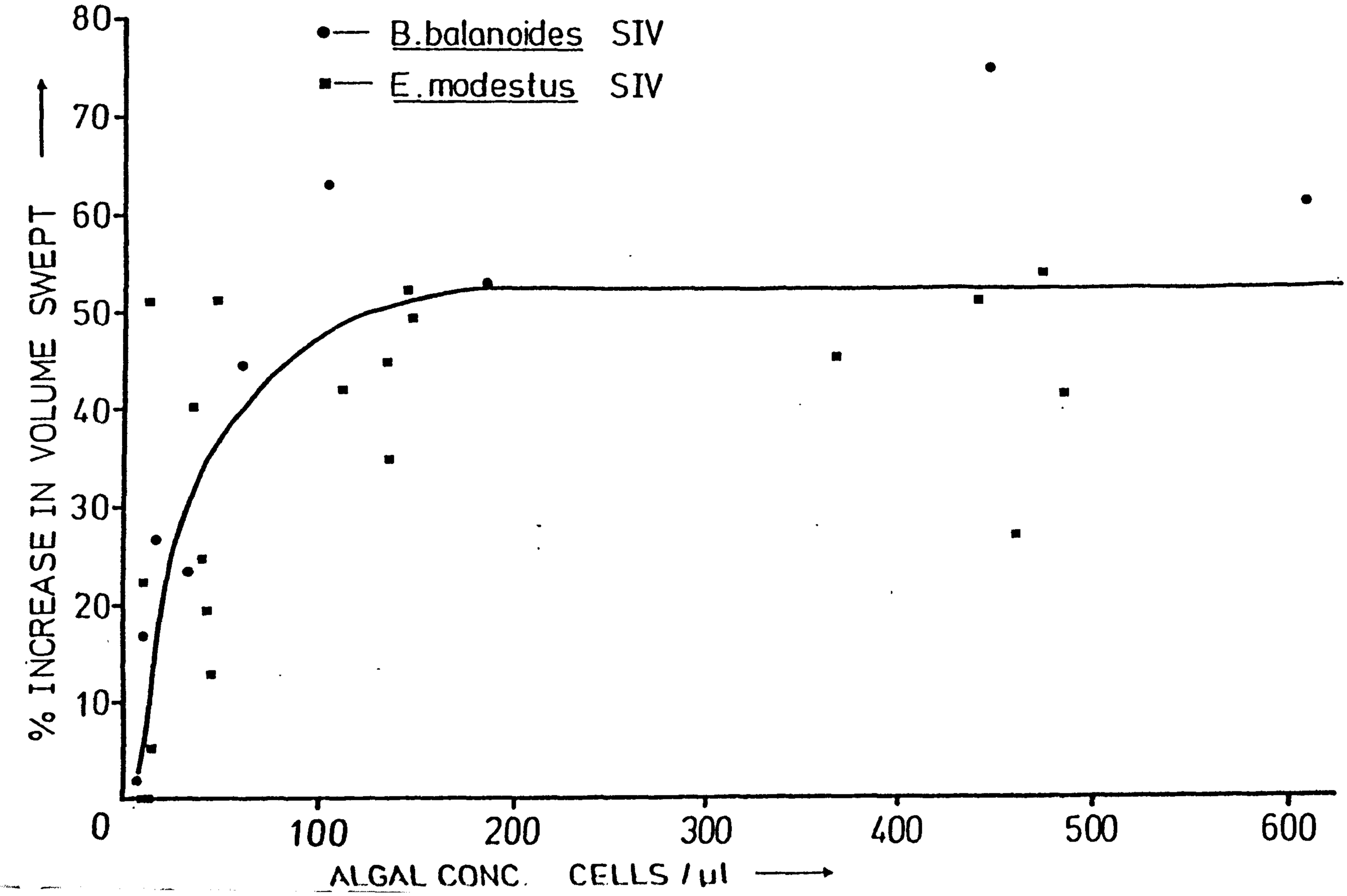


Fig 4.12

The percentage increase over controls for the volume swept by the antennae of B.balanoides (●) and E.modestus (■) stage IV nauplii, plotted against the concentration of S.costatum in the observation chamber. The curve was fitted to the data points by eye.

Section 5

The Response of E.modestus Nauplii Presented with Various Dissolved Organic Substances

The Response of E.modestus Nauplii Presented with Various Dissolved Organic Substances.

Introduction

Observations have been made on substances which evoke feeding responses in adult barnacles (Crisp 1967, Allison and Dorset 1977). Incremental concentrations of dissolved organic substances, such as amino acids and sugars, were presented to adult barnacles and the threshold for the feeding response, normally based on enhanced cirral activity (Crisp and Southward 1961), was measured. No comparable study has been made with barnacle nauplii. The techniques for handling and observation of these small organisms have always presented too great a stumbling block to detailed experimentation.

The use of a suction restraint technique and a flowing seawater observation system now makes such observation possible. In Section 4 above it was shown that the amount of water handled by the antennae of E.modestus and B.balanoides nauplii was increased in the presence of food organisms. It was suggested that the increase in antennal movement may represent a type of feeding response and that the magnitude of this response may be correlated with the concentration of food algae present. The investigation reported below was performed to ascertain whether dissolved amino acids and sugars could cause an increase in the amount of water handled by E.modestus stage IV nauplii similar in magnitude to the increases demonstrated in the presence of food algae.

Methods

A series of concentrations of four organic substances were made up in fresh, fine-filtered seawater which had been irradiated with ultra-violet light to discourage bacterial growth. The disaccharide sugar Sucrose was prepared in concentrations within the range 1.46×10^{-4}M to 2.92×10^{-3}M, the monosaccharide sugar Glucose from 1.25×10^{-4}M to 3.5×10^{-3}M and the amino acids Glutamic acid and Glycine from 1.33×10^{-4}M to 3.8×10^{-3}M and 1.25×10^{-4}M to 3.57×10^{-3}M respectively.

Stage IV Elminius modestus nauplii, which had been reared in the laboratory (See 'Methodology' above), were restrained in a flowing seawater system (Fig 1.2 'Methodology') with the nauplii facing into the seawater current. A fine plastic tube was drawn out to an internal diameter of approximately 80 μm. and held in a micromanipulator. The other end of the tube was attached to a standard agla syringe. The fine end of the tube was then easily manouvered into a position in front of, and slightly above, the restrained nauplius.

Initially, Methylene Blue dye was delivered from the syringe to ascertain that the dose supplied completely covered the nauplius and was then quickly lost from the observation dish, without mixing with the bulk of the seawater in the chamber. The dose was not delivered as a jet from the plastic delivery tube but as a droplet which bathed the nauplius for a short time while being carried in the current towards the outlet pipe. When the test routine had been performed satisfactorily the syringe was filled with each concentration of each substance in turn and small doses offered to the larvae. With each change in concentration or substance, the syringe and

plastic tube were washed thoroughly with distilled water and then with fresh seawater.

The experimental routine was as follows. A stage IV E.modestus larva was attached to the suction tube and allowed at least 45 minutes settling time in a seawater flow of 0.5 - 0.75 cm s^{-1}. The temperature within the observation chamber was maintained at approximately 20°C and checked periodically by thermistor. The behaviour of the nauplius was recorded on video tape (See 'Methodology' above) for one minute, then a small dose of a particular concentration of one of the substances was presented. The behaviour of the nauplius was then recorded for one minute following the presentation. A minimum of five minutes was allowed, before the procedure was repeated. After five repetitions the concentration of presented substance was changed and the procedure repeated until all the concentrations of that substance had been tested. Finally, using the same animal and procedures, doses of fresh, fine-filtered seawater were presented to the larva as a control experiment. New animals were used for each new substance.

The results for sucrose are somewhat different, in that three animals were used instead of one. This occurred because two of the animals came off the suction tube after only one complete run at one concentration and another came off after only two runs.

The behaviour of the nauplii was analysed from the video recordings as described in 'Methodology' above. In each recorded minute 15 measures of antennal beat frequency were taken by stopwatch and 15 measures of antennal beat amplitude measured by ruler. The number of breaks in swimming in each

minute were timed. The results for each concentration, therefore, consisted of six groups of 15 frequency and 15 amplitude measurements, along with the respective duration of breaks in normal swimming. These measurements were then combined, as described in Section 4 above, to obtain the antennal swept volume and thus an estimate of the amount of water handled by the nauplii.

Results

The initial results are shown in Tables 5.1 - 5.4, where the mean frequency and amplitude of antennal beat along with their associated standard errors is given for each substance used. The means before and after presentation were compared by small sample t-Tests. The t-value for the comparison and the probability that the means were drawn from the same population of means is given. A * next to a probability figure shows that there was a significant difference between the mean value before, and that after, the presentation. Not all significant results reflect an increase after presentation, thus a positive t-value shows a decrease and a negative t-value shows an increase in the mean value after presentation. In general significant increases in beat frequency were confined to the higher concentrations used. Significant increases in beat amplitude were more widespread and appear to be the major contributor to the response.

The results show that at concentrations of Glycine, Glutamic acid, Glucose and Sucrose, in the order of 10^{-4} to 10^{-3} M, an increase in beat amplitude occurred accompanied by an increase in beat frequency. Sucrose is the least effective, significant increases in amplitude were found only above 10^{-3} M. The variability in the sign of frequency and amplitude changes makes further interpretation of the initial results difficult (see

Table 5.1

conc^{n}	Before presentation			After Presentation			t-Value		Probability	
x10^{-3}M	Freq.Hz	Ampl.µm	St	Freq.Hz	Ampl.µm	St	Freq	Ampl	Freq	Ampl
	5.01±0.09	134±3.2	12	5.21±0.07	152±3.5	3	+1.639	+3.407	0.113	0.002*
	4.76±0.06	135±2.8	4	5.31±0.09	155±2.2	4	+5.198	+5.661	0.001*	0.001*
3.8	5.06±0.05	126±2.1	2	5.38±0.09	141±4.6	4	+3.109	+2.889	0.004*	0.007*
	4.95±0.04	119±2.7	6	5.50±0.17	139±4.4	2	+3.161	+4.063	0.004*	0.001*
	4.90±0.07	115±1.7	11	5.18±0.11	131±3.6	4	+2.126	+4.013	0.042*	0.001*
	5.06±0.05	110±1.6	1	5.24±0.07	123±3.0	2	+2.062	+3.792	0.049*	0.001*
	5.10±0.09	114±3.1	5	5.14±0.11	126±3.0	3	+0.230	+2.769	0.820	0.010*
1.84	5.04±0.03	109±2.7	4	5.06±0.07	127±2.7	4	+0.278	+4.773	0.783	0.001*
	4.98±0.05	103±2.7	6	5.37±0.05	120±3.2	2	+5.466	+4.009	0.001*	0.001*
	5.12±0.06	103±2.1	4	5.45±0.06	123±3.5	2	+3.815	+4.790	0.001*	0.001*
	7.65±0.05	87±2.1	8	7.60±0.04	125±6.0	11	-0.722	+5.932	0.477	0.001*
	7.55±0.03	85±1.6	10	7.60±0.04	114±5.4	10	+1.510	+5.129	0.142	0.001*
0.74	7.09±0.04	94±2.9	8	7.12±0.07	119±5.5	8	+1.050	+4.081	0.303	0.001*
	7.42±0.08	149±4.4	15	7.33±0.06	181±7.5	12	-0.865	+3.666	0.394	0.001*
	7.34±0.07	151±3.2	10	7.37±0.08	183±5.7	14	+0.214	+4.899	0.831	0.001*
	5.18±0.04	101±2.4	4	5.25±0.05	118±2.8	4	+1.152	+4.830	0.259	0.001*
	4.91±0.08	106±1.9	4	5.21±0.05	108±2.6	3	+3.257	+0.620	0.003*	0.540
0.463	5.26±0.05	102±1.5	1	5.64±0.12	111±2.5	1	+2.843	+3.421	0.008*	0.003*
	5.34±0.06	101±2.4	5	5.26±0.09	112±3.1	2	-0.661	+2.690	0.514	0.012*
	5.57±0.08	104±2.3	2	5.71±0.08	115±2.6	0	+1.230	+3.153	0.229	0.004*
	5.18±0.04	105±2.1	3	5.13±0.05	105±2.4	0	-0.737	+0.188	0.467	0.852
	5.29±0.07	105±3.1	1	5.44±0.08	105±3.5	0	+1.380	+0.043	0.179	0.916
0.133	5.43±0.06	100±1.3	4	5.51±0.06	100±2.2	4	+1.007	-0.129	0.322	0.898
	5.45±0.05	108±3.6	3	5.48±0.05	107±2.5	2	+0.434	-0.275	0.668	0.786
	5.06±0.07	132±2.9	5	4.81±0.07	145±3.1	10	-2.422	+3.060	0.022*	0.005*
	4.25±0.15	143±3.5	12	4.68±0.14	138±3.0	7	+2.138	-1.060	0.041*	0.298
cont.	5.36±0.10	121±1.4	2	5.57±0.07	117±1.7	6	+3.224	-2.012	0.003*	0.054
	6.03±0.03	112±1.9	3	6.07±0.05	108±1.7	2	+0.692	-1.396	0.494	0.174
	6.24±0.07	106±2.1	8	6.26±0.07	104±1.7	3	+0.212	-0.533	0.833	0.598

Table 5.1 : The mean frequency and amplitude (± standard error) of the antennal beat of E.modestus nauplii before and after presentation with Glutamic acid. Freq = frequency (Hz). Ampl = amplitude (µm). St = duration of swimming breaks. t-Values are for small sample t-test on mean beat parameters (after the stimulus - before) and probability = the probability of obtaining the observed differences by chance. cont. = seawater control.

Table 5.2

conc^{n}	Before Presentation			After presentation			t-Value		Probability	
x10^{-3}M	Freq.Hz	Ampl.µm	St	Freq.Hz	Ampl.µm	St	Freq	Ampl	Freq	Ampl
	3.79±0.03	125±2.7	2	4.17±0.10	148±3.7	1	+3.420	+5.159	0.002*	0.001*
	3.83±0.03	126±1.9	2	4.12±0.06	150±4.2	2	+4.210	+5.240	0.001*	0.001*
3.75	3.88±0.03	118±2.2	0	4.23±0.07	141±4.7	1	+4.516	+4.379	0.001*	0.001*
	4.18±0.06	119±2.1	1	4.51±0.09	145±3.8	2	+3.090	+5.899	0.004*	0.001*
	4.36±0.03	114±3.0	1	4.67±0.12	137±3.7	2	+2.490	+4.700	0.019*	0.001*
	5.31±0.15	104±3.4	7	5.23±0.20	125±2.7	6	−0.305	+4.823	0.763	0.001*
	5.55±0.15	107±3.8	8	5.95±0.26	135±3.5	10	+1.322	+5.498	0.197	0.001*
1.67	6.78±0.10	120±2.1	11	6.78±0.09	136±4.6	16	+0.059	+3.293	0.953	0.004*
	6.76±0.09	113±2.9	6	6.59±0.10	127±2.5	10	−1.208	+3.710	0.237	0.001*
	6.52±0.05	128±2.1	6	6.44±0.07	149±2.2	0	−1.046	+7.003	0.304	0.001*
	4.15±0.06	125±3.2	4	4.24±0.09	150±3.4	0	+0.795	+5.269	0.433	0.001*
	4.27±0.06	132±2.0	1	4.47±0.05	148±2.6	2	+2.648	+4.958	0.013*	0.001*
0.862	4.46±0.03	128±1.9	1	4.84±0.14	144±3.4	2	+2.690	+4.275	0.012*	0.001*
	4.40±0.05	126±1.7	0	4.99±0.10	144±2.8	5	+5.233	+5.669	0.001*	0.001*
	4.49±0.03	134±2.1	2	5.07±0.11	142±4.2	5	+4.917	+1.816	0.001*	0.080
	4.63±0.04	130±2.3	4	4.58±0.05	144±2.4	0	−0.671	+4.108	0.508	0.001*
	4.58±0.04	125±1.6	0	4.75±0.06	144±2.2	5	+2.458	+7.044	0.020*	0.001*
0.435	4.42±0.05	128±2.5	0	4.77±0.10	147±3.1	3	+3.225	+4.774	0.003*	0.001*
	4.35±0.04	130±2.0	0	4.94±0.13	146±3.6	1	+4.410	+3.862	0.001*	0.001*
	4.57±0.03	126±2.0	1	5.01±0.09	141±2.4	2	+4.531	+4.397	0.001*	0.001*
	4.49±0.03	129±1.7	0	4.64±0.11	128±3.5	0	+1.390	−0.136	0.176	0.893
	4.71±0.03	124±2.0	2	4.93±0.07	122±2.2	6	+2.893	−0.721	0.007*	0.477
0.125	4.76±0.03	130±2.2	0	4.54±0.05	129±1.9	0	−3.980	−0.437	0.001*	0.666
	4.63±0.05	127±1.7	0	4.75±0.11	126±2.0	1	+0.974	−0.602	0.338	0.552
	4.73±0.04	122±1.8	1	4.82±0.08	123±1.7	1	+1.041	+0.531	0.307	0.600
	5.38±0.05	122±1.6	4	5.30±0.05	123±2.2	0	−1.112	+0.546	0.276	0.589
	5.23±0.04	122±2.0	0	5.24±0.06	121±2.1	2	+0.133	−0.274	0.895	0.786
cont.	5.26±0.05	122±1.7	0	5.39±0.07	118±2.5	3	+1.533	−1.360	0.136	0.185
	5.16±0.04	121±2.3	0	5.16±0.04	120±1.5	1	−0.045	−0.406	0.965	0.688
	5.19±0.03	122±1.7	1	5.30±0.04	122±1.6	4	+2.207	+0.229	0.036*	0.821

Table 5.2 : The mean frequency and amplitude (± standard error) of the antennal beat of E.modestus nauplii before and after presentation with Glycine. Freq = frequency (Hz). Ampl = amplitude (µm). St = duration of swimming breaks. t-Values are for small sample t-test on mean beat parameters (after the stimulus - before) and probability = the probability of obtaining the observed differences by chance. cont. = seawater control.

Table 5.3

Conc^{n} x10^{-3}M	Before Presentation Freq.Hz	Ampl.μm	St	After Presentation Freq.Hz	Ampl.μm	St	t-Value Freq	Ampl	Probability Freq	Ampl
	6.35±0.04	103±1.9	4	6.58±0.16	136±3.6	6	+1.445	+8.288	0.160	0.001*
	6.58±0.06	102±2.3	3	6.74±0.07	124±2.6	0	+1.706	+6.070	0.099	0.001*
3.5	6.82±0.07	103±2.2	0	7.18±0.12	129±3.6	1	+2.624	+6.047	0.014*	0.001*
	7.07±0.10	104±2.0	2	7.45±0.07	135±1.7	1	+3.109	+11.83	0.004*	0.001*
	7.31±0.07	108±2.0	0	7.59±0.05	128±2.7	1	+3.150	+5.895	0.004*	0.001*
	7.53±0.08	108±2.5	2	7.62±0.08	127±3.0	1	+0.787	+4.962	0.438	0.001*
	7.54±0.06	106±2.1	0	7.65±0.05	122±3.0	1	+1.299	+4.731	0.205	0.001*
1.72	7.50±0.03	107±2.6	0	7.61±0.07	126±3.7	1	+1.499	+4.279	0.158	0.001*
	7.70±0.08	107±2.0	2	7.82±0.06	123±2.2	1	+1.180	+5.337	0.248	0.001*
	7.65±0.06	106±2.5	1	7.79±0.07	122±2.8	1	+1.506	+3.992	0.143	0.001*
	4.87±0.12	170±3.6	13	5.02±0.12	208±5.2	10	+0.888	+5.931	0.382	0.001*
	4.68±0.11	187±5.4	10	4.69±0.09	217±6.2	16	+0.094	+4.113	0.925	0.001*
0.694	4.57±0.04	128±3.9	9	4.67±0.03	146±4.4	4	+1.685	+3.055	0.103	0.005*
	4.81±0.04	132±2.5	8	5.08±0.07	149±3.9	2	+3.373	+3.625	0.002*	0.001*
	6.13±0.08	91±1.4	0	6.15±0.07	112±3.2	0	+0.184	+6.131	0.855	0.001*
	7.59±0.09	108±2.6	6	7.82±0.12	129±4.9	10	+1.514	+3.796	0.141	0.001*
	7.14±0.04	108±2.8	0	7.40±0.15	122±4.8	1	+1.678	+2.489	0.104	0.019*
0.435	7.30±0.04	107±2.6	1	7.33±0.13	132±4.9	13	+0.896	+4.366	0.851	0.001*
	6.37±0.07	130±2.6	12	6.67±0.21	139±3.9	3	+1.373	+1.905	0.181	0.067
	6.18±0.10	131±3.9	15	6.33±0.15	150±5.3	12	+0.836	+2.933	0.410	0.007*
	5.43±0.09	142±4.1	8	6.11±0.15	155±3.5	14	+3.891	+2.428	0.001*	0.022*
	5.77±0.07	135±3.1	0	5.75±0.20	153±3.5	14	−0.107	+4.020	0.916	0.001*
0.125	4.75±0.12	151±3.6	14	4.92±0.13	155±3.6	12	+0.994	+0.999	0.329	0.326
	4.81±0.11	159±2.5	7	4.75±0.11	163±3.4	10	−0.424	+1.302	0.675	0.311
	4.96±0.08	154±2.0	4	4.89±0.13	156±3.0	8	−0.464	+0.539	0.646	0.594
	5.34±0.07	148±3.4	3	5.33±0.05	150±3.0	2	−0.109	+0.473	0.914	0.640
	5.49±0.05	148±3.2	0	5.32±0.10	152±3.6	9	−1.522	+0.841	0.139	0.407
cont.	4.69±0.14	157±2.3	7	4.92±0.12	152±2.6	6	−1.257	−1.563	0.213	0.129
	4.87±0.06	156±2.6	5	4.91±0.13	157±2.5	10	+0.251	+0.488	0.804	0.629
	4.96±0.09	153±3.4	3	4.86±0.07	154±2.4	6	−0.902	+0.290	0.375	0.774

Table 5.3 : The mean frequency and amplitude (± standard error) of the antennal beat of E.modestus nauplii before and after presentation with Glucose. Freq = frequency (Hz). Ampl = amplitude (μm). St = duration of swimming breaks. t-Values are for small sample t-test on mean beat parameters (after the stimulus - before) and probability = the probability of obtaining the observed differences by chance. cont. = seawater control.

Table 5.4

Conc^{n} x10^{-3}M	Before Presentation Freq.Hz	Ampl.µm	St	After Presentation Freq.Hz	Ampl.µm	St	t-Value Freq	Ampl	Probability Freq	Ampl
	6.26±0.05	136±3.4	8	6.42±0.12	156±3.9	5	+1.264	+3.846	0.217	0.001*
	6.27±0.07	131±3.0	5	6.53±0.08	161±4.0	7	+2.498	+5.914	0.019*	0.001*
2.92	6.31±0.11	143±3.3	10	6.46±0.07	169±3.7	9	+1.147	+5.212	0.261	0.001*
	6.22±0.09	142±2.8	11	6.61±0.10	170±3.0	10	+2.950	+6.863	0.006*	0.001*
	6.22±0.07	146±4.3	10	6.75±0.10	182±2.8	14	+4.584	+7.007	0.001*	0.001*
	6.82±0.08	145±1.7	12	6.86±0.09	176±3.7	14	+0.290	+7.580	0.774	0.001*
	6.50±0.11	150±4.1	11	6.53±0.10	182±4.0	12	+0.209	+5.560	0.836	0.001*
1.46	6.70±0.07	151±3.9	15	6.55±0.09	173±2.7	14	-1.304	+4.556	0.209	0.001*
	6.49±0.07	143±3.5	10	6.98±0.07	156±4.1	18	+4.943	+2.469	0.001*	0.020*
	6.78±0.11	152±3.2	14	6.75±0.06	174±2.8	13	-0.291	+4.966	0.773	0.001*
	6.83±0.07	136±3.8	8	6.67±0.08	167±2.5	10	-2.438	+6.682	0.021*	0.001*
	6.96±0.06	174±3.1	15	6.86±0.07	204±5.6	14	-1.143	+4.691	0.263	0.001*
0.731	7.01±0.04	184±3.5	16	6.77±0.06	203±2.5	14	-3.279	+4.285	0.003*	0.001*
	6.88±0.07	181±2.4	16	6.74±0.07	210±5.3	17	-1.412	+5.063	0.169	0.001*
	6.99±0.04	180±2.6	14	6.72±0.08	205±4.3	19	-3.176	+4.853	0.004*	0.001*
	7.12±0.06	184±2.1	16	6.92±0.09	201±4.2	17	-1.842	+3.172	0.076	0.001*
	7.06±0.05	174±3.7	14	6.91±0.09	197±4.3	13	-1.434	+3.930	0.163	0.001*
0.365	7.18±0.06	171±3.4	16	6.96±0.08	189±4.7	17	-2.266	+3.164	0.031*	0.004*
	7.08±0.05	171±3.9	12	6.95±0.09	198±6.0	12	-1.293	+3.865	0.206	0.001*
	6.73±0.08	180±3.6	14	6.81±0.08	198±4.5	15	+0.778	+3.084	0.443	0.001*
	7.03±0.08	121±4.7	5	7.11±0.05	117±1.6	6	+0.910	-0.696	0.375	0.492
	7.01±0.07	114±2.2	4	6.94±0.07	116±1.9	4	-0.712	+0.565	0.483	0.577
0.146	6.96±0.08	112±1.3	2	6.78±0.09	113±2.4	2	-1.509	+0.142	0.142	0.683
	7.08±0.04	110±1.5	1	6.93±0.09	113±3.1	2	-1.510	+0.878	0.142	0.387
	7.05±0.05	114±2.1	6	7.02±0.05	113±1.8	4	-0.468	-0.194	0.643	0.848
	6.83±0.08	113±2.1	3	6.91±0.05	114±2.0	5	+0.821	+0.301	0.419	0.766
cont.	6.89±0.08	119±2.3	3	6.85±0.07	116±2.4	2	-0.248	-0.776	0.806	0.444
	6.85±0.08	115±2.1	1	6.94±0.07	114±2.4	2	+0.940	-0.252	0.355	0.803

Table 5.4 : The mean frequency and amplitude (± standard error) of the antennal beat of E.modestus nauplii before and after presentation with Sucrose. Freq = frequency (Hz). Ampl = amplitude (µm). St = duration of swimming breaks. t-Values are for small sample t-test on mean beat parameters (after the stimulus - before) and probability = the probability of obtaining the observed differences by chance. cont. = seawater control.

especially Table 5.4 for Sucrose). However, by combining frequency, amplitude and the amount of time spent swimming by the nauplius into a measure of the volume of water moved through by the antennae (the swept volume) a clear picture emerges.

Tables 5.5 - 5.8 give the results for each concentration of each substance of the swept volume per minute. Paired sample t-Tests were performed comparing the mean difference in swept volume before presentation with that after presentation. A probability (p) less than 0.05 indicates that the mean difference significantly exceeds zero. The results of Tables 5.5 - 5.8 are plotted in Figure 5.1. The mean difference in swept volume has been plotted against compound concentration. The plots for all the substances tested were very similar. They are reminiscent of a normal dose response curve where a rapid increase in response was observed with increasing concentration followed by a levelling off at higher concentrations. Sucrose was not as powerful in eliciting the response in E.modestus larvae as the three other compounds, no significant response below 1.4×10^{-3} M being detectable.

The highest concentration of any compound used during the investigations was 3.8×10^{-3}M for glutamic acid. A solution of this strength would have an osmotic concentration of approximately 4 milliosmoles. The osmotic concentration of seawater at 34ppt. is close to 1000 milliosmoles. A change in salinity of 1 ppt. would thus alter the osmotic concentration of the seawater by some 7 - 8 times more than the highest concentration of organic compounds used during this investigation. The control presentations of seawater and the associated mechanical effects never elicited a significant response in swept volume from the larvae

Table 5.5

Conc $x10^{-3}$		Swept Volume mm^3					Mean Diff.	St Error	Paired t-value	prob
	Before	6.7	7.3	6.6	5.7	7.7				
3.8	After	9.1	9.2	9.1	7.8	8.7	+1.98	0.267	7.410	0.002
	Difference	+2.4	+1.9	+2.5	+2.1	+1.0				
	Before	6.9	6.7	6.5	5.8	6.2				
1.84	After	7.8	7.7	7.4	7.8	8.1	+1.34	0.250	5.356	0.006
	Difference	+0.9	+1.0	+0.9	+2.0	+1.9				
	Before	7.4	6.7	7.4	10.0	11.1				
0.739	After	9.6	9.1	9.3	12.2	11.9	+1.90	0.286	6.635	0.003
	Difference	+2.2	+2.4	+1.9	+2.2	+0.8				
	Before	6.2	6.1	6.7	6.3	7.1				
0.463	After	7.2	6.8	7.8	7.2	8.2	+1.00	0.078	12.91	0.001
	Difference	+1.0	+0.7	+1.1	+1.1	+1.1				
	Before	6.5	6.9	6.4	7.1					
0.133	After	6.8	7.2	6.5	7.3		+0.23	0.048	4.700	0.018
	Difference	+0.3	+0.3	+0.1	+0.2					
	Before	7.6	6.0	7.8	8.0	7.3				
cont.	After	7.1	7.0	7.4	8.0	7.8	+0.12	0.282	2.129	0.101
	Difference	-0.5	+1.0	-0.4	0.0	+0.5				

Table 5.5 Paired sample t-Test analysis of the calculated swept volume (mm^3) for *E.modestus* nauplii After - Before the presentation of Glutamic acid. Mean diff. = mean difference, St error = standard error and prob = the probability that the magnitude of the mean difference will exceed zero by chance. cont. = seawater control.

Table 5.6

Conc $x10^{-3}$		Swept Volume mm^3					Mean Diff.	St Error	Paired t-value	prob
3.57	Before	5.7	5.8	5.7	6.0	6.1	+1.60	0.045	35.78	0.001
	After	7.4	7.4	7.2	7.7	7.6				
	Difference	+1.7	+1.6	+1.5	+1.7	+1.5				
1.67	Before	6.2	6.4	6.1	8.6	9.3	+1.56	0.438	3.564	0.024
	After	7.3	8.2	8.6	8.7	11.6				
	Difference	+1.1	+1.8	+2.5	+0.1	+2.3				
0.862	Before	6.0	6.8	7.0	6.9	7.2	+1.18	0.139	8.472	0.001
	After	7.7	7.8	8.2	8.0	8.1				
	Difference	+1.7	+1.0	+1.2	+1.1	+0.9				
0.435	Before	7.0	7.1	7.0	7.0	7.2	+1.06	0.175	6.060	0.004
	After	8.0	7.6	8.1	8.6	8.3				
	Difference	+1.0	+0.5	+1.1	+1.6	+1.1				
0.125	Before	7.1	7.0	7.7	7.3	7.1	-0.02	0.128	0.156	0.883
	After	7.4	6.8	7.3	7.3	7.3				
	Difference	+0.3	-0.2	-0.4	0.0	+0.2				
cont.	Before	7.6	8.0	8.0	7.8	7.7	-0.14	0.166	0.421	0.695
	After	8.1	7.6	7.6	7.6	7.5				
	Difference	+0.5	-0.4	-0.4	-0.2	-0.2				

Table 5.6 Paired sample t-Test analysis of the calculated swept volume (mm^3) for *E.modestus* nauplii After - Before the presentation of Glycine. Mean diff. = mean difference, St error = standard error and prob = the probability that the magnitude of the mean difference will exceed zero by chance. cont. = seawater control.

Table 5.7

Conc $x10^{-3}$		Swept Volume mm^3					Mean Diff.	St Error	Paired t-value	prob
	Before	7.7	8.1	8.9	9.0	10.0				
3.50	After	9.9	10.3	11.2	11.1	11.8	+2.08	0.097	21.45	0.001
	Difference	+2.2	+2.2	+2.3	+1.9	+1.8				
	Before	9.9	10.0	10.0	10.0	10.1				
1.72	After	11.8	11.5	11.6	11.4	11.6	+1.58	0.086	18.37	0.001
	Difference	+1.9	+1.5	+1.6	+1.4	+1.5				
	Before	7.6	8.4	6.2	6.8	7.1				
0.694	After	9.6	8.1	7.7	8.9	8.7	+1.38	0.435	3.171	0.034
	Difference	+2.0	-0.3	+1.5	+2.1	+1.6				
	Before	9.3	9.7	9.4	8.2	7.5				
0.434	After	10.4	11.0	9.7	10.8	9.2	+1.4	0.377	3.715	0.021
	Difference	+1.1	+1.3	+0.3	+2.6	+1.7				
	Before	8.2	9.6	6.6	8.1	8.6				
0.125	After	8.7	8.1	7.4	7.6	7.9	-0.28	0.418	0.671	0.539
	Difference	+0.5	-1.5	+0.8	-0.5	-0.7				
	Before	9.1	9.9	7.8	8.3	8.7				
cont.	After	9.3	8.3	8.1	7.7	8.1	-0.46	0.347	1.341	0.251
	Difference	+0.2	-1.6	+0.3	-0.6	-0.6				

Table 5.7 Paired sample t-Test analysis of the calculated swept volume (mm^3) for *E.modestus* nauplii After - Before the presentation of Glucose. Mean diff. = mean difference, St error = standard error and prob = the probability that the magnitude of the mean difference will exceed zero by chance. cont. = seawater control.

Table 5.8

Conc $x10^{-3}$		Swept Volume mm^3					Mean Diff.	St Error	Paired t-value	prob
	Before	9.1	9.0	9.1	8.8	9.2				
2.92	After	11.0	11.6	10.9	11.0	10.8	+2.02	0.174	11.59	0.001
	Difference	+1.9	+2.6	+1.8	+2.2	+1.6				
	Before	9.6	9.6	9.2	9.4	9.5				
1.46	After	10.8	11.0	10.2	9.2	10.7	+0.92	0.287	3.205	0.033
	Difference	+1.2	+1.4	+1.0	-0.2	+1.2				
	Before	9.9	10.6	10.9	10.6	11.2				
0.731	After	10.8	11.9	10.7	11.1	11.7	+0.60	0.249	2.410	0.074
	Difference	+0.9	+1.3	-0.2	+0.5	+0.5				
	Before	11.1	11.0	10.6	11.4	10.8				
0.365	After	11.1	12.0	10.8	12.4	11.3	+0.54	0.204	2.648	0.0571
	Difference	0.0	+1.0	+0.2	+1.0	+0.5				
	Before	9.7	9.3	9.4	9.6					
0.146	After	9.4	9.4	9.3	9.5		-0.10	0.099	1.011	0.387
	Difference	-0.3	+0.1	-0.1	-0.1					
	Before	9.1	9.2	9.7	9.7					
cont.	After	9.3	9.1	9.6	9.6		-0.03	0.069	0.3612	0.742
	Difference	+0.2	-0.1	-0.1	-0.1					

Table 5.8 Paired sample t-Test analysis of the calculated swept volume (mm^3) for E.modestus nauplii After - Before the presentation of Sucrose. Mean diff. = mean difference, St error = standard error and prob = the probability that the magnitude of the mean difference will exceed zero by chance. cont. = seawater control.

Fig 5.1

The response of stage IV E.modestus nauplii to various dissolved organic substances. The mean difference, ± standard error bars, in the antennal swept volume one minute before minus one minute after the application of sugars and amino acids is plotted against molar concentration. All curves have been fitted by eye.

(a).....Glutamic acid.

(b).....Glycine.

(c).....Glucose.

(d).....Sucrose.

FIG. 5.1

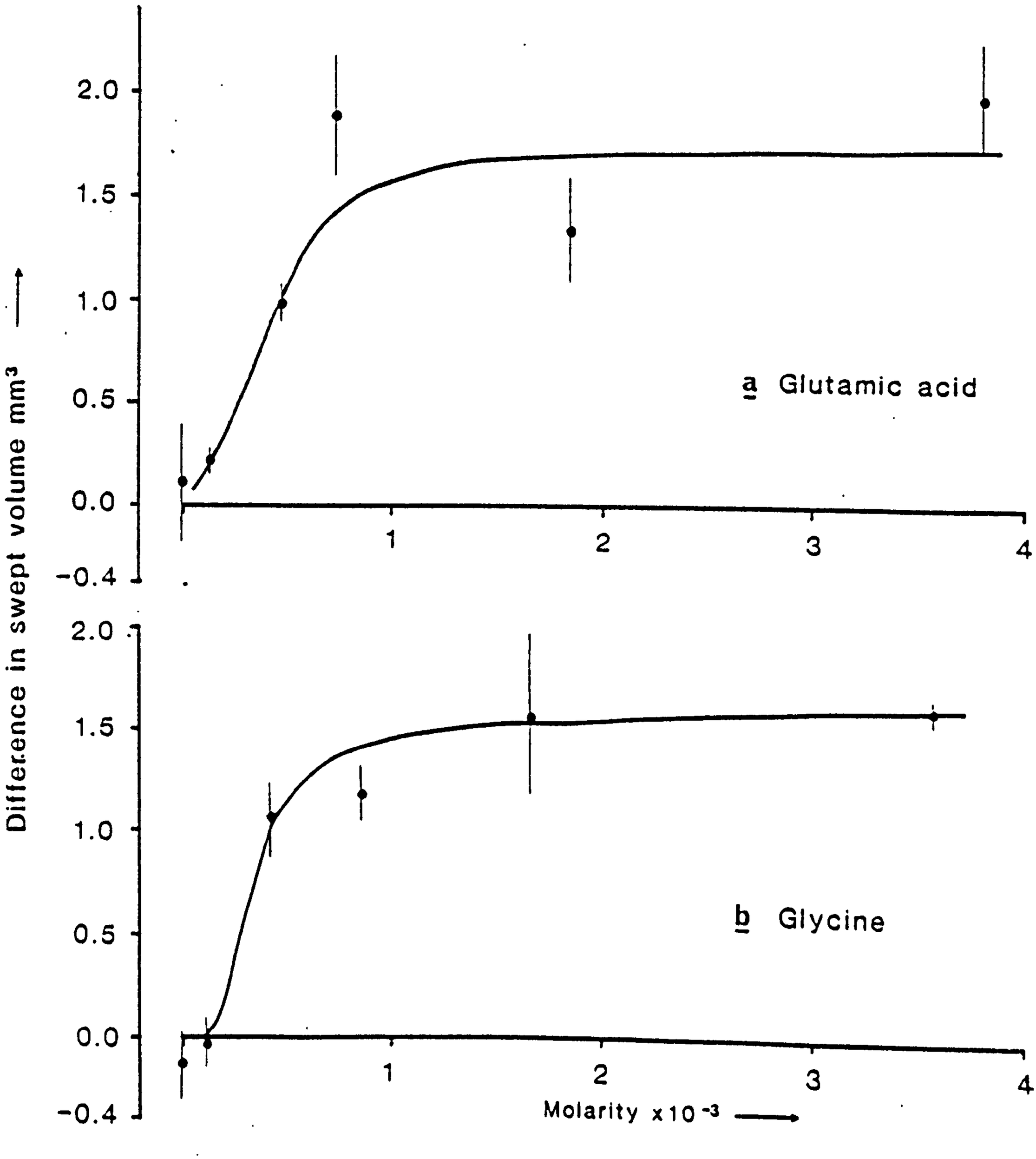
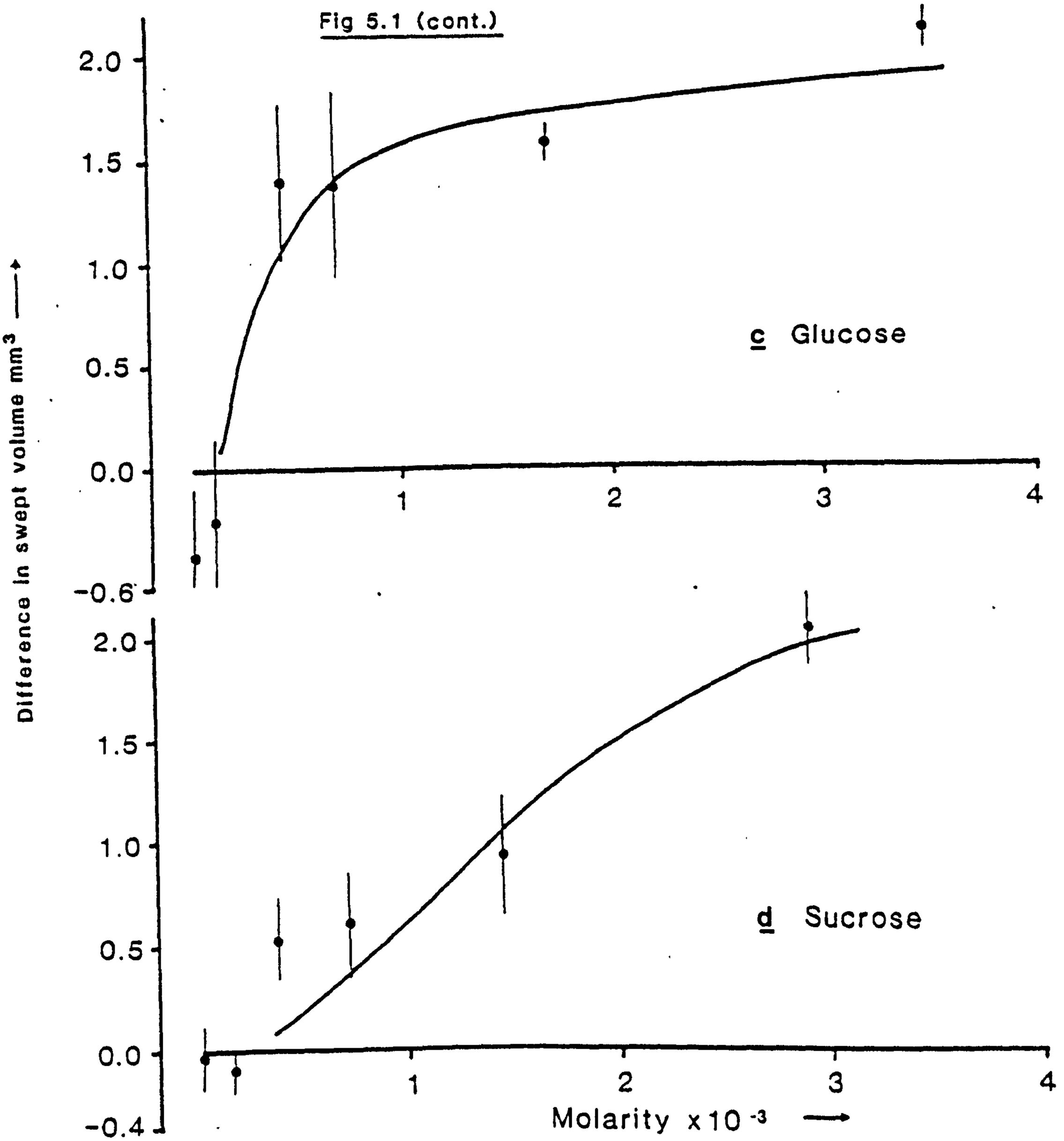

Difference in swept volume mm³
2.0
1.5
1.0
0.5
0.0
-0.4
1
2
3
4
a Glutamic acid
b Glycine
Molarity x10⁻³

Fig 5.1 (cont.)


Difference in swept volume mm³ ⟶
2.0
1.5
1.0
0.5
0.0
-0.6
1
2
3
4
c Glucose
2.0
1.5
1.0
0.5
0.0
-0.4
1
2
3
4
d Sucrose
Molarity x10 -3 ⟶

although occassional differences in beat frequency and amplitude were observed (see for example Table 5.1). The larvae of E.modestus have been shown to be very tolerant of salinity changes (Bhatnagar and Crisp 1965) and it therefore seems very unlikely that the response shown in this investigation was a result of colligative changes in the seawater due to the presence of the dissolved organic compounds.

Discussion

The results presented in Fig 5.1 show that stage IV nauplius larvae of E.modestus will increase the volume through which their antennae move (swept volume) in response to the concentration of certain dissolved organic molecules. Such a response is considered to relate to feeding, as an increase in swept volume is likely to be mirrored by a proportional increase in the amount of water handled by the larvae, and thus an increased probability of algal capture. Food particles striking the nauplius might be thought to trigger a feeding response by purely mechanical means, but this can be discounted since carmine granules on their own, often in concentrations sufficient to clog the limbs of the nauplius, do not trigger a response (see Section 3 above). The nauplius does not appear to ingest such inert particles, except in the presence of an acceptable food organism. Thus, since the larva does not complete the series of movements resulting in particle ingestion other than in the presence of suspended food, it must employ one of several methods to differentiate between food particles and other material suspended in the water column.

The larvae could sample the water at regular intervals. If such a sampling burst rewarded the larvae with algal ingestion a more prolonged feeding behaviour pattern could be embarked on. Such a random approach might lead to situations where the larvae expended energy in feeding though only a few algae were available, while larger and more dense patches of algae could be missed because the larvae had passed through them without sampling. A more efficient stimulus for such feeding behaviour would be a chemical sense which linked the presence of algae to particular organic compounds. Such a feeding stimulus from natural seawater has been shown for oysters, although the carbohydrate suspected as the triggering molecule was not identified (Collier et al 1950).

The nauplii of E.modestus do indeed show a response which could be equated to feeding behaviour when presented with the kinds of organic molecules which would be associated with food organisms in seawater. The increases in swept volume caused by dissolved organic compounds was at most some 20 - 30 % of the swept volume prior to the presentation, whereas increases in swept volume caused by the presence of algal could be in excess of 50% of the swept volume without algae in the seawater. Probably, stimulating organic substances encountered in the medium will elicit a limited feeding response, such as that described above, which is then reinforced by the ingestion of algae. The question therefore arises whether such a mechanism could operate in the sea.

Seawater contains a great deal of organic matter, Cauwet(1978) reports some 0.8g m^{-3} on average, present in seawater, exclusive of living plants and animals. By far the largest proportion of this material is in dissolved or colloidal form (Cauwet 1978), living organisms providing only 2% of the

organic matter in seawater. Non-living organic material in the sea ranges from high molecular weight organic molecules such as polypeptides, complex humic acids, carbohydrates and simpler long chain fatty acids and hydrocarbons, to low molecular weight molecules such as amino-acids, monosaccharides, carboxylic acids etc. (Duursma 1965). Although the presence of dissolved organic matter has been recognised since the early eighteen ninties (Natterer 1892), the individual compounds are often present in such low concentrations that quantitative data on the level of many groups of compounds in seawater is very difficult to obtain. Of the organic molecules used in the above investigation, Table 5.9 sets out representitive concentrations of Glutamic acid and Glycine and the range for Glucose, found in various oceans.

Sucrose has been reported freely dissolved in seawater (Vallentyne 1957) but no quantitative data is forthcoming. All four compounds used in the present investigation have thus been reported as dissolved in seawater but at concentrations below 10^{-6} Molar. Such measurements have rarely been performed during phytoplankton blooms and could be considered as background levels in seawater. Of the few long term measurements of the dissolved organic content of seawater that have been made, those by Wangersky (1959) and by Riley and Segar (1970) suggest that increases in dissolved amino acids or carbohydrates in seawater are detectable towards the end of, rather than during, phytoplankton blooms.

The occurrence of dissolved organic compounds in seawater can be attributed to several causes (Vallentyne 1957, Duursma 1965, Cauwet 1978). A wealth of data has been accumulated to show that phytoplankton, grown in bacterial free cultures, excrete various organic substances (Fogg 1962).

Table 5.9

The concentration of dissolved Glutamic acid, Glycine and Glucose reported from natural seawaters.

Reference	Substance	Concentration	Location
Degens et al (1964)	Glutamic acid	7.48×10^{-9}M.	Offshore California
	Glycine	8.67×10^{-8}M.	Offshore California
Chau & Riley (1966)	Glutamic acid	4.08×10^{-8}M.	Irish Sea
	Glycine	1.33×10^{-7}M.	Irish Sea
Clark et al (1972)	Glutamic acid	1.53×10^{-7}M.	Inshore California
	Glycine	4.62×10^{-7}M.	Inshore California
Vaccaro et al (1968)	Glucose	1.25×10^{-7}M.	West to East Atlantic
		1.08×10^{-6}M.	

The range of results for the concentration of dissolved Glucose in seawater shown were obtained by Vaccaro et al (1968) during a trans-Atlantic research cruise in low latitudes.

Hellebust (1965), for instance, lists amino acids, sugar alcohols and small amounts of carbohydrate as excretory products of phytoplankton. In particular he states that the diatom Skeletonema excretes quantities of glycollic acid, glycerol, glucose and amino acids, including glycine and glutamic acid. Tolbert and Zill (1956) have shown that, in acid culture, Chlorella pyrenoidza excretes quantities of sucrose. Estimates of the amount of algal excretion vary from 5 - 60 % (Fogg 1952) to 10 - 45 % (Allen 1956) of primary production. Apart from the account of Anderson and Zeutschel (1970) there appears to be little evidence to show that such excretory products persist in the natural environment. It would be expected that an increase in the dissolved organic content of seawater could be obtained during a phytoplankton bloom comparable to that shown by laboratory observation but this is not supported by long term field measurments. If, however, as Wangersky (1959) suggests, such dissolved matter can be utilised directly by plants and animals almost as fast as it is being excreted, increases in the dissolved organic content of seawater may not be apparent at a distance from the algal cells or for long after the bloom.

Duursma (1965) believes that most dissolved organic material comes from the breakdown, spontaneously or by bacterial action, of particulate organic matter in seawater. This particulate matter consists primarily of the dead bodies of phyto- and zooplankton. Certainly at the end of a phytoplankton bloom much particulate matter may be undergoing mineralisation while the organic molecules released may not be utilised to the same extent as substances excreted during the growth of the bloom. They may, therefore, be different from those released during the bloom, being those more resistent to bacterial attack. Such processes may account for the increase in dissolved organic matter noted at the end of a phytoplankton bloom.

It has been noted that zooplankton also excrete organic molecules. Johannes and Webb (1965) estimated the release of amino acid carbon by zooplankton in the Seragasso and Gulf stream as high as 25% of secondary production. The above considerations show that considerable amounts of organic matter can be released from aggregations of plankton, either living or dead. Although the largest measured increases in dissolved organic matter occur at the end of phytoplankton blooms, it seems likely that some transient amounts of organic matter, in excess of the measured background levels, accompany phytoplankton blooms as excretory products of phytoplankton and zooplankton or as breakdown products of dead organisms. Zooplankton grazing on phytoplankton blooms will also break up many of the algal cells without ingesting them, thus adding to the dissolved organic matter associated with phytoplankton patches. Even during the growth of a plankton bloom the ideal situation, exponential growth as in some laboratory cultures, is probably never achieved. Vallentyne's (1957) view that "Planktonic and other organisms spend more of their time dead than alive, and the healthy cell is the exception rather than the rule." may well be exaggerated, yet considerable numbers of damaged or dead plants and animals must always be undergoing mineralization in the euphotic zone so releasing organic matter, some of which will remain in solution. Accordingly, in most investigations of dissolved organic matter in seawater, the highest concentrations are found in the most productive areas and tend to decrease with depth (Duursma 1965, Vaccaro et al 1968, Anderson and Zeutschel 1970).

The above discussion has shown that the recorded levels of dissolved organic matter in seawater are generally below that required to ellicit a response from E.modestus larvae. However, higher levels of dissolved organic matter can be expected in the region of a phytoplankton bloom,

although these may be of a transitory nature. The majority of dissolved organic matter found in association with phytoplankton patches must however come from the algal cells or the zooplankters. Dissolved matter will thus diffuse from the region of high concentration within the cell towards the reduced background level of the surrounding water. The concentration of such dissolved matter very close to the food particle must, therefore, be considerably in excess of the background concentration. As the nauplius must come into very close contact with the food particles before ingesting them, the high concentration of the shell of organic matter surrounding the cell will doubtless stimulate the larva to commence or continue feeding. Furthermore, the presence or absence of a shell of dissolved substances, at sufficient concentration, around floating particles may inform the nauplius whether the particles are edible and can be ingested or whether they are inedible and can be rejected.

Section 6

A Quantitative Study of Algal Grazing by E.modestus Stage II Nauplii

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Introduction

Zooplanktonic organisms make up the greater part of the herbivorous community of the sea. Many are primarily herbivorous, providing the first link in the food chain from the primary producers, the phytoplankton, to higher trophic levels which include commercially exploited fish stocks. The first step towards unravelling the complex relationships between organisms within food webs is to study, quantitatively, the feeding of one organism on another lower down the chain.

The best studied planktonic herbivores are the copepods, particularly Calanus and Pseudocalanus (Marshall and Orr 1955 (a), Harris and Paffenhofer 1976, Corkett and McLaren 1978). Techniques employed to estimate copepod feeding rates have been as varied as the results obtained. The majority of investigations have relied on feeding animals with laboratory maintained cultures of phytoplankton, measuring the change in algal concentration after the herbivores have fed for a known period of time. These results are normally corrected for the change in a control culture containing no herbivores. Results have been expressed either as feeding rates, viz. the number of algal cells taken by an animal in unit time, or as the volume of water that the animal would need to clear of cells in unit time to account for the observed change in algal concentration. The latter representation of grazing is a measure of the animals capacity to move water and has been termed the "volume swept free" by Fuller (1937) and Harvey (1937), the "volume swept clear" by Gauld (1951) and Frost (1972), the "grazing rate" by Paffenhofer (1971) and is equivalent to the "filtration rate" as used by

bivalve physiologists (e.g. Caughlan 1969, Foster-Smith 1975, Hildreth and Crisp 1976). Frost (1972) pointed out that the volume swept free was often confused with the volume of water handled by the organism to obtain its food (e.g. Mullin and Brookes 1967, 1970), whereas the two quantities are equivalent only if the efficiency of particle retention on the filtering surfaces is 100%. The equivalent term in bivalve physiology for the volume of water handled by the organism is the "pumping rate" or the "ventilation rate".

The concentrations of algal cultures have been assessed in a number of different ways. Harvey (1937), Fuller (1937) and Gauld (1951) all used visual methods of counting algae in known volumes of seawater. Paffenhöfer (1971) and Frost (1972) used a Coulter Counter whereas Marshall and Orr (1955 (b)) counted the radioactivity of labelled algae, a technique also used by Walne (1965) and Adams and Steele (1966). Methods of maintaining the algae in suspension during an experiment have also varied. Simple systems where cultures were left undisturbed or gently aerated have been used by Fuller (1937) and Gauld (1951), whereas more complicated rotating devices have been used by Corner et al (1972) and continuous culture systems attempted by Paffenhöfer (1971) and Frost (1972).

Initial observations of algal grazing by zooplankton claimed that the volume swept free by the herbivores was unaffected by the food density available (Fuller and Clarke 1936, Fuller 1937, Gauld 1951), resulting in an increasing feeding rate with increasing food concentration. More recently however, observations from many planktonic organisms have shown a fall in the volume swept free as the available algal concentration was increased (Ryther 1954 (Daphnia), Reeve 1963 (Artemia), Mullin 1963 (Calanus), Haq

1967 (Metridium), Paffenhöfer 1971 (Calanus), Frost 1972 (Calanus), White 1976 (Ostrea larvae), Jones 1978 (Elminius larvae). Such inverse relationships between the volume swept free and the algal density may persist over a wide range of algal densities or may be confined to a plateau in food intake at higher concentrations. At still higher algal concentrations a decline in feeding rate has been reported in some cases(e.g. Mullin 1963).

The true filter feeding organism will continue to remove all the algal cells from the water independantly of cell concentration up to the point where the filtering surfaces, or capacity to ingest, become overloaded. More widespread amongst planktonic herbivores, perhaps, is the alternative strategy whereby the organism ingests a fixed ration of algal cells and controls its intake up to satiation (see Conover 1968).

Many factors have been shown to influence the quantity of food taken in by planktonic herbivores. Marshall and Orr (1955 (b)) and Frost (1972, 1977) have shown that copepods will select larger rather than smaller algal cells. Wilson (1973) suggested that Acartia exhibited a complex selective feeding behaviour pattern whereby the size range of the particles ingested was adjusted to the particle size distribution offered. However, selective feeding, determined by size or shape of algal cells, is more commonly considered to be a passive consequence of the size of the holes on the filtering surfaces, the "leaky sieve" analogy proposed by Boyd (1976).

Harvey (1937) reported that Calanus ingested Lauderia in preference to Chaetoceros from mixtures of the two algae. Riley et al (1949) argued that Harvey's result could be explained by the difference in size of the algae,

however, Gauld (1951) suggested that size alone was not the major factor involved. Both Gauld (1951) and Petipa (1959) have suggested that copepods can actively reject filtered algae, possibly on the grounds of taste or the strength of the cell wall.

The determination of the grazing rates of marine planktonic herbivores has also been shown to be affected by the size of the containers and the animal density within the containers. Marshall and Orr (1955 (b)), Cushing (1958) and Anraku (1964) have all shown that the volume swept free by copepods is reduced in small containers. Furthermore, Hargrave and Geen (1970) showed that in vessels of the same shape and capacity the volume swept free per copepod was reduced at increased animal densities.

Studies of the grazing rates of meroplanktonic larvae have been mainly confined to commercially exploitable species of bivalve (Ostrea : Walne 1956,1965, Mytilus : Bayne 1965, Ostrea : White 1976). Jones (1978) successfully investigated the grazing by Elminius modestus larvae on the flagellate Rhodomonas baltica though the work of Moyse (1963), aimed at assessing the value of different micro-algal species as food, suggests that for many barnacle larvae, including E.modestus, flagellates are not as good as certain diatoms, notable Skeletonema costatum. It seemed pertinent therefore to extend the work of Jones to include Skeletonema as a food source and to investigate quantitatively any preference shown by stage II E.modestus larvae for different algal species.

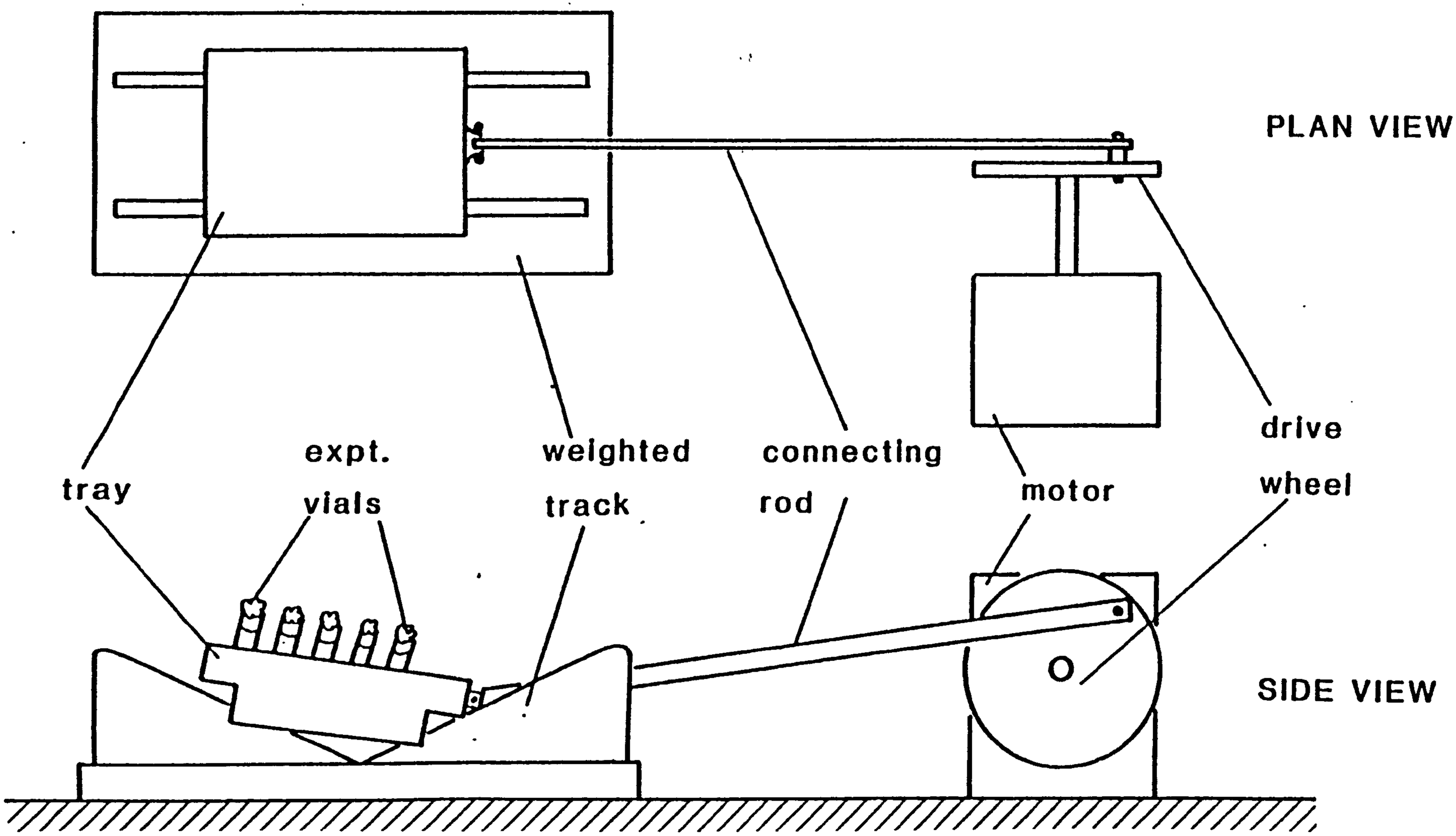
Materials and Methods

Adult Elminius modestus were collected from the shores of the Menai Strait, either at Beaumaris or Menai Bridge. The larvae were released in the laboratory (see 'Materials' Section 1 above) as stage I nauplii. The moult from stage I to stage II normally occurs within a few hours of release (Knight-Jones and Waugh 1949). All the larvae were maintained with gentle aeration in 3 litre glass jars of fine-filtered seawater which had been irradiated with ultra-violet light to reduce bacterial numbers. The nauplii were maintained without food for 24 hours before the majority of the experiments.

In nearly all experiments 30 nauplii were used in 20 mls. of seawater with algal concentrations adjusted to suit the experiment. The experimental vials were of glass, 10 cm. high and 2.5 cm. in diameter. Normally 20 vials were used for each experiment, 5 controls and 15 experimental replicates. Once the vials had been filled for an experiment they were secured to a tray and shaken, in the apparatus shown in Fig 6.1, to maintain the algal cells in suspension. All the vials were fitted with cotton wool plugs to prevent contamination.

The following experimental routine was adhered to, unless otherwise specified :- Suitable concentrations of algae were made up from semi-continuous stock cultures maintained in the laboratory. The initial algal concentration was assessed using a Hawkesley B.S. 748 haemocytometer, counting 20 x 0.1 µl. samples. The nauplii were then counted into vials and as much excess water as possible removed with a glass pipette. 20 mls. of algal culture were dispensed to each vial and the

Fig 6.1 Apparatus used to agitate the culture vessels during feeding experiments



shaker set in motion. Good mixing of the culture was achieved with gears 8 - 12 on a Palmer Electric 12 Kymograph motor (Fig 6.1). The experiments were run between 10.00 and 16.00 hrs. during early summer when there was natural illumination and room temperatures were fairly constant, between 18 - 20°C.

After four hours the algal concentration in each control and experimental vial was again measured using an haemocytometer. The time required for measurement was allowed for in later calculations, as it was significant. It took approximately 1.25 - 1.5 hours to measure the algal concentration in all 20 vials.

Three species of phytoplankton were used, Skeletonema costatum, a chain forming diatom on which E.modestus are known to develop well (Moyse 1963); Tetraselmis suecica and Pavlova lutheri, both flagellate algae. The 20 ml. cultures containing flagellates were treated with an insignificant drop of strong formalin to kill the cells before counting.

The grazing rates of E.modestus nauplii were calculated using the generally accepted assumption that in the presence of a steadily grazing herbivore the algal concentration of the culture will decline exponentially.

$$\text{i.e.} \qquad C_t = C_o e^{-kt} \ldots\ldots (1) \qquad \text{(Harvey 1937)}$$

where C_t is the algal concentration at time t and C_o is the initial algal concentration. k is a constant which represents the fraction of the total water volume available swept free of algae by all the animals present. If F = the volume swept free per animal in unit time and n the number of animals in the experimental volume V then

$$k = Fn/V \ldots\ldots (2)$$

substituting (2) into (1) and rearranging

$$F = \frac{V}{nt} \log_e \frac{C_o}{C_t} \quad \ldots\ldots(3)$$

Equations of the form of equation (3) have been used by Fuller and Clarke (1936), Fuller (1937), Gauld (1951) and Paffenhöfer (1971) to measure the volume swept free by copepods in situations where no growth or settling out of the algal culture was anticipated. The inclusion of a modification to eq.(3) incorporating control concentrations is necessary where factors other than animal grazing affect the algal concentration over the time period of the experiment. Such a modification has been employed by Adams and Steele (1966) for Calanus and White (1976) for Ostrea larvae as follows

$$F = \frac{V}{nt} \log_e \frac{C^e_o \cdot C^c_t}{C^c_o \; C^e_t} \quad \ldots\ldots(4)$$

C^c_o and C^e_o are the initial concentrations of the control and experimental cultures respectively. C^c_t and C^e_t are the concentrations of the control and experimental cultures at time t. The term C^e_o/C^c_o reduces to 1 if control and experimental cultures come from the same, thoroughly mixed, stock culture as they do in most of these investigations. A fuller discussion of equation (4) can be obtained from Crisp (1971), Rigler (1971) and White (1976). Hildreth and Crisp (1976) discuss a similar type of equation used in the study of feeding by adult bivalve molluscs and point out that such equations are valid only if agitation prevents the build up of concentration gradients in the experimental vessel.

The calculation of the volume swept free gives an indication of how the animal deals with the fluid around it and whether the concentration of food particles influences the rate of filtration. It does not directly estimate

the quantity of food eaten by the animal. Fuller (1937) estimated the number of cells eaten by a copepod in x hours (Nx) as :-

$$Nx = (V/N)(C_1 - C_2) \quad \ldots\ldots(5)$$

where V is the experimental volume, N is the number of copepods and C_1 and C_2 the algal concentration initially and after x hours respectively. Equation (5) is a direct measure of feeding rate, although a correction for control concentration is necessary. The feeding or ingestion rate (Ir) can also be obtained from the volume swept free as follows :-

$$Ir = \int_{Co}^{Ct} FC \, dC$$

where F is the volume swept free and C the algal concentration. Co and Ct represent the initial concentration and the concentration at time t respectively. Over short time periods the mean algal concentration multiplied by the volume swept free will approximate to the average ingestion rate thus

$$Ir = F \times [C^e_o + C^c_t]/2 \quad \ldots\ldots(6)$$

where Ir = the ingestion rate in algal cells / animal / hour, F = the volume swept free in µl / animal / hour and C^e_o, C^e_t = the experimental culture concentration in cells / µl at time o and t respectively. This measure of ingestion rate has been used as an estimate of grazing by planktonic herbivores by several workers (Frost 1972, Corner et al 1972, White 1976, Jones 1978). Occasionally F x C^e_o has been used to estimate the ingestion rate of planktonic herbivores (Mullin 1963, Reeve 1963) and, as explained by Hildreth and Crisp (1976), similar types of mistake have been made in studies of feeding in adult molluscs (e.g. Walne 1972). The use of C^e_o instead of the actual or mean concentration will lead to overestimates of ingestion rates, since algal concentration decreases over the

experimental period. The organisms are feeding at concentration C_o only at the beginning of the experiment. A better estimate of ingestion rate will be obtained by using the arithmetic mean concentration, $\bar{C}^e = [C^e_o + C^e_t]/2$ (see Rigler 1971).

Detailed Methods and Results

Experiments using mono-cultures of Skeletonema

(a) The effect of algal concentration on grazing in E.modestus nauplii

A series of experiments were performed to assess the influence of algal concentration on the grazing rate of stage II E.modestus larvae. Measurements of the volume swept free (F equation (4)) by the nauplii were made over a range of concentrations of Skeletonema costatum from 50 - 500 cells per µl.

Fig 6.2 shows a plot of F (µl/larva/hour) against the mean concentration of S.costatum in cells per µl. Each point represents F calculated for one experiment and the points marked with squares (■ Fig 6.2) represent the mean F for groups of up to 40 replicates at similar algal concentrations. Vertical bars represent one standard deviation about the mean. There is noticeably more variation in F at lower algal concentrations, due to the loss of accuracy involved in counting fewer numbers of cells in the same volume of seawater. The regression line (Fig 6.2) was fitted to the data by the least squares method as follows

$$F = 98.71 - 0.137\,\bar{C}^e \quad \mu l/larva/hour \quad \ldots\ldots(7)$$

where F = the volume swept free (µl) per nauplius per hour and $\bar{C}^e$ = the mean experimental algal concentration in cells per µl. The product moment

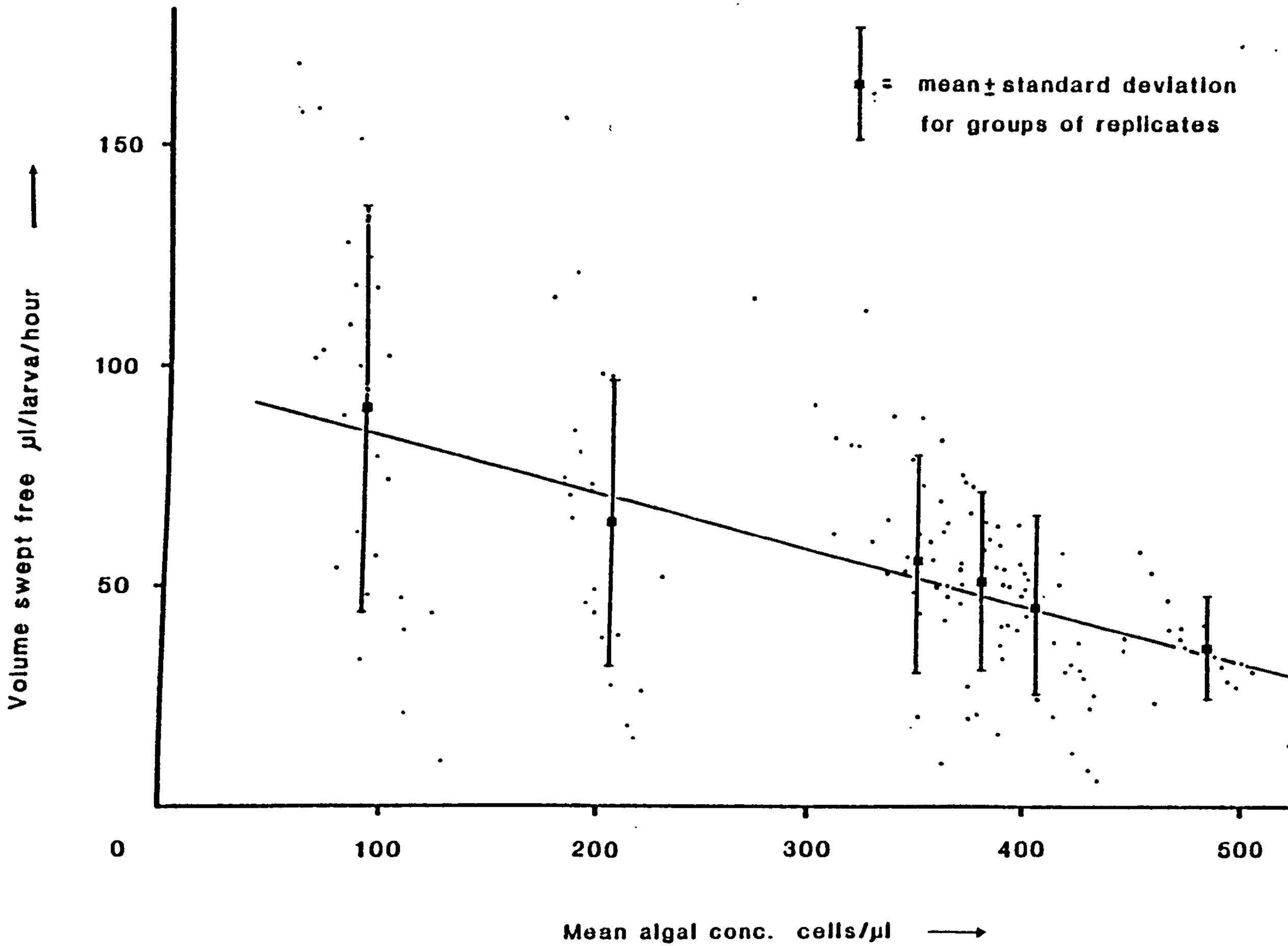
Fig 6.2

The volume of seawater swept free (v.s.m) of S.costatum by E.modestus stage II nauplii plotted against mean S.costatum concentration. Dots represent replicate results and squares the mean of up to 40 replicates at similar algal concentrations ± standard deviation bars. The line was fitted by least squares regression resulting in the equation :-

$$v.s.m. = 98.71 - 0.137\ conc^{n}\ \mu l/larva/hour$$

with a correlation coefficient of -0.5561 for 154 degrees of freedom.

Fig 6.2 E.modestus S II nauplii grazing on S.costatum



correlation coefficient for (7) was -0.5561 for 154 degrees of freedom ($p<0.001$). Therefore, despite the large scatter of points, a highly significant negative regression exists for the dependancy of F on mean algal density ($\bar{C}^e$).

Fig 6.3 shows a plot of the ingestion rate (Ir eq.(6)), in cells of S.costatum eaten per E.modestus nauplius per hour, against mean food density in cells per µl. Each point represents Ir calculated for one experiment and points marked with squares (■ Fig 6.3) are mean ingestion rates for groups of up to forty replicates at similar food concentrations. Vertical bars represent one standard deviation about the mean.

Several types of function have been fitted to feeding rate data of this type. Frost (1972) used a simple rectilinear model with regession lines fitted to parts of the data. The underlying assumption of Frost's model of copepod feeding was that at low food concentrations F is independant of algal concentration, hence Ir increases proportionally to food density. Beyond some critical algal concentration F decreases with algal concentration such that the copepod feeding rate (Ir) remains constant. Inspection of Fig 6.2 shows that, for E.modestus stage II nauplii feeding on S.costatum, F was negatively correlated with food density over the range investigated. Frost's (1972) model was, therefore, not applicable to the data of Fig 6.3 since F was not shown to be independant of food density over any part of the range.

Ivlev (1955) used complex asymptotic functions to model fish feeding. Similar functions have been suggested by Parsons et al (1967) as applicable to data of zooplankton grazing whilst Sushcheyna (1962, 1964) and Corner

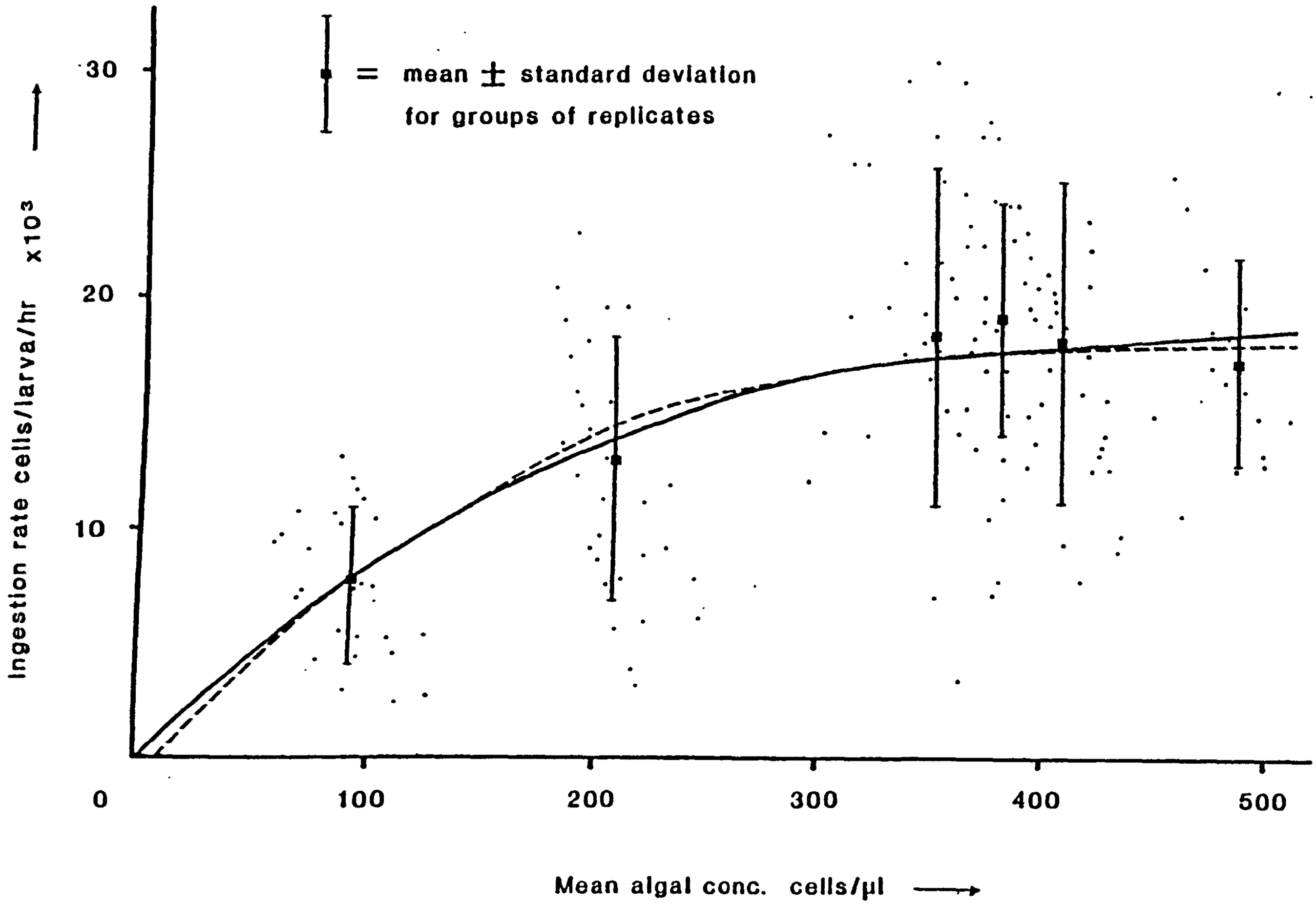
Fig 6.3

The ingestion rate (Ir) by E.modestus stage II nauplii of S.costatum cells (cells/larva/hour) plotted against mean algal concentration (cells/µl). Dots represent replicate results and squares the means for groups of up to 40 replicates at similar concentrations ± standard deviation bars. The lines were fitted by a least squares iteration technique as follows:-

solid line....Ir = 19818$(1 - e^{-0.0056conc})$ c/l/hr

dashed line...Ir = 19314$(1 - e^{0.052}.e^{-0.006conc})$ c/l/hr

Fig 6.3 Ingestion rate by S II E.modestus nauplii of S.costatum cells



et al (1972, 1976) fitted such curves to data of feeding by Artemia and Calanus respectively. The form of the scattergram, Fig 6.3, suggests that a general fit to all the data can be arrived at with a two constant asymptotic model. The solid curve and the very similar dashed curve in Fig 6.3 have been fitted to the data, assuming that the amount of food eaten by the nauplius is at first proportional to $\bar{C}^e$ but will eventually approach some maximum level (Imax). A suitable equation to model the situation is given by

$$Ir = Imax\ (\ 1 - e^{-k\bar{C}^e}\ ) \quad \ldots\ldots(8)$$

with boundaries as $\bar{C}^e \longrightarrow \infty$, $Ir \longrightarrow Imax$ and as $\bar{C}^e \longrightarrow 0$, $Ir \longrightarrow 0$,

where Imax is the asymptotic constant, k is the constant of proportionality defining the initial slope and e the base of natural logarithms.

Equation (8) is a special case of a more general series of curves developed by Von Bertalanffy (e.g. 1934) as part of a general application of open system theory to biological situations. A more general form of equation (8) can be given as

$$Ir = Imax\ (\ 1 - e^{kCm}.e^{-k\bar{C}^e}\ ) \quad \ldots\ldots(9).$$

Parsons et al (1967) suggested that a curve of type (9) might fit their data on zooplankton feeding where Cm represented a food density below which the animals did not feed. Bertalanffy curves have a wide application to biological systems, particularly in the field of fisheries research where length or weight, and time, are substituted for Ir and $\bar{C}^e$ respectively. Methods of fitting such curves are time consuming and many rely on the inexact graphical methods of Beverton and Holt (1957). Stevens (1951) developed a least squares method for fitting a limited selection of asymptotic curves and this method has been elaborated by Allen (1966) to encompass a much wider selection with less limitation on the type of data

required. Allen's model can be used to fit curves of type (8) or (9) by a least squares technique employing Newtons method (iteration) to estimate k. The process is very time consuming hence I have developed a computer programme to facilitate the fitting of both types of curve. The programme, written in D.E.C. 10 Algol 60, incorporating graphics subroutines, is given in Appendix I.

The fit to the data of curve type (8) is shown by the solid line of Fig 6.3 where k = 0.00562 and Imax = 19818.2 cells per larvae per hour with an estimated standard error of $\pm$ 2644.8 cells per animal per hour. The fit of curve type (9), where a value of Cm is calculated below which feeding does not take place, is shown by the broken line in Fig 6.3 where k = 0.00631, Imax = 19319.4 with an estimated standard error of $\pm$ 2196 cells per larva per hour and Cm = 8.107 with approximate standard error of $\pm$37.496 cells per µl.

Both curves are very similar (Fig 6.3) although the deviations sum of squares from the curve for type (9) curve (6.03218×10^9) was smaller than that for the type (8) curve (6.03594×10^9). There was no significant reduction in the deviations sum of squares between the two fitted curves (F-value =0.0953 for 1 and 153 degrees of freedom). Also the value of Cm for curve type (9) was small and comparing it to zero gives a t-value of 0.216 for 153 degrees of freedom, where t = Cm / standard error. The value of Cm was not significantly different from zero. Thus the equation which best expressed the relationship between feeding rate and food concentration in E.modestus stage II nauplii feeding on Skeletonema was

$$Ir = 19818.2 \left(1 - e^{-0.00562\bar{C}^e} \right) \text{ cells/animal/hr} \ldots\ldots (10).$$

At high food levels nauplii of E.modestus will feed until a maximum

ration is obtained and then stop feeding. At the lowest food concentrations the feeding effort of the nauplius is not sufficient to obtain the maximum hourly ration during the course of the experiment and the food gathering activity is maximised, the rate of intake being limited by particle concentration.

(b)Choice of Skeletonema chain length by Elminius nauplii

In a series of 5 experiments the chain length distributions of S.costatum were assessed to ascertain the effect of grazing by E.modestus nauplii on the distribution of chain lengths in experimental culture. A preference shown by the larvae for particular chain lengths should be reflected in a difference between control and experimental distributions after a period of grazing. The chain length distributions were determined as numbers of chains 1, 2, 3, 4, 5 or 6 cells long, noted from haemocytometer counts after a 4 - 5 hour grazing experiment. The longest chains noted in any of the experiments were six cells long. Although S.costatum can occur in longer chains, they are rarely noted from the cultures at Menai Bridge. The distribution of chain lengths in control vials where no grazing had taken place, but sedimentation or cell growth might have altered the cell concentration, were used to calculate the expected numbers of each chain length in the experimental situation had the nauplii fed indescriminately on Skeletonema chains, and the control and experimental distributions compared by Chi squared analysis. The results are shown in Table 6.1 where the chain length distributions for all five experiments are given and a Chi squared analysis of the combined data. From Table 6.1 it can be seen that the chain length distributions do not differ significantly (Chi-squared = 4.8152 for 4 degrees of freedom, p = 0.3).

Table 6.1

Chi-squared analysis for the preference shown by E.modestus stage II nauplii for different cell chain lengths of the diatom Skletonema costatum.

Chain length distributons : Experimental data.

	Experiment No. 1		2		3		4		5		
cells/chain	Co	Ex	Co	Ex	Co	Ex	Co	Ex	Co	Ex	Totals
1	26	52	25	52	18	46	29	55	21	43	367
2	79	159	71	176	58	141	70	147	65	160	1126
3	15	38	20	39	14	36	29	43	22	34	290
4	10	17	9	16	10	12	8	15	17	44	158
5	4	1	4	6	1	2	1	-	1	2	26
6	-	-	-	-	-	-	-	-	1	3	
							Grand total				1967

Co = control Numbers, Ex = Experimental numbers

Combined results from above 5 experiments at the same algal concentration.

cells/chain	Control	Experimental	Totals
1	119 (117.171)	248 (249.829)	367
2	343 (359.496)	783 (766.504)	1126
3	100 (92.588)	190 (197.412)	290
4	54 (50.444)	104 (107.556)	158
5+	12 (8.301)	14 (17.699)	26
Totals	628 (628)	1339 (1339)	1967

Figures in brackets represent the expected frequencies calculated as (row total x column total)/grand total.

Chi-squared = the sum of (observed - expected values)^2/expected value. i.e. Chi-squared above = 4.8152 for a 5 x 2 analysis with four degrees of freedom. The probability of obtaining such a high Chi-squared value by chance was 0.30 hence the distributions do not differ significantly.

Thus, with a range of chain lengths to choose from, E.modestus nauplii show no significant preference for, or greater feeding efficiency on, long chains or short chains of Skeletonema.

Experiments with monocultures of Flagellate algae

Two experiments were performed with monocultures of flagellate algae. In one experiment E.modestus nauplii were fed Pavlova lutheri at a mean concentration of 567.6 cells per µl. and in the other E.modestus larvae were fed Tetraselmis suecica at a mean concentration of 89.1 cells per µl. The experiments were performed with 5 control and 15 replicate culture vials in each case. The results are shown in Table 6.2 where the volume swept free per larva per hour and the feeding rate per larva per hour are detailed for each replicate. In experiment 1 the concentration of Pavlova was high and the volume swept free low (37.26 µl/larva/hr.), whereas in experiment 2, where the concentration of Tetraselmis was low, the swept volume was high (92.47 µl/larva/hr.) reflecting the feeding behaviour already noted for E.modestus with Skeletonema.

A direct comparison of the feeding rate of E.modestus nauplii on different algal species cannot be made on the basis of cell numbers eaten in unit time, as the algae differ widely in size. The volume of algae eaten in unit time gives a more accurate basis on which to compare the feeding performance of planktonic herbivores. A series of measurements were therefore made on the three algal species used in this investigation. Table 6.3 gives the mean values $\pm$ standard errors of 25 measurements, made at random, of the linear dimensions of samples taken from the algal cultures at Menai Bridge. The cell volume of Skeletonema was calculated as that of a

Table 6.2

Results of the feeding of E.modestus stage II larvae on monocultures of flagellate algae.

	Expt 1 P.lutheri initial conc. 681.5 cells/µl				Expt 2 T.suecica initial conc. 141.5 cells/µl		
Rep	Algal conc cells/µl	F µl/a/hr	Ir cells/a/hr	Rep	Algal conc cells/µl	F µl/a/hr	Ir cells/a/hr
1	550.8	37.19	20484	1	93.25	33.45	3119
2	555.8	33.27	18491	2	90.75	53.08	4817
3	563.3	27.55	15519	3	95.75	15.89	1512
4	580.8	38.88	22581	4	88.25	119.60	10555
5	573.3	43.86	25145	5	90.75	98.61	8948
6	623.3	13.30	8290	6	90.75	98.61	8948
7	525.8	70.38	37006	7	90.75	60.07	5451
8	573.3	36.52	20937	9	80.75	162.80	13146
9	543.3	56.99	30963	9	85.75	102.70	8806
10	580.8	34.78	20200	10	83.25	154.20	12837
11	580.8	34.78	20200	11	85.75	128.60	11027
12	570.8	40.76	23266	12	90.75	88.23	8006
13	558.3	33.68	18803	13	88.25	101.60	8968
14	560.8	32.15	18030	14	88.25	101.60	8968
15	573.3	24.79	14212	15	93.25	68.11	6351
Mean	567.58	37.26	20940		89.08	92.47	8097
error	±5.63	±3.41	±1745		±1.03	±10.62	±859

Figures are given for each replicate (Rep) of the mean algal concentration over the experimental period (algal conc), the volume swept free (F) in µl per animal per hour and the feeding rate (Ir) in cells per animal per hour for the two flagellate algae Pavlova and Tetraselmis. The mean value, ± the standard error of the mean are tabulated at the foot of the appropriate column.

cylinder and the cell volume of the flagellates Pavlova and Tetraselmis as that of a sphere. The cell volume of Skeletonema was more than twice that of Pavlova, while that for Tetraselmis was six times that of Skeletonema.

The feeding rate of E.modestus nauplii can thus be expressed as algal cell volume eaten per larva in unit time. To compare the feeding rate of the nauplii on flagellates and diatoms, an estimate of algal consumption from cultures with the same cell volume concentration is required. An estimate of the feeding rate on Skeletonema can be obtained from equation (10). In experiment 1 the mean cell volume concentration of Pavlova was 12,845 $\mu m^3/\mu l$. (567.58 cells/µl). The algal concentration of Skeletonema with the same cell volume concentration is 252.81 cells/µl. Similarly for experiment 2, a Skeletonema culture at 531.18 cells/µl. has the same cell volume concentration as that used for the Tetraselmis experiment (26,989 $\mu m^3/\mu l$.) Table 6.4 shows the comparison between the cell volume ingestion rates of flagellates by Elminius nauplii with those estimated for Skeletonema cultures from equation (10) x Skeletonema cell volume.

The result shows that Elminius nauplii ingest a smaller volume of Pavlova from a monoculture than the algal volume which can be estimated to be taken from a monoculture of Skeletonema at the same cell volume concentration. The opposite is true for experiment 2 where a larger volume of Tetraselmis was eaten than could be estimated for Skeletonema from cultures at the same cell volume concentration. Elminius stage II nauplii can, therefore, ingest a greater volume from monocultures of larger algae than from smaller algae at the same cell volume concentration.

Table 6.3

Measurements of the cell volumes of 3 species of micro-algae.

Alga	Shape	Diameter	Length	Volume
Skeletonema costatum	Cylindrical	3.3±0.1128 µm	5.64±0.345µm	50.81±5.306 μm^3
Tetraselmis suecica	Spherical	8.0±0.3152 µm	---	302.98±37.37 μm^3
Pavlova lutheri	Spherical	3.4±0.1330 µm	---	22.63±2.720 μm^3

all values are means from 25 observations ± standard error

Table 6.4

The feeding rate of E.modestus nauplii on T.suecica and P.lutheri compared to an estimate of their feeding rate on Skeletonema at equivalent concentrations. Feeding rates are expressed as cell volume ingested per nauplius per hour.

Alga	Mean algal Concentration cells/µl	μm^3/µl	Mean Feeding Rate μm^3/larva/hr	Range of 95% confidence limits of Mean μm^3/larva/hour
Experiment 1				
Pavlova	567.58	12,845	473,914	389,774 - 558,054
Skeletonema (Estimate)	252.81		831,548	568,159 - 1,094,937
Experiment 2				
Tetraselmis	89.08	26,989	2,453,197	1,898,590 - 3,007,803
Skeletonema (Estimate)	531.18		954,056	690,667 - 1,217,445

Results for Skeletonema were estimated from the relationship between food concentration and ingestion rate, equation (10).

Experiments with mixed cultures of Diatoms and Flagellates

Two experiments were performed to investigate the feeding rate of E.modestus stage II nauplii on cultures of mixed algae. In each case 15 experimental and 5 control cultures were used containing mixtures of Skeletonema, Pavlova and Tetraselmis. The mean algal concentrations for experiment 1 were :- S.costatum 112 cells/µl, P.lutheri 119 cells/µl, T.suecica 53 cells/µl, and for experiment 2 :- S.costatum 104 cells/µl, P.lutheri 135 cells/µl, and T.suecica 37 cells/µl.

The total algal cell ingestion rates by E.modestus larvae from both experiments are shown in Table 6.5. The algal cell volume has again been used to give a better comparison between different sized phytoplankton. The cell concentrations were similar in both experiments, although the cell volume concentration was higher for experiment 2, where more of the large Tetraselmis cells were present. The mean feeding rates measured for each experiment were very similar, with a wide overlap in the 95% confidence limits. Table 6.5 also shows an estimate of the cell volume that an E.modestus nauplius might ingest per hour from a monoculture of Skeletonema at the same cell volume concentration as the experimental mixtures (from eq.(10) above). In both cases the algal cell volume ingestion rate measured for the mixed cultures was very close to the estimated ingestion rate from a Skeletonema culture, though slightly greater in both experiments. There was, however, wide overlap in the 95% confidence limits which indicates that the differences were not significant at the 5% level of probability.

Table 6.5

A comparison of the total feeding rate of E.modestus stage II nauplii on mixed algal cultures to that estimated from equivalent monocultures of Skeletonema.

		Mixed Culture (Total Algae)	Skletonema (Estimates)
Experiment 1			
Mean Algal conc^{n}	cells/µl	275.83	382.52
	µm^3/µl	19,436	(19,436)
Feeding Rate	cells/larva/hr	13,507	17,509±5,184
	µm^3/larva/hr	1,168,967±317,905	889,632±263,389
Experiment 2			
Mean Algal conc^{n}	cells/µl	284.75	481.6
	µm^3/µl	24,470	(24,470)
Feeding rate	cells/larva/hr	12,267	18,495±5184
	µm^3/larva/hr	1,153,228±429,132	939,733±263,389

The figures shown are mean values from 15 replicates ± 95% confidence limits of the mean where appropriate.

The values estimated from Skeletonema cultures were calculated from equation (10) i.e. $Ir = 19818.2(1 - e^{-0.00562\bar{C}^{e}})$ cells/larva/hour ± 95% confidence limits of Imax.

Table 6.6 shows a breakdown of the results for both experiments for Elminius nauplii feeding on each algal species from the mixture. The mean algal concentrations, volumes swept free and the feeding rates from the 15 replicates are given ± the 95% confidence limits of the mean. The figures in brackets represent algal cell volumes (μm^3) for either concentration or consumption. As in the observations described above the volume swept free of algae by the nauplii tends to be highest at the low cell concentrations. In both experiments Tetraselmis was present in the highest cell volume concentration and accounted for the largest share of the algal cell volume ingested by the larvae. Pavlova represented the smallest proportion of the available algal cell volume, even though Pavlova was present at the highest cell concentrations in both experiments, and likewise accounted for the smallest share of the ingested ration.

An investigation of whether one or more algal species are captured and ingested preferentially to other species requires an analysis of the results similar to that used above for investigating the effect of grazing by nauplii on different Skeletonema chain lengths. If it is assumed that the nauplii will feed indiscriminately on the algae available, then algal cells would be ingested in proportion to their abundance in the culture. It could, therefore, be expected that, from cultures of mixed algae from the same, well stirred stock, the proportion of each species in a culture, which had been grazed by nauplii for four hours, would be the same as that in a control culture with no animal grazing over the same time period. Table 6.7 shows a Chi-squared analysis of 30 cell counts for the control and experimental algal cultures of experiments 1 and 2. The numbers of cells counted for each species have been totalled for the control and experimental cultures to construct a 3x2 contingency table (Table 6.7 a, c). In both

Table 6.6

A summary of the grazing performance of E.modestus nauplii on mixed cultures of phytoplankton.

	Algal concentration cells/µl ($\mu m^3/\mu l$)	Volume swept free µl/larva/hr	Feeding rate cells/larva/hr (μm^3/larva/hr)
Experiment 1			
Skeletonema	104.25±6.17 (5297±313)	70.77±21.13	6988±1763 (355,081±89,594)
Tetraselmis	36.58±2.89 (11084±875)	73.23±39.87	2377±1081 (720,154±327,479)
Pavlova	135.0±4.02 (3055±91.0)	31.28±12.53	4142±1650 (93,732±37,333)
Experiment 2			
Skeletonema	112.42±7.92 (5712±402)	63.95±25.48	6617±2015 (336,171±102,297)
Tetraselmis	53.0±2.77 (16058±687)	48.93±26.76	2458±1291 (744,811±22,228)
Pavlova	119.33±4.33 (2701±98.0)	27.71± 9.12	3192±1785 (72,246±22,228)

The values shown are mean values from 15 replicates ± the 95% confidence limits of the mean.

Table 6.7

Selection of algae by E.modestus stage II nauplii from a mixed phytoplankton culture. A Chi-squared analysis of cell counts in ungrazed cultures (Controls) against grazed cultures (Experimental).

Experiment 1.

Alga (a)	Controls Observed(O)	Controls Expected(E)	Experimental Observed	Experimental Expected	Total
Skeletonema	378	(359.8)	264	(282.2)	642
Tetraselmis	131	(119.9)	83	(94.1)	214
Pavlova	438	(467.3)	396	(366.7)	834
Totals	947	(947)	743	(743)	1690

	(O - E)	$(O - E)^2/E$	(O - E)	$(O - E)^2/E$	Chi-squared	
Skeletonema	+18.2	1.83713	-18.2	2.34112		D.F. = 2
Tetraselmis	+11.1	1.02761	-11.1	1.30935	8.6061	p= 0.014
Pavlova	-29.3	0.92062	+29.3	1.17378		

(b)	Observed	Expected	Observed	Expected	Total
Skeletonema	378	(381.75)	264	(260.25)	214
Tetraselmis	131	(127.25)	83	(86.75)	642
Totals	509	(509)	347	(347)	856

	(O - E)	$(O - E)^2/E$	(O - E)	$(O - E)^2/E$	Chi-squared	
Skeletonema	-3.75	0.03684	+3.75	0.05403		D.F. = 1
Tetraselmis	+3.75	0.11051	-3.75	0.16210	0.3635	p= 0.547

Experiment 2

Alga (c)	Observed	Expected	Observed	Expected	Total
Skeletonema	422	(417.5)	328	(332.5)	750
Tetraselmis	181	(154.2)	96	(122.8)	277
Pavlova	380	(411.3)	359	(327.6)	739
Totals	983	(983)	783	(783)	1766

	(O - E)	$(O - E)^2/E$	(O - E)	$(O - E)^2/E$	Chi-squared	
Skeletonema	+4.5	0.04850	-4.5	0.06090		D.F. = 2
Tetraselmis	+26.8	4.65785	-26.8	5.84886	15.9886	p= 0.001
Pavlova	-31.3	2.38194	+31.3	2.99051		

(d)	Observed	Expected	Observed	Expected	Total
Skeletonema	422	(440.4)	328	(309.6)	750
Tetraselmis	181	(162.6)	96	(114.4)	277
Totals	603	(603)	424	(424)	1027

	(O - E)	$(O - E)^2/E$	(O - E)	$(O - E)^2/E$	Chi-squared	
Skeletonema	-18.4	0.76876	+18.4	1.09354		D.F = 1
Tetraselmis	+18.4	2.08216	-18.4	2.95944	6.9039	p= 0.009

D.F. = degrees of freedom, p = probability of obtaining Chi-squared by chance.

experiments the calculated Chi-squared value exceeds the tabulated value for Chi-squared at the 5% probability level. The probability of obtaining such a high Chi-squred value by chance was 0.02 in experiment 1 and 0.001 in experiment 2. The proportions of each algal species in mixed cultures subjected to grazing by the nauplii were thus significantly different from the algal cultures where no grazing took place.

Analysis of the deviations from the expected values (Table 6.7 a and c) shows a consistent pattern in both experiments. The cultures which had been grazed by nauplii were deficient in Skeletonema and Tetraselmis cells and the numbers of Pavlova cells exceeded expectation. Table 6.7 (b) and (d) shows a further Chi-squared analysis between the proportions of Tetraselmis and Skeletonema in the cultures. In experiment 1 the probability of obtaining the Chi-squared value by chance exceeded 0.5, hence there were no significant differences between the proportion of Skeletonema and Tetraselmis cells between grazed and ungrazed cultures. The concentration of Tetraselmis in experiment 2 was higher than in experiment 1 and from Table 6.7 (d) the probability of obtaining the Chi-suared value for the comparison of the proportions of Tetraselmis and Skeletonema by chance was 0.01. The proportions of Tetraselmis and Skeletonema in mixtures which had been grazed by nauplii were, therefore, significantly different from the proportions in ungrazed cultures. Again, inspection of the deviations from expectation (Table 6.7 (d)) shows that grazed cultures were deficient in Skeletonema cells relative to Tetraselmis cells.

The result of this analysis indicates that the nauplii do not capture and ingest different algal species in proportion to their abundance in mixed cultures. The smallest alga, Pavlova, was consistently in excess in the

cultures after grazing, indicating that the nauplii either do not capture Pavlova as readily as Tetraselmis and Skeletonema or possibly even that Pavlova, once captured, is more readily rejected by the nauplii. In experiment 2 the result indicates that the larger Tetraselmis cells were more readily captured, or less readily rejected, than Skeletonema, although both algae were preferred, by one mechanism or another, to Pavlova.

The feeding rate of E.modestus nauplii measured over longer time periods

Mullin (1963) found that the feeding rates of Calanus were often higher over the first four to six hours of an experiment than they were after 24 hours. Two experiments were performed to investigate the feeding rates of E.modestus nauplii over 24 hours and, simultaneously, to compare the feeding rates of animals which had been maintained without food prior to the experiment with those which had been fed.

The experimental method also differed from that used in the experiments already described in that screw top vessels of 100 ml + capacity were used with between 60 and 120 larvae per vessel. The algae were maintained in suspension by fixing the vessels to a wheel rotating vertically at 6 r.p.m.. Skeletonema costatum was once again used as the food organism. E.modestus stage II nauplii were pipetted into the experimental vessels and as much excess water as possible removed. 101 mls of Skeletonema culture were added to each of 12 experimental vessels, containing nauplii, and to 6 control vessels without larvae. A 1ml sample was removed from each well shaken vessel to assess the initial algal concentration by haemocytometer counting. Once filled, each vessel was immediately fixed to the rotating wheel. After four hours each vessel was removed in turn and a 1 ml sample removed to

assess the algal concentration. The remains of this 1 ml sample, usually about 0.8 ml, was made up to 1 ml again with formalin to roughly estimate the defaecation rate of the nauplii by faecal pellet counts. The experiment was allowed to run for a further twenty hours and the algal cell counts repeated. 1 ml of strong formalin was added to the cultures at the end of the experiment to facilitate counting of the nauplii. A further 1 ml sample was taken for a faecal pellet count. The number of nauplii in each experimental vessel was counted at the end of the experiment and only the numbers of larvae with full guts were used for the calculation of feeding rates.

The algal concentrations assessed by haemocytometer counts were used to calculate the volume swept free and hence the feeding rate of the nauplii from equations (4) and (6) discussed above i.e.

$$\text{Volume swept free (F)} = \frac{V}{nt} \log_e \frac{C^e_o}{C^c_o} \cdot \frac{C^c_t}{C^e_t}$$

$$\text{Feeding rate (Ir)} = F \times (C^e_o + C^e_t) \times 0.5$$

where C^c_o and C^e_o represent the initial control and experimental algal concentrations, C^c_t and C^e_t represent the control and experimental concentrations respectively, at time t. V = the experimental volume and n = the number of nauplii which had fed during the experiment. The defaecation rates were roughly assessed by counting all the faecal pellets in the 1 ml samples on a counting slide. Allowances were made for any dilution incurred and the results expressed as numbers of faecal pellets released per larva per hour. Many of the faecal pellets encountered were broken, hence during counting, parts of faecal pellets judged to be less than half a pellet were ignored and only whole pellets or pieces larger than half a full pellet were

Table 6.8

The feeding and defaecation rates for E.modestus stage II larvae feeding on Skeletonema averaged over 4 and 24 hours for nauplii which had been maintained without algal food, and those maintained with algal food prior to the experiment.

	Algal conc cells/µl	F µl/a/hr	Ir cells/a/hr	Estimate cells/a/hr	Def rate p/a/hr	Expt time
Experiment A						
Larvae not fed	237.1±8.37	67.72±5.98	16,525±1912	14,590±2645	4.71±1.05	4 hrs
Larvae fed	264.0±11.5	35.90±9.79	9,045±2184	15,323±2645	5.52±0.73	4 hrs
Larvae not fed	222.9±8.33	18.12±3.06	4,013±653		1.56±0.23	24 hrs
Larvae fed	249.1±5.51	10.00±1.27	2,485±313		1.76±0.19	24 hrs
Experiment B						
Larvae not fed	123.3±2.29	77.58±16.9	9,639±2159	9,907±2645	2.30±0.24	4 hrs
Larvae fed	126.1±3.27	18.66±8.33	2,457±1134	9,896±2645	1.01±0.21	4 hrs
Larvae not fed	127.3±2.86	25.62±2.85	3,246±376		1.62±0.13	24 hrs
Larvae fed	117.7±5.27	25.01±2.36	2,959±328		1.53±0.14	24 hrs

F = the volume swept free in µl/animal/hour. Ir = ingestion rate in cells/animal/hour. Def rate = defaecation rate in faecal pellets/animal/hour and Expt time = the experimental duration in hours. The figures represent mean values ± standard error of the mean of six replicates. The estimates of Skeletonema ingestion by E.modestus nauplii were calculated from equation (10) as before with ± the standard error of Imax.

counted. This practice may have led to underestimation of the defaecation rate, yet it was considered preferable to overestimation, particularly when low numbers of pellets were being counted.

The two experiments performed differed only in the initial concentrations of Skeletonema. In both cases six experimental vessels contained stage II E.modestus nauplius larvae maintained without food from release and six experimental vessels contained larvae from the same larval release which were maintained in a culture of Skeletonema at approximately 350 cells/µl for up to 9 hours before the experiment. The initial algal concentration for experiment A was approximately 250 cells/µl and that for experiment B approximately 140 cells/µl.

The results are shown in Table 6.8 where the result has been expressed as a mean from the six replicate experimental cultures ± the standard error of the mean. An estimate of the feeding rate of E.modestus nauplii on Skeletonema based on the results from the previous experiments and calculated from equation (10) has been given for comparison. Over the first four hours of the experiment the feeding rates of larvae which had not been fed prior to the experiment show very good agreement with the results obtained from the earlier, small volume, culture experiments. The feeding rates of larvae which had been fed prior to the experiment were, however, considerably lower. In both experiments the larvae which were fed before the experiment fed at a lower rate than those which had not fed prior to the experiment. A small sample t-Test comparison between the mean feeding rates of previously fed and unfed larvae over 4 and 24 hours for both experiments gave the following t-values.

	Experiment A		Experiment B	
Feeding rates averaged over	4 Hours	24 hours	4 Hours	24 hours
Small sample t	2.4831	2.1096	2.9445	0.5745
Degrees of freedom	10	10	10	10
Probability of obtaining the observed difference between means by chance	0.0324	0.0611	0.0147	0.5783

Over the first four hours, the feeding rate of the larvae which had been fed prior to the experiment was, therefore, significantly lower than for those which had been maintained without food prior to the experiment. The difference measured over 24 hours, however, was not significant, although the result for experiment A approached the 5% level of significance. As might be expected, therefore, hungry animals eat faster.

In general the defaecation rate follows the feeding rate, in that more faecal pellets were released when the feeding rate was high than when the feeding rate was low. Over the first 4 hours of experiment A, however, the defaecation rate was slightly higher for animals which had been fed before the experiment, although their feeding rate was lower. A series of measurements were therefore made to determine whether faecal pellets produced by animals which had been fed before the experiment differed in size from those produced by animals which had not been fed during the experiment. The samples of culture taken after 4 hours for faecal pellet counting were retained and measurements of length and breadth made on twenty whole pellets from animals which had been fed and those which had not fed before the experiment. The result is shown in Table 6.9. Neither the mean length nor the mean breadth of faecal pellets from animals fed and those not fed prior to the experiment showed any significant difference (Table 6.9).

Table 6.9

Measurements of faecal pellet sizes from stage II *E.modestus* nauplii, after a four hour period feeding on *S.costatum*.

	Mean length (μm)	t	Mean breadth (μm)	t
Larvae fed before experiment	55.00±5.441	0.3136	27.19±2.492	0.5338
Larvae not fed before experiment	54.19±5.796		27.875±3.065	

Mean measurements ± standard deviation from 20 observations. t = Small sample t-test value for comparison between mean length and breadth of pellets from previously fed and unfed larvae. t = 2.101 for significance at the 5% probability level with 18 degrees of freedom.

Table 6.10

The volume reduction from ingestion to defaecation of Skeletonema cells eaten by stage II *E.modestus* nauplii.

	Larvae fed before experiment			Larvae not fed before experiment		
	μm^3 Ir /1/hr	μm^3 Dr /1/hr	Dr/Ir	μm^3 Ir /1/hr	μm^3 Dr /1/hr	Dr/Ir
Experiment A						
Over 4 hours	459,576	180,316	0.3924	839,635	153,857	0.1832
Over 24 hours	126,263	57,492	0.4553	203,901	50,959	0.2499
Experiment B						
Over 4 hours	124,840	32,993	0.2643	489,758	75,132	0.1534
Over 24 hours	150,347	49,979	0.3324	164,929	58,680	0.3558

Ir= ingestion rate(μm^3/larva/hour). Dr= defaecation rate(μm^3/larva/hour). Ingestion and defaecation rates are shown as the mean volume of algae ingested and the mean volume of faecal pellets released respectively. Dr/Ir is the ratio of defaecation rate to ingestion rate.

The mean volume of the E.modestus faecal pellet while feeding on Skeletonema will approximate to that of a cylinder as the pellets were shaped like a very blunt cigar. Thus the volume of the pellet could be estimated as 32,666 μm^3 for a cylinder of radius 13.8 µm (half the mean breadth) and height 54.6 µm (the mean pellet length).

The contents of the faecal pellet were seen as a granular mass which was coloured a light browney - green. Very few whole Skeletonema cells were noticed in any of the faecal pellets counted. The formidable looking gnathobases of the antennae and mandibles described by Rainbow and Walker (1976) for the morphologically similar nauplii of B.balanoides, are responsible for forcing the food into the mouth of the nauplius and are obviously quite efficient at cracking open siliceous algal cells, even such small cells as those of Skeletonema.

The reduction in volume from the ingested ration of S.costatum to the production of faecal pellets by E.modestus nauplii has been roughly assessed in Table 6.10. The ratio of the faecal pellet volume released per larva per hour to the volume of the hourly Skeletonema ration per nauplius (Dr/Ir Table 6.10) was lower for animals which had not fed prior to the experiment, when measured over the first four hours. This result was most probably due to the fact that these larvae started the experiment with completely empty guts which needed to be filled with algae before faecal pellets could be released. Animals which had been fed before the experiment, and therefore had full guts, could defaecate from the start of the experiment and their measured defaecation rate would tend to be higher. Such a difference would thus be more noticeable when measured over 4 hours, when total volumes involved were less, than over 24 hours when the initial discrepancy in gut

contents would represent a much smaller proportion of the total volume involved in the calculations. Inspection of Table 6.10 would suggest, therefore, that the ingested Skeletonema cell volume was reduced to one third, due to assimilation of nutrients, crushing and packing of the silica cell walls, and other losses during its passage through the nauplius' gut.

Conclusions

The results of this study on the feeding of E.modestus stage II nauplii on Skeletonema concur with the investigations of Sushchenya (1962, 1964) on Artemia and Corner et al (1972, 1976) on Calanus to show that the feeding rates of several zooplanktonic herbivores vary with food concentration in a manner that is best described by an asymptotic function, similar to that described for fish feeding by Ivlev (1945, 1955). Jones (1978) gives the only other detailed study of the feeding rate of E.modestus larvae and her results are tabulated, with the results from this study for comparison, in Table 6.11. The measurements of Jones (1978) showed a constant feeding rate for Elminius feeding on Rhodomonas baltica over the range of concentrations used. The rates were measured over an 18 hour period, hence the most direct comparison with the data reported in the present study can be found from the measurements made over 24 hours, described in Table 6.8. Jones calculated the carbon content of R.baltica cells from the equation of Strathmann (1967) and her results can thus be expressed as a rate of carbon ingestion per nauplius (Table 6.11). Cushing and Nicholson (1958) measured the carbon content of Skeletonema cells by a radioactive labelling technique and related the carbon content to cell volume. The cell volumes were calculated from cellular dimensions and the relationship between carbon content and cell volume given as follows

Table 6.11

Comparison of the feeding rates of E.modestus stage II nauplii feeding on Skeletonema and Rhodomonas.

	Jones (1978)	This Study
Food alga	Rhodomonas baltica	Skeletonema costatum
Temperature ^{o}C	20	18 - 20
Experimental duration (hours)	18 - 24	24 (Expts A+B Tab. 6.8)
Algal concentration (cells/µl)	86 - 240	117 - 250
Mean feeding rate (cells/larva/hour)	371±110	3176±1153
Carbon content/cell (g Carbon)	5.7×10^{-11}	6.6×10^{-12}
Carbon ingestion rate (gC/larva/hour)	$2.115 \pm 0.627 \times 10^{-8}$	$2.096 \pm 0.76 \times 10^{-8}$

The ingestion rates are given as means ± standard deviation of the mean. The carbon content of Skeletonema was derived from the equation of Cushing and Nicholson (1958) and that for Rhodomonas from the equations of Strathman (1967).

$$\text{Carbon } (g \times 10^{-12})/\text{cell} = \text{Cell volume } (\mu m^3)/7.7 \quad .....(a)$$

Equation (a) was used to estimate the carbon content of the Skeletonema cells in Table 6.11 and hence the rate of carbon ingestion by the nauplius. As can be seen from Table 6.11 there was close agreement between the results obtained by Jones (1978) using Rhodomonas as the algal food and those reported here using Skeletonema.

Selection of algal sizes by Elminius

When E.modestus stage II nauplii were fed on mixtures of algal species the larger cells, i.e. Tetraselmis and Skeletonema, were selected in preference to the smaller cells, i.e. Pavlova. It has commonly been reported that planktonic herbivores, such as Acartia (Hargrave and Geen 1970, Wilson 1973), Diaptomus (McQueen 1970) and Calanus (Frost 1977), select larger algal cells when feeding on mixtures of algal species. As part of a study of mixed plankton cultures feeding on natural phytoplankton populations from Southampton Water, Barlow and Monterio (1979) have shown that when stage III and IV larvae of Elminius predominate in the mixed zooplankton cultures larger algal species tend to be selected. Barlow and Monterio (1979) considered that the theoretical filtration efficiency (see Nival and Nival 1976) based on the intersetular distances of the net formed by the innermost setae of the antennae and mandibles, could account for the observed selection of larger algal cells from natural phytoplankton cultures. From the two mixture experiments described in the present study the general trend was for Tetraselmis (cell volume 303 μm^3) to be selected to a slightly greater degree than Skeletonema (cell volume 50 - 300 μm^3 i.e. 1 - 6 cells per chain) and both to be selected in preference to Pavlova

(cell volume 21 μm^3). No preference for Skeletonema chains of different lengths was demonstrable, however, suggesting perhaps that cells greater than 50 - 100 μm^3 in volume are equally well handled, yet cells below 50 - 100 μm^3 are not so readily captured by E.modestus stage II larvae.

Opportunistic feeding by Elminius larvae

The Elminius nauplius is a pelagic disseminating vehicle designed to acquire sufficient energy from the environment to maintain it's dispersive exsistance and to complete metamorphosis to the post larval stage on arrival (see Crisp 1976). E.modestus nauplii feed on the phytoplankton of coastal waters from the second stage nauplius through four further moults, before finally moulting to the non-feeding settlement stage, the cypris larva. The food gathered by the various naupliar stages provides sufficient stored material, probably in the form of neutral lipid as is the case for the larvae of B.balanoides (Holland and Walker 1975), to enable the cypris larvae to explore possible settlement sites, cement themselves to suitable substrates, and metamorphose into juvenile barnacles. The larvae have to feed and grow in a hostile environment where predators abound and the patchiness of the food supply is often quite marked (Cushing 1962, Parsons et al 1967, Strickland 1968). The feeding strategy of E.modestus nauplii would appear to serve them well in such an uncertain environment. They can feed at a fast rate when conditions are favourable yet can adjust their feeding rate as they become satiated or if conditions change dramatically. Although the larvae select larger algal cells from mixtures, diatoms and flagellates within a range of cell volumes from 20 - 300 μm^3 can be ingested from monocultures of algae by E.modestus larvae in significant quantities. Crisp (pers. comm.) has observed barnacle nauplii feeding on the planktonic

eggs of the lugworm, Arenicola, thus further emphasising the variety of food sources which may be available to the opportunistic barnacle nauplius.

Section 7

Swimming Movements of the Cypris Larvae of Balanus balanoides

Swimming Movements of the Cypris Larvae of Balanus balanoides

Introduction

The barnacle nauplius is the first dispersive phase of the animal's life history. Nauplii feed whilst in the plankton and when sufficient food reserves have been accumulated the final nauplius stage moults to a non-feeding cypris stage. Cypris larvae can remain planktonic for some considerable time in the laboratory (Holland and Walker 1975), although their capacity for settlement and successful metamorphosis to the adult form decreases after 3 to 4 weeks as the reserves diminish. As the presettlement stage of the barnacle, cyprids possess sensory and physical capabilities which enable them to locate settlement sites and to hold fast to carefully selected sites before cementing themselves to the substrate prior to metamorphosis (Crisp 1974).

Many of the previous studies of barnacle larvae have been directed towards the cypris stage, particularly at settlement (e.g. Knight-Jones 1953, Crisp 1961, Crisp and Meadows 1962,1963). The direction of such studies was initially dictated by a great interest in the fouling of ships and marine installations (Knight-Jones and Crisp 1953). Studies of the planktonic life of the cyprid are less relevant to commercial problems and have been more scanty. Much anatomical information has been gathered using light and electron microscopy suggesting that cypris larvae carry many sensory structures which are particularly densely packed on, and around, the antennular attachment discs (Nott 1969, Nott and Foster 1969, Gibson and Nott 1971, Walker 1974, Walker and Lee 1976). The compound eyes are a prominent feature and the animals can respond to very low light intensities

(Crisp and Ritz 1973). Free swimming cyprids have a distinct pressure sense which enables the animals to respond to absolute and pressure pulses (Knight-Jones and Qasim 1967). As with all but a few of the studies on planktonic organisms, group responses have been used to elucidate the planktonic behaviour of the cyprid. The settlement behaviour of the exploring cyprid has been well documented (Knight-Jones and Stevenson 1950, Knight-Jones 1953, Crisp 1961) but little is known of the swimming capabilities of cypris larvae, apart from the ingenious swimming velocity measurements of Crisp (1955). The swimming behaviour of planktonic organisms may be recorded by ciné cameras, but they rely for the most part on fortuitous footages of film where the organism has performed the behaviour pattern required within the microscope field of view at exactly the right moment (e.g. Lowndes 1935, Vlymen 1970, Strickler 1977). In the following account the swimming behaviour of barnacle cyprids has been investigated using video-tape recording as an alternative to ciné film and a suction restraint technique for more convenient and accurate observation of the swimmming movements of the larvae.

Methods and Results

Cypris larvae of the barnacle B.balanoides were collected from the Menai Strait, North Wales, by medium mesh plankton nets. The animals were maintained in the laboratory at 15°C in 3 litre glass jars of vigorously aerated, fine-filtered, ultra-violet irradiated seawater (see 'Materials' Section 1 above).

(1) Free swimming larvae

Varying numbers of cypris larvae were placed in a perspex vessel with clean fine-filtered seawater. The temperature of the vessel was maintained at 15°C by circulating water from a constant temperature bath around a water jacket enclosing the observation vessel. All observations were made in a darkroom and the vessel was illuminated from either side by two 12" fluorescent strip lights establishing even lighting conditions suitable for television recording. Video-recordings of cyprid swimming behaviour were made over several hours using the highest magnification of the camera's zoom lens. It was found that cypris larvae swam only occasionally in still water, hence lengthy periods of recording were necessary to ensure that as wide a range of movement as possible was recorded. Crisp (1955) also noted that cyprids were relatively inactive in still or slow moving water.

The video recordings were analysed at slow playback speeds. Events were timed by stopwatch, distances measured by vernier calipers, and angles by protractor. Great care was exercised to ensure that the movements analysed were all in the same plane. Records for cyprids moving out of focus on the T.V. monitor were discarded, as such movements represented a deviation in the horizontal plane towards or away from the camera. Measurements were made only on animals moving in the visual plane of the camera. The necessary high magnification of the zoom lens reduced the depth of focus to a few millimeters, hence animals moving out of the focal plane to which the camera was set could quickly be detected.

The resolution of the T.V. monitor image was such that only basic features of cyprid behaviour could be noted, namely, whether the animal was swimming or not, and whether the carapace was open or closed. Movements of the antennules or caudal appendages etc. could not be clearly observed under these conditions. The following aspects of cyprid movement were determined for free moving larvae.

(a) Passive descent in the water column

The angle at which the long axis of the cyprid was inclined to the vertical while sinking passively in the water column was determined from video-recordings. Suitable sections of tape were played back frame by frame and lines were drawn, on a perspex sheet covering the monitor screen, through the long axis of the images of cyprids as they sank. The long axis of the cyprid was defined as a line joining the posterior to anterior tips of the carapace. The observation vessel was aligned to the vertical with a spirit level before observation, hence the side wall of the vessel was taken as the vertical reference. The angle between the extrapolation of the cyprid long axis and the vertical reference was measured with a protractor. A note was made for each observation describing the attitude of the cyprid's thoracic limbs; either protruding from, or enclosed within, the carapace.

All the cyprids observed sank in the water column with the body inclined at an angle to the vertical and led by the posterior tip of the carapace which was always below the anterior tip. Many of the cyprids appeared to spiral down in the water, rotating about the leading posterior end of the carapace. The angles were, therefore, always measured when the long axis of the cyprid was parallel to the plane of the T.V. camera lens

(see Fig 7.1). The results of the observations are shown in Fig 7.1. Unbroken lines outline a cyprid with carapace open and limbs protruding, drawn with the long axis (ANT to POST) inclined to the vertical with the mean angle measured from 25 animals. The broken lines show a similar outline for a cyprid with carapace closed and limbs withdrawn, with the long axis inclined to the vertical with the mean angle measured from 18 animals. Animals with carapace open sank with the long axis at a slightly greater angle (θ) to the vertical. The difference between the means for animals showing limbs protruding from the carapace and those with limbs enclosed within the carapace was significant at $p < 0.001$ (Table 7.1 (a)).

The rate of passive descent of live cyprids in the water column was also measured from video-recordings. The distances over which the cyprids were timed varied from 5 to 20 mm. Times were taken only of cyprids that had fallen some 20 cm. straight from a mark at the top of the observation vessel. They were again classified into two groups, either with carapace open and limbs protruding or with carapace closed and limbs withdrawn. The result is shown in Table 7.1 (b). The rates of descent are shown as the mean velocity in cm s^{-1} of 14 animals for larvae with carapace closed and 21 larvae with carapace open. A small sample t-test of the difference between means shows that the probability of obtaining the observed difference by chance was < 0.001. Thus, although the difference between means was only 1 mm s^{-1}, larvae descending at a steeper angle with the carapace closed sank faster than larvae with limbs protruding from an open carapace.

Fig 7.1

A diagram showing the attitude of B.balanoides cyprids sinking passively in the water column. Unbroken lines denote a cyprid sinking with carapace open and the long axis of the cyprid (ANT - POST) inclined at an angle (θ) of 57^{o}, the mean of 25 observations. Broken lines denote a cyprid sinking with carapace closed at an angle of 45^{o}, the mean of 18 observations.

a = antennule.

ca = caudal appendages.

ce = compound eye.

ls = setae of the thoracic limbs.

Fig 7.1 Attitude of B.balanoides cyprids sinking in the water column

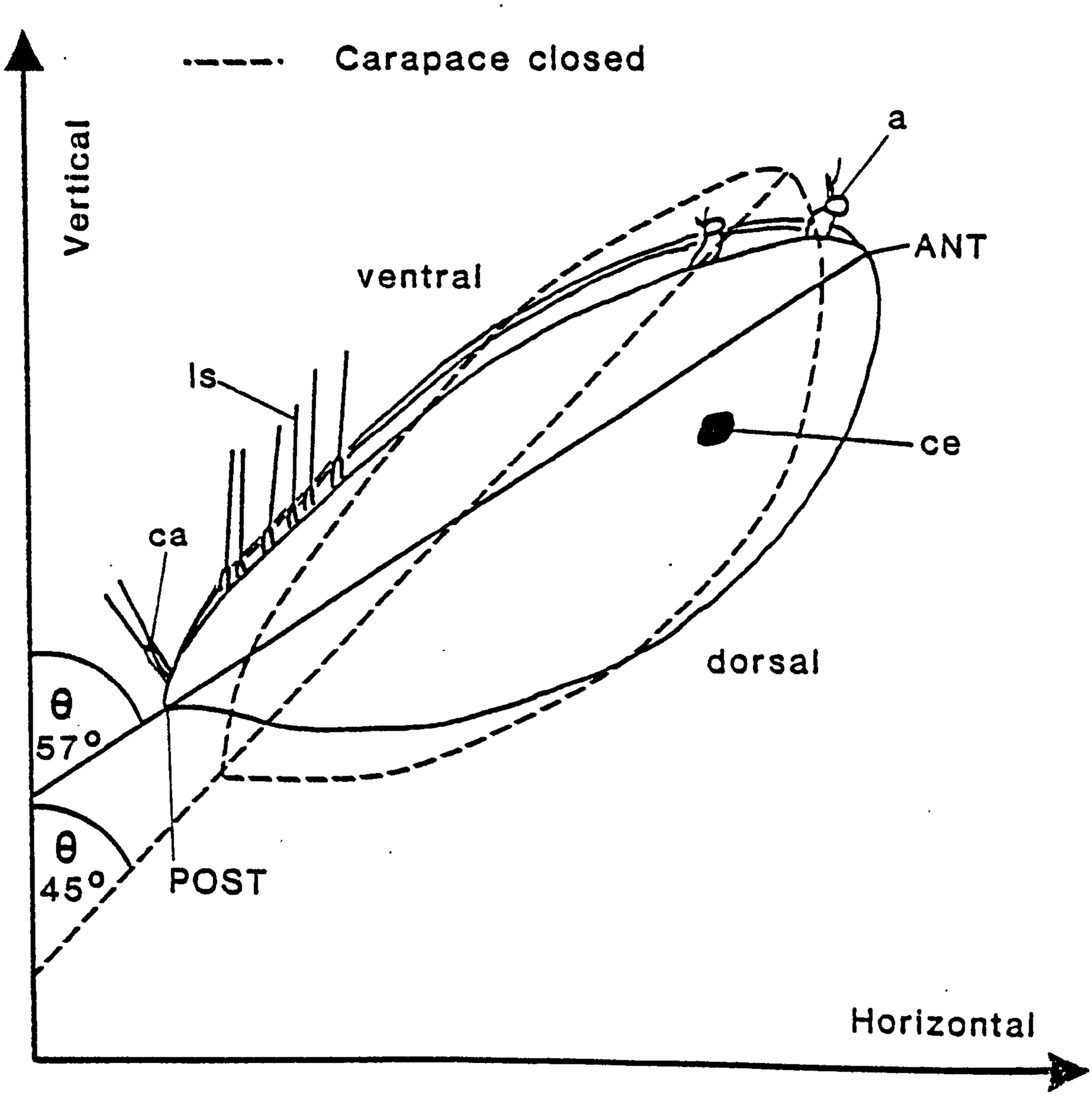


Table 7.1

Observations on free swimming B.balanoides cyprids.

(a) Inclination to the vertical of sinking cyprids

Mean angle between long body axis and vertical (See Fig 7.1)

Carapace open θ = 57.34±6.54° (±St. deviation)

Carapace closed θ = 44.7±5.19° (± St. deviation)

Small sample t-test between means :- t = 6.767 for 41 degrees of freedom ($p<0.001$).

(b) Passive rate of descent

Sinking rate

Carapace open = 0.44±0.05 cm s^{-1} (±St. deviation)

Carapace closed = 0.54±0.05 cm s^{-1} (±St. deviation)

Small sample t-test between means :- t = 5.9143 for 33 degrees of freedom ($p<0.001$).

(c) Velocity measurements

(1) Large displacement measurements

Average speed = 3.124 cm s^{-1} (46 observations of 23 larvae)

Range of speeds observed = 1.33 - 6.04 cm s^{-1}

(2) Small displacement measurements

Average speed = 5.346 cm s^{-1} (59 observations of 5 larvae)

Range of speeds observed = 1.5 - 9.48 cm s^{-1}

Range of acceleration observed = 9.0 - 183 cm s^{-2}

(b) Active swimming

The swimming speeds of B.balanoides cyprids were measured using slow motion playback facilities on the video-recorder. The position of the anterior tip of the cyprid carapace was marked, at timed intervals, with a fine tipped pen on a clear perspex sheet covering the monitor screen. The distances between the reference marks were measured under a binocular microscope using a micrometer eyepiece.

Initially, observations were made over relatively large distances with the cyprids moving along irregular paths. Later, observations were made over 3 - 5 T.V. frames. The distance moved by the cyprids in less than 3 - 5 frames could not be accurately measured using this technique. The time interval between frames was established as 0.012 seconds by timing, with a stopwatch, a number of frames played back at slow speed. In all cases where small distances were measured, observations were restricted to cyprids moving in straight lines and rising in the water column.

Swimming speed was measured as the average velocity between two reference marks viz. distance between marks divided by time, and expressed in cm s^{-1}. The acceleration of cyprids could also be estimated from several of the small displacement measurements as

$$a = (v_2 - v_1)/(t_1 - t_2) \quad \text{cm } s^{-2}.$$

where a = acceleration, v_1 and v_2 represent the average cyprid velocity at times t_1 and t_2 respectively. Table 7.1 (c) gives a resumé of the results obtained. The initial observations over larger distances gave a mean speed from 46 observations of 3.124 cm s^{-1} and the maximum observed cruising speed was 6.04 cm s^{-1}. The speeds observed for the shorter bursts

over smaller distances were greater, with a mean of 5.346 cm s^{-1} and a maximum speed of 9.48 cm s^{-1}. Cyprid swimming bursts tend to be quite short and irregular, especially in still water, thus a short time period between displacement measurements will give a more accurate assessment of the cyprids' swimming capabilities. The analysis of the results over larger distances was therefore carried no further.

The results for smaller displacement measurements are shown in detail for the movements of four cyprids in Figs 7.2 - 7.5. The original data consists of a series of displacement measurements from the larvae at different times. These displacements have been plotted cumulatively from zero, which has been taken as the position of the cyprid when measurement started (Figs 7.2 - 7.5 ▲). The average velocities of the cyprids between points are shown as dots (Figs 7.2 - 7.5 ●) joined by a broken line. Estimates of average velocity over small distances are by nature discontinuous but the velocity of the cyprid between displacement measurements is probably continuously variable. Lehman (1977) suggested that more appropriate estimates of continuous changes in velocity with time for small planktonic organisms could be obtained from the first differential of the cumulative displacement curve. The solid curve fitted through the points of cumulative displacement (Figs 7.2 - 7.5 ▲) is a polynomial expression fitted by a computer multiple regression analysis of displacement on time. Solid curves fitted through the points calculated for average velocities (Figs 7.2 - 7.5 ●) represent the first differential of the polynomial fit to the cumulative displacement points, i.e. cyprid velocity against time.

Figs 7.2 to 7.5

The swimming performance of B.balanoides cyprids in still water. The cumulative displacement curve (left hand axis) is shown (▲), and the curve fitted to a polynomial regression of cyprid displacement against time. The right hand vertical axis shows cyprid velocity, where dots (●), joined by broken lines, indicate the average velocities measured between displacement points, and the solid curve represents the first differential of the fitted displacement curve. The cumulative displacement and first differential curves have been drawn by computer.

Fig 7.2

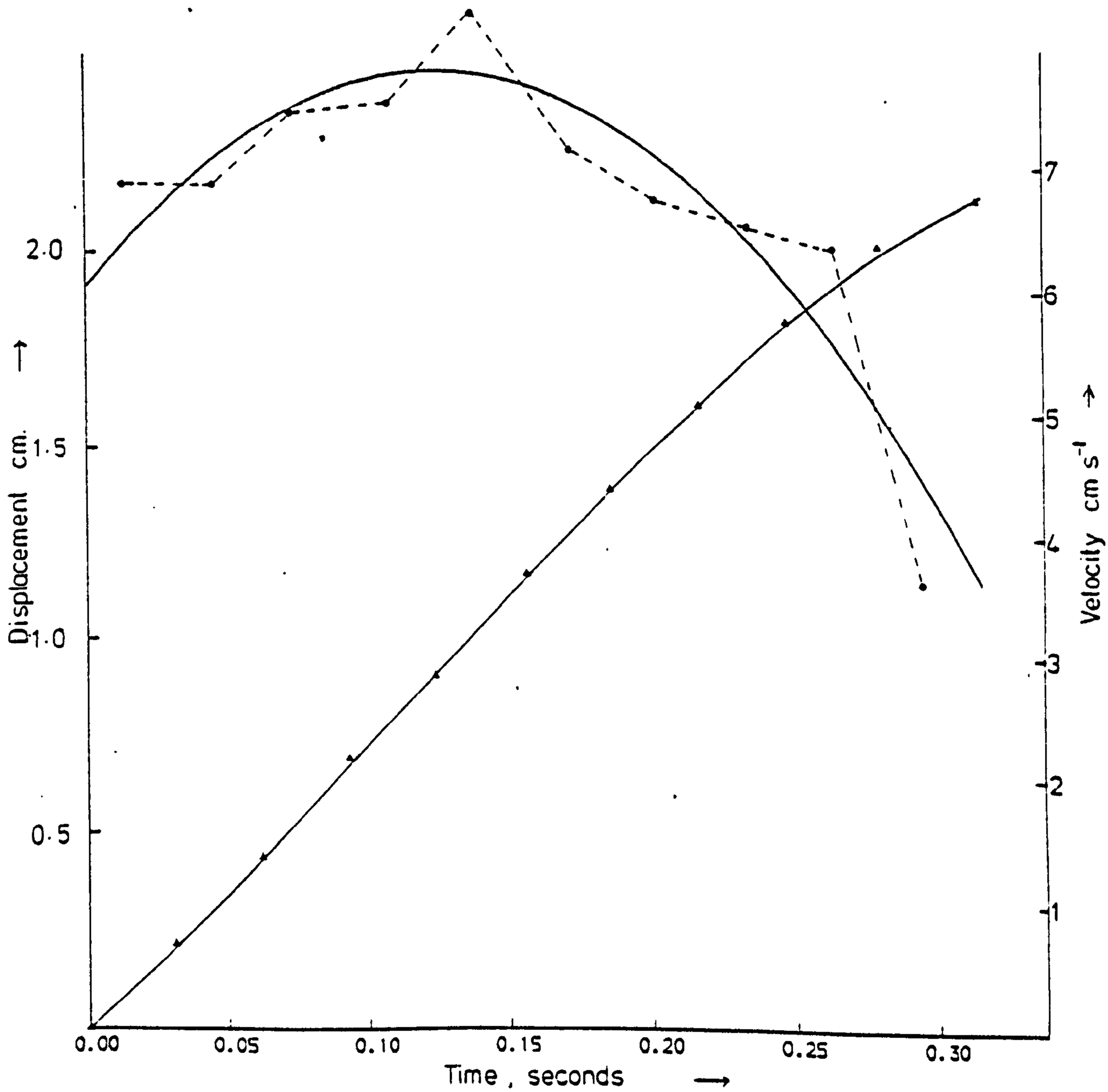

Displacement cm. →
2·0
1·5
1·0
0·5
Velocity cm s⁻¹ →
7
6
5
4
3
2
1
0.00
0.05
0.10
0.15
0.20
0.25
0.30
Time , seconds ⟶

Fig 7.3

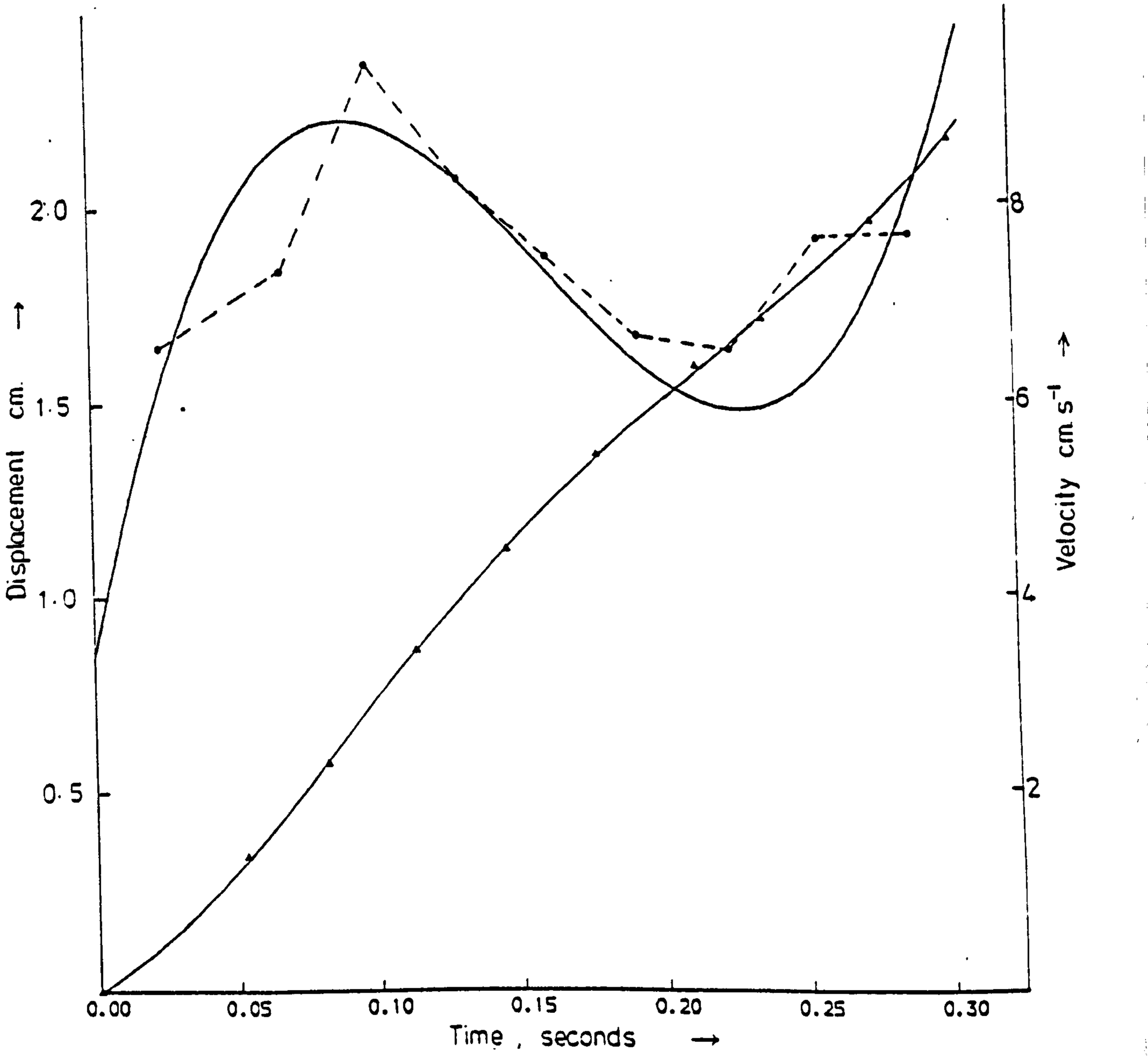

2·0
1·5
1·0
0·5
Displacement cm. →
8
6
4
2
cm s⁻¹ →
Velocity
0.00
0.05
0.10
0.15
0.20
0.25
0.30
Time , seconds →

Fig 7.4

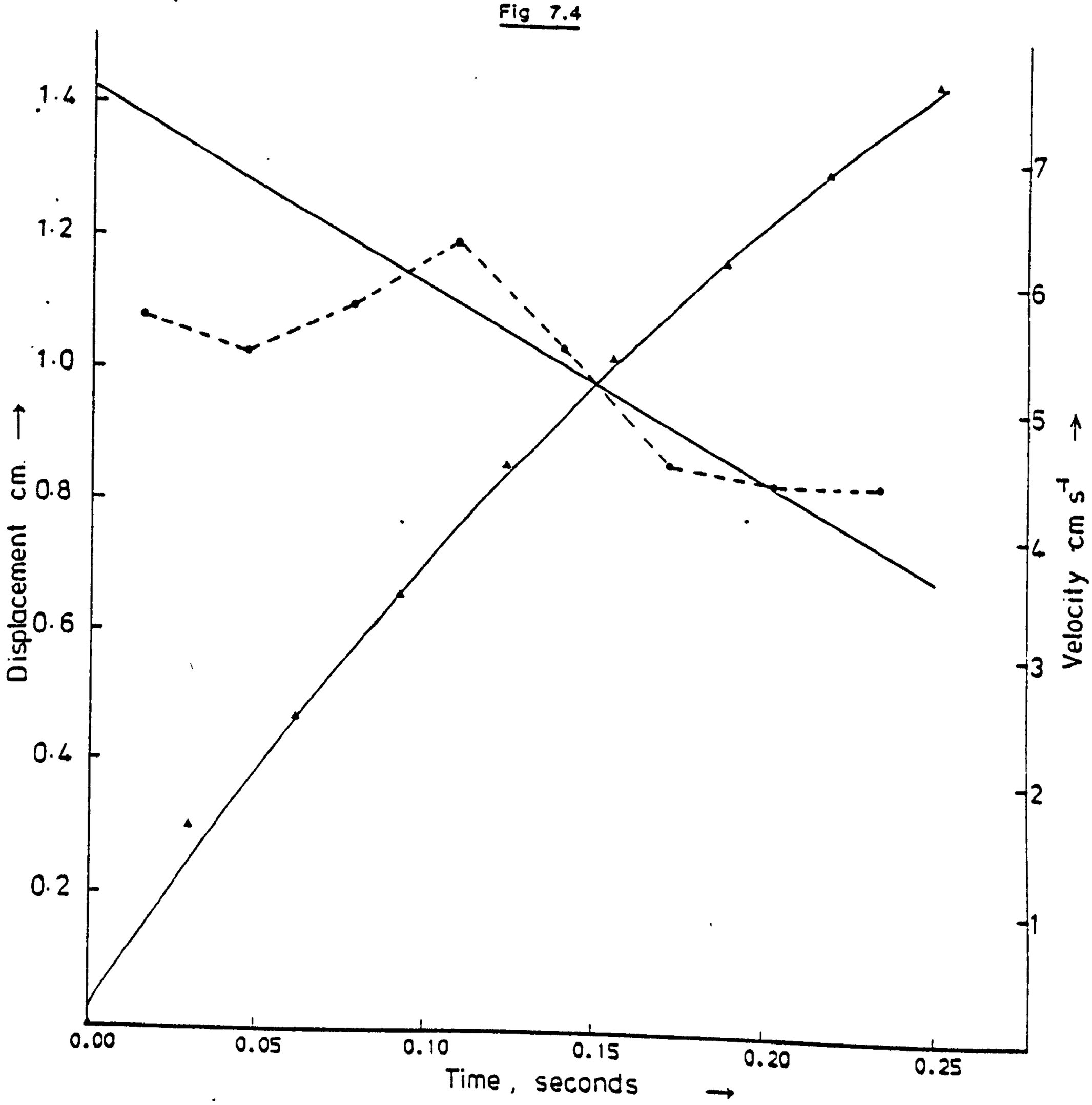
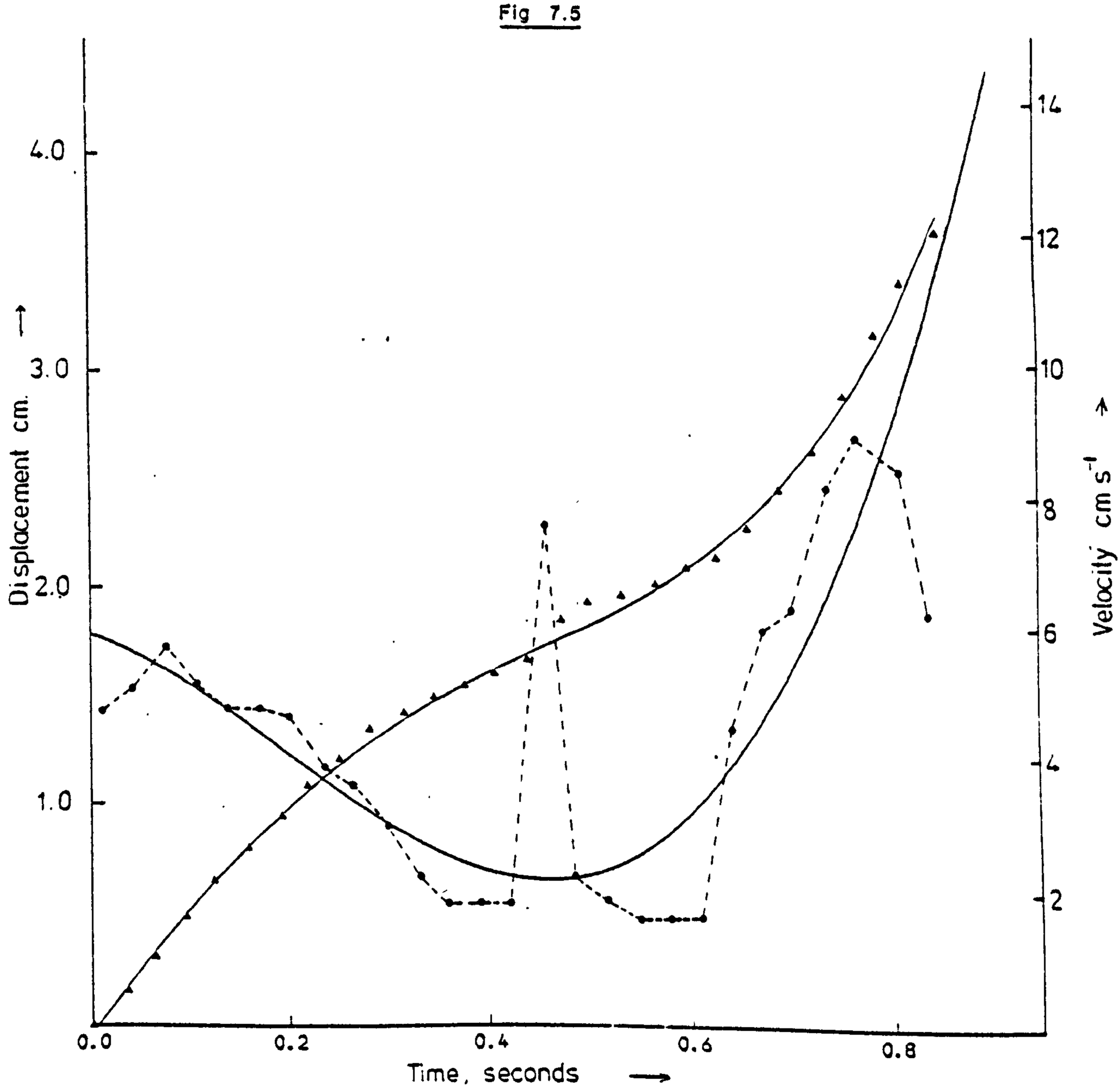

1.4
1.2
1.0
0.8
0.6
0.4
0.2
Displacement cm →
7
6
5
4
3
2
1
Velocity cm s⁻¹ →
0.00
0.05
0.10
0.15
0.20
0.25
Time , seconds →

Fig 7.5


Displacement cm. →
Velocity cm s⁻¹ →
4.0
3.0
2.0
1.0
14
12
10
8
6
4
2
0.0
0.2
0.4
0.6
0.8
Time, seconds ⟶

In some instances this statistical approach to characterising the swimming velocities of cyprids gives good agreement with the points calculated for average velocities i.e. Figs 7.2 and 7.3. The second differential of the polynomial displacement curve would then give a very reliable estimate of the acceleration of the cyprid at any time. In other cases, however, the variation in velocity with time was too large to be adequately described statistically by these methods e.g. Fig 7.5. The sudden acceleration of the cyprid shown by the rapid increase in velocity halfway through the record (Fig 7.5) was based on only a pair of points. Had the resolution of the video-recording allowed for measurements of even smaller displacements over shorter time periods, more points on the cumulative displacement curve could have been obtained and separate sections of the data more adequately handled by Lehman's (1977) method.

The jerky nature of cyprid swimming is well illustrated by the result of Fig 7.5. Swimming activity occurs in bursts with periods of rapid acceleration and equally rapid deceleration. The greatest acceleration measured for any of the cyprids observed was 183 cm s^{-2} which is also that shown in Fig 7.5.

(2) Restrained larvae

In order to observe the swimming movements of barnacle cyprids more closely, B.balanoides cyprids were restrained within the field of view of a microscope using a suction device (Section 1, 'Methodology' above). They were maintained in flowing seawater at 15°C and their behaviour recorded on video-tape. The suction restraint technique was suitable to use on the hard cyprid carapace, but occasionally a smear of silicone grease around the end

of the suction tip was necessary to ensure a lasting seal. Unlike the nauplius, the cyprid can partially isolate itself from the environment by curling up and closing it's bivalve carapace. The use of anaesthesia to subdue the organism prior to attachment to the suction tip (Section 1, 'Methodology' above), therefore, became very unpredictable and was eventually dispensed with. It was found more convenient to develop a rapid attachment procedure during the few seconds taken by the cyprid to recover from being dropped into the observation chamber.

A micro-impedance pneumograph technique (Section 1, 'Methodology' above) was used to obtain recordings of the movements of the posterior pair of thoracic limbs. The two wires of the pneumograph electrode were placed on either side of the posterior end of the cyprid carapace such that the limbs passed between, yet were not obstructed by, the electrodes. It was quickly discovered that the very powerful swimming bursts of the cyprid moved the electrodes, hence, the system was not reliable for long term recording. All long term records of swimming activity were therefore made with video-tape recordings.

(a) General observations of the swimming stroke

Fig 7.6 shows a cypris larva of B.balanoides attached to a suction tube within the field of view of a microscope. Initially, general observations were made by plotting the position of the limbs on a clear perspex sheet laid over the monitor screen. The position of the thoracic limb bases, limb tips and the tips of the setae were marked at the beginning and end of the stroke and followed as closely as possible throughout the stroke. Where organisms propel themselves through the water by a rowing type action the

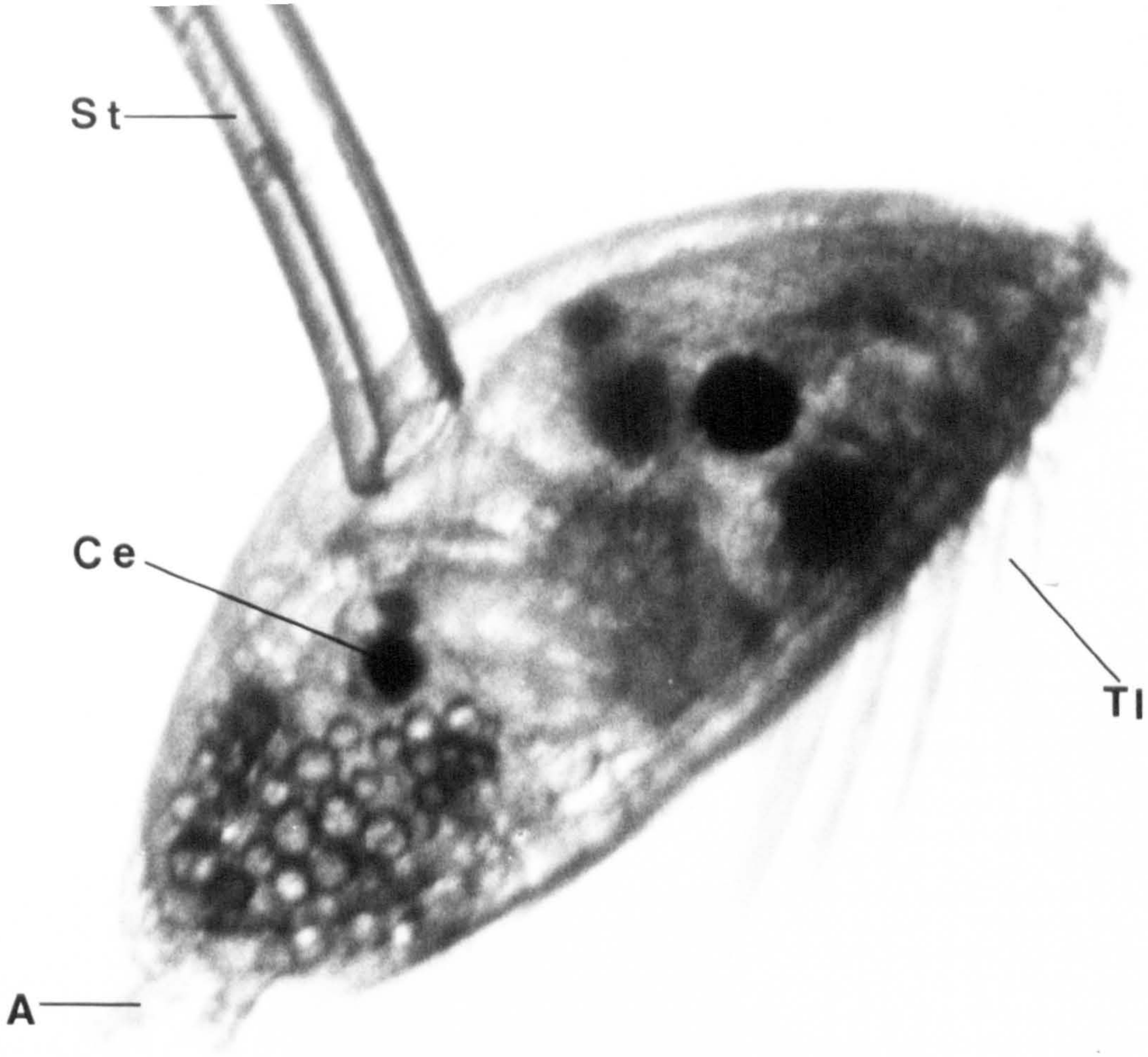


Fig 7.6 Restrained B.balanoides cyprid

A : antennule Ce : compound eye

St : suction tip Tl : thoracic limbs

stroke involves two major components; an initial backward push of the limb with the maximum surface area presented to the water, followed by a recovery phase where the limb is returned to it's original position with a feathering action to reduce the surface area presented to the water.

Fig 7.7 (a) shows a diagram of the external anatomy of the cypris larva of Balanus balanoides and the usual position of the six pairs of biramous thoracic limbs at the start of a swimming stroke. Fig 7.7 (b) shows a side view diagram of 5 successive positions of the posterior pair of thoracic limbs during the propulsive phase of the swimming stroke. The limbs were thrust backwards, starting with the posterior pair and rapidly followed by each succeeding limb pair in turn. The limbs appeared to be held rigid during the stroke while the tips of the setae described an arc about the base of the limb. Five successive positions of the posterior pair of thoracic limbs during the recovery stroke are shown in side view in Fig 7.7 (c). The limb was bent in such a way that the setae were feathered, presenting less surface area to the water than they did during the propulsive stroke. Feathering of the setae was more pronounced over the first half to two thirds of the stroke and the limbs were nearly straight again over the final phase. When viewed from underneath the limbs did not appear to deviate from the medial plane of motion in either the propulsive or recovery strokes.

(b) Impedance pneumograph recordings of the cyprid swimming stroke

The cyprid swimming stroke as described above was not a regular beat and activity occurred in irregular bursts. Fig 7.8 and 7.9 show impedance pneumograph traces recorded from the posterior pair of thoracic limbs. Fig

Fig 7.7

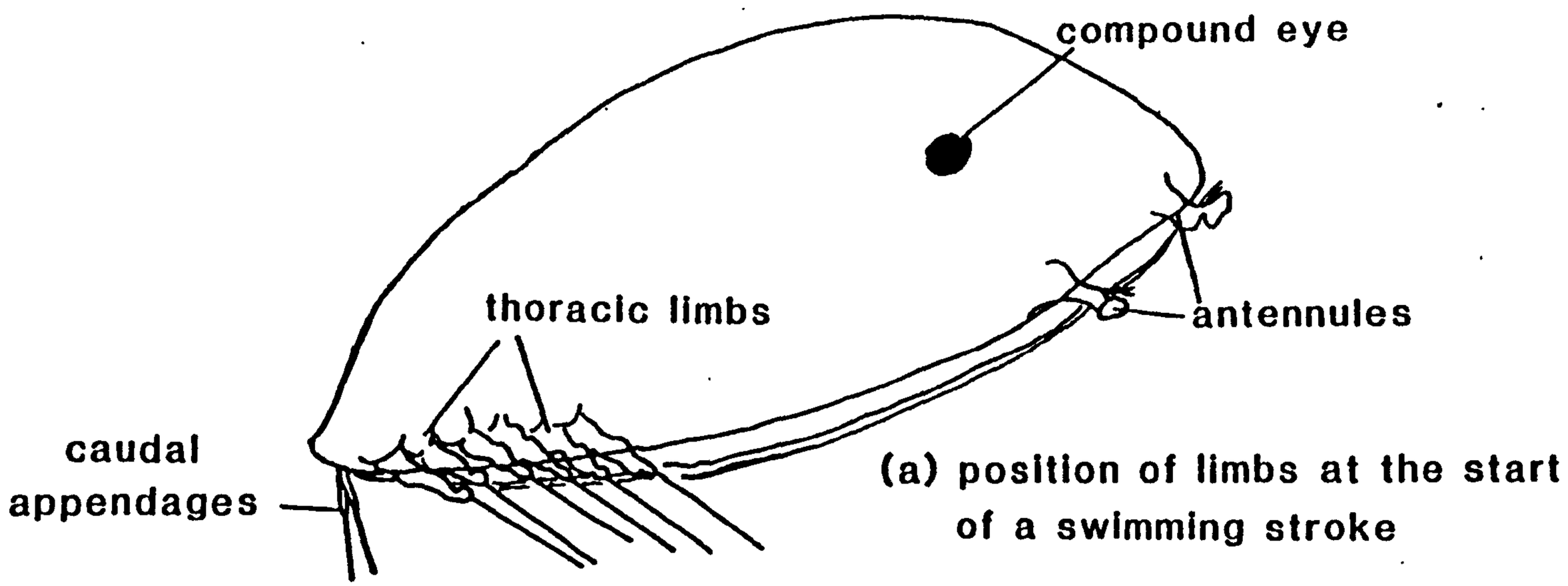
A diagram of the swimming stroke of B.balanoides cyprids.

(a)..the normal position of the cyprid thoracic limbs at the start of the swimming stroke.

(b)..5 successive positions of the posterior thoracic limb during the propulsive phase of the stroke.

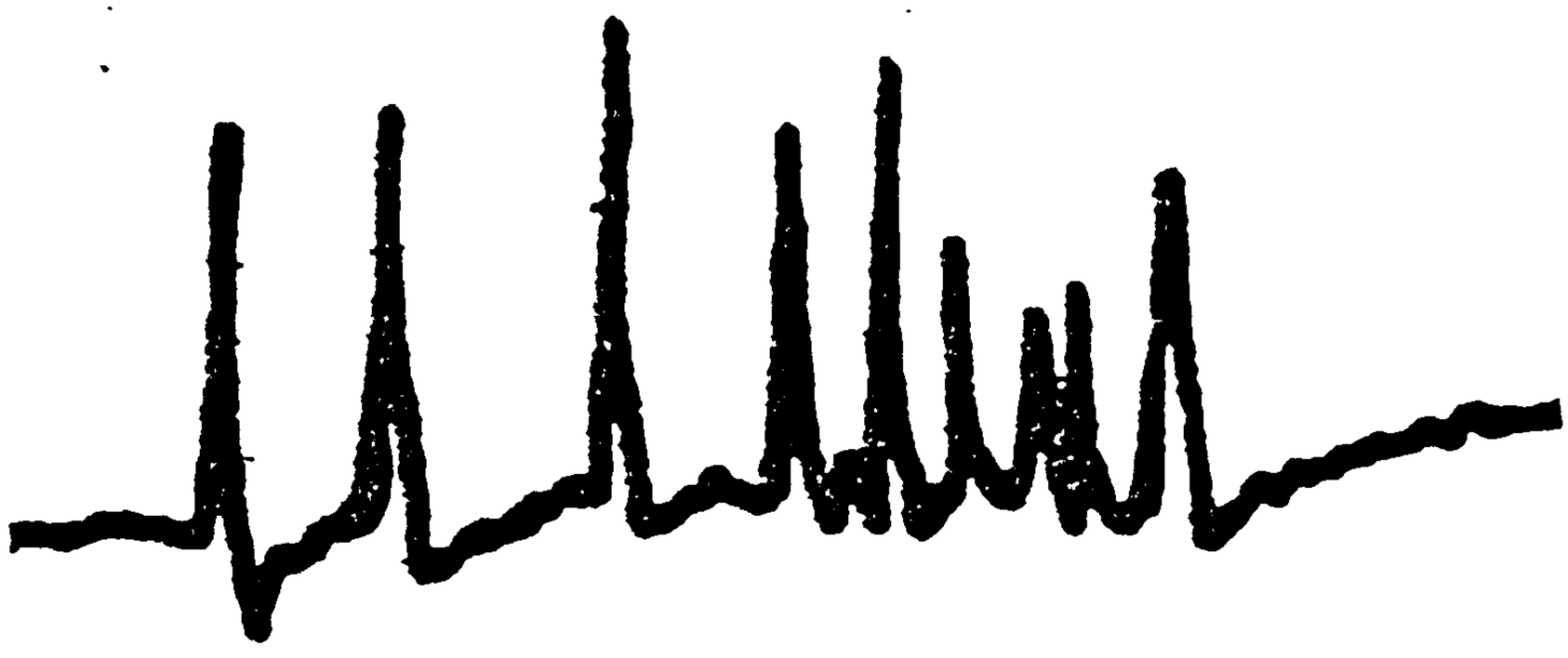
(c)..5 successive positions of the posterior thoracic limb during the recovery phase of the stroke.

Fig 7.7 The swimming stroke of B.balanoides cyprids

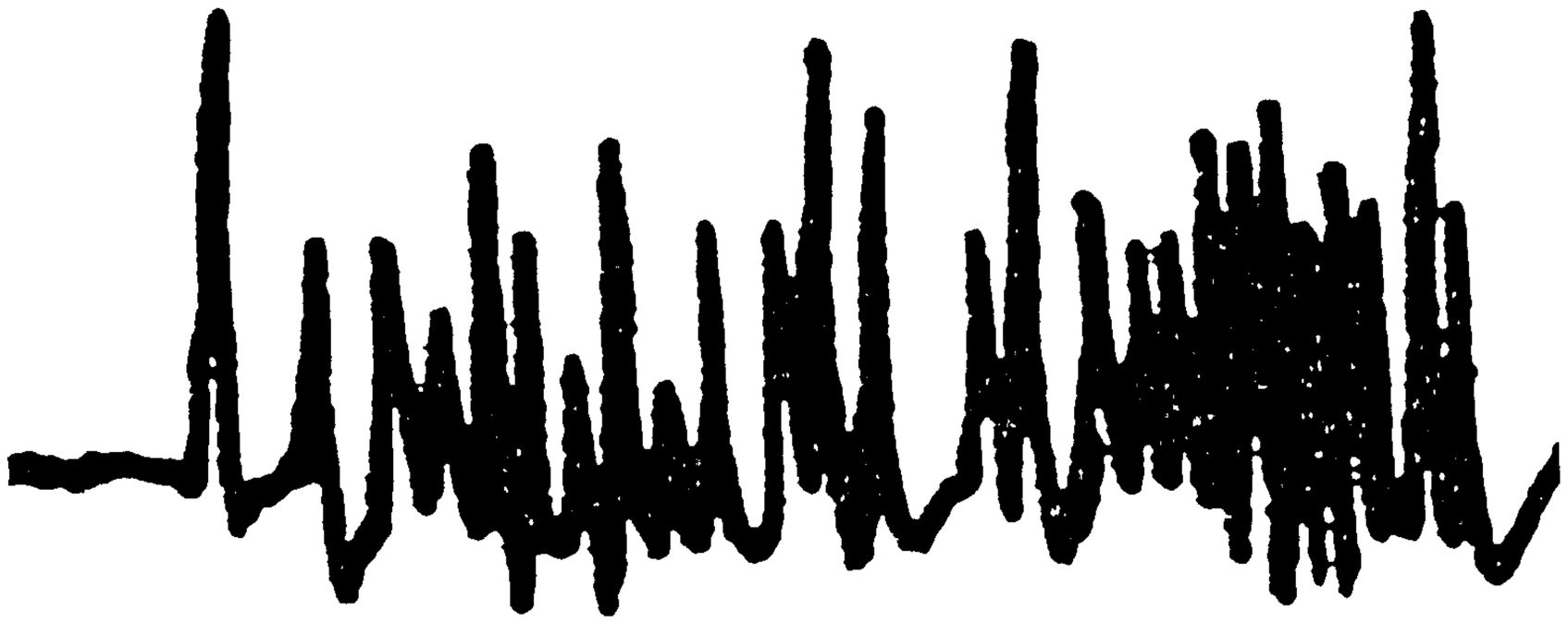


7.8 shows two traces demonstrating the variability in the swimming bursts of the cyprids. Fig 7.8 (a) represents about 2 seconds of swimming activity with only 9 irregularly spaced swimming strokes, whereas Fig 7.8 (b) represents about 2.5 seconds of activity with 26 swimming strokes rapidly following one another. The traces run from left to right with time, hence the initial rise in the trace corresponds to the propulsive stroke and the succeeding fall to the recovery stroke. It was possible to use the traces to obtain a rough estimate of the relative duration of the propulsive and recovery strokes. Fig 7.9 shows yet another trace of the limb movements of a B.balanoides cyprid magnified some 4.2 times from the original trace. The trace runs from left to right with time and the scale is marked in seconds. Again the variability of the swimming activity is shown with a short burst at the beginning of the trace and two isolated strokes towards the end. The pen of the chart recorder moves in an arc, hence the relationship between time and position on the trace could not be simply measured by ruler. A scale model of the recorder pen was made and used to mark the positions of the start of the power stroke (a Fig 7.9), the end of the power stroke (b Fig 7.9) and the end of the recovery stroke (c Fig 7.9). Positions a, b and c were extrapolated to a reference base parallel to the base line of the trace (Fig 7.9). The distances between a, b and c were measured by calipers and thence calibrated in seconds, relative to the recorder time scale. The analysis was made on six traces in all, ranging from 26 measurments per trace for a long period of rapid swimming, to only 4 observations per trace from a short period of moderate activity. The results are shown in Table 7.2 where the mean time for propulsive and recovery strokes measured from each trace is shown $\pm$ the standard error of the mean. A paired sample t-test was performed on the results and in each case the propulsive phase of the stroke was shown to be significantly slower than the recovery phase

FIG 7.8: Pneumograph recordings from the posterior pair of thoracic limbs of B.balanoides cypris larvae.



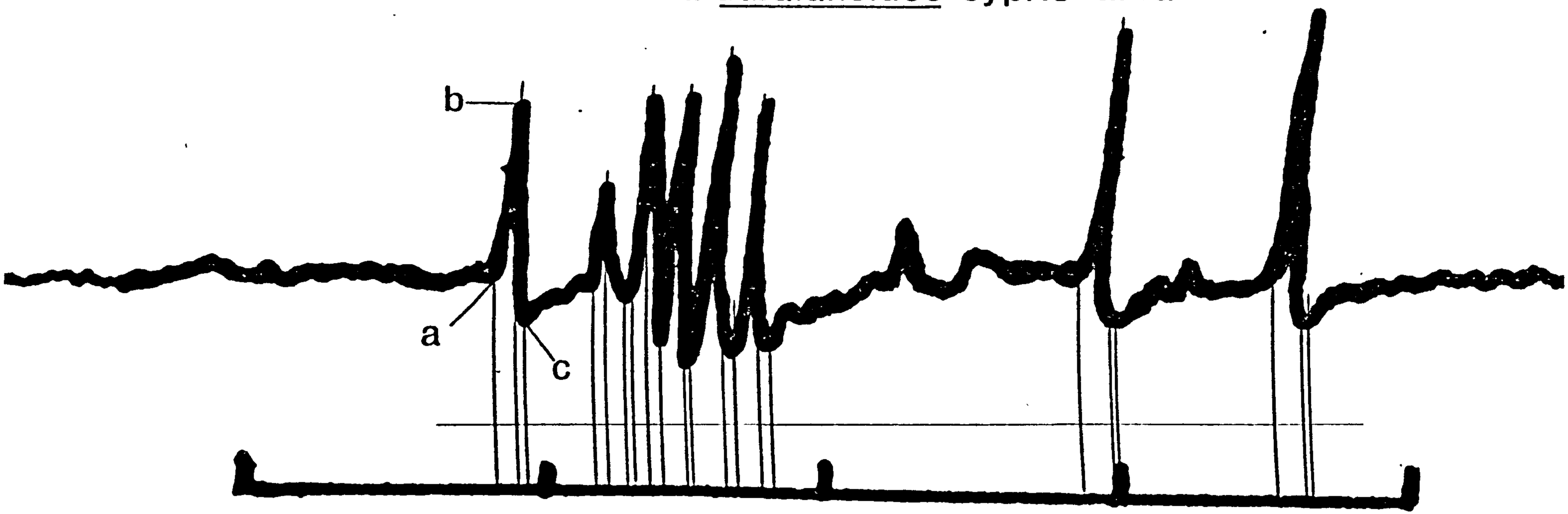
(a)



(b)

1 Second

FIG.7.9 Measurement of a pneumograph recording trace from a B.balanoides cypris larva



a- start of propulsive stroke. b- start of recovery stroke

c- end of recovery stroke

Table 7.2

Analysis of the swimming stroke of the cypris larvae of Balanus balanoides :- the duration of the propulsive and recovery phases of the swimming stroke as estimated from impedance pneumograph recordings.

Trace	Propulsion (seconds)	Recovery (seconds)	Difference (seconds)	t	df	prob	Ratio
1	0.051±0.0033	0.030±0.0024	0.021±0.0043	4.8697	25	0.001	0.600
2	0.073±0.0080	0.033±0.0047	0.040±0.0114	3.5121	8	0.008	0.452
3	0.082±0.0088	0.038±0.0060	0.044±0.0132	3.3417	7	0.012	0.463
4	0.087±0.0085	0.037±0.0067	0.050±0.0136	3.6709	7	0.008	0.425
5	0.075±0.0095	0.029±0.0068	0.046±0.0109	4.2160	6	0.006	0.387
6	0.071±0.0055	0.035±0.0040	0.036±0.0081	4.4482	3	0.021	0.492
						Mean ratio =	0.470
						St. error =	0.003

Values given are means for propulsive and recovery stroke duration on each trace. The Difference is the duration of propulsive stroke - the duration of the recovery stroke. Each mean is shown ± its standard error.

Ratio duration of recovery stroke/duration of propulsive stroke.

t = the value obtained from a paired sample t-test between the duration of propulsive and recovery stroke means.

df = degrees of freedom for the t-test.

prob = the probability of obtaining the observed differences by chance.

(Table 7.2). From Table 7.2 it can be seen that, on average, the duration of the recovery stroke was only half that of the propulsive stroke.

Table 7.3 shows an analysis of variance performed on the mean stroke times per trace for both recovery and propulsive phases of the swimming stroke. The result for the propulsive phase (Table 7.3 (A)) shows that the probability that the means were drawn from the same population of means was low ($p = 0.002$). In particular, a sequential comparison between means showed that the only significant difference was that between the mean for trace 1 (0.051 s) and that for trace 4 (0.087 s). The mean duration of the recovery phase of the stroke, however, showed no significant differences between traces (Table 7.3 (B)), as the probability that the means were drawn from the same population of means was high ($p = 0.669$).

Figs 7.8 and 7.9 show records of cyprid limb beat movements used in the above analysis. The frequency of limb beat was much faster for trace 1 (Fig 7.8 (b)) than for the burst of activity shown in either trace 2 (Fig 7.8 (a)) or trace 3 (Fig. 7.9). The duration of the recovery stroke from all traces has been shown to be more or less constant (Table 7.3 (B)). Thus the cyprid appears to increase the speed of the propulsive phase only of the swimming stroke to increase the frequency of limb beat within any burst of swimming activity.

(c) The force produced by the propulsive phase of the swimming stroke

Many of the restrained cyprids were able to move the suction tube tip during swimming bursts. It was possible, from the video-recordings, to mark the anterior edge of the suction tip before a burst of swimming activity and

Table 7.3

Analysis of the swimming stroke of the cypris larvae of B.balanoides :- analysis of variance for the relative duration in seconds of the recovery and propulsive strokes measured from six pneumograph traces of the limb movements.

(A) The Propulsive Phase

Trace	1	2	3	4	5	6
Mean duration (s)	0.051	0.073	0.082	0.087	0.075	0.071
Standard deviation	0.017	0.024	0.025	0.024	0.025	0.011
No. of observations	26	9	8	8	7	4

Analysis of variance table

Source of variation	Sum of squares	df	Mean square	F	prob
Between traces	1.2412053×10^{-2}	5	2.4824106×10^{-3}	5.8312	0.002
Within traces	2.3839943×10^{-2}	56	4.2571328×10^{-4}		
Total	3.6251997×10^{-2}	61			

Sequential Q comparison between means (e.g. Snedecor and Cochran (1967)

Test	$Q_{0.05}$	(a)	(f)	Diff. required for 5% significance	Observed Difference
Trace 4 - Trace 1	4.16	6	56	0.03470	0.03579
Trace 4 - Trace 6	3.98	5	56	0.05029	0.01575
Trace 3 - Trace 1	3.98	5	56	0.03320	0.03109

Only the difference between the mean for Trace 1 and that for Trace 4 exceeds the calculated difference for significance at the 5% level.

Table 7.3 (cont.)

(B) The Recovery Phase

Trace	1	2	3	4	5	6
Mean duration (s)	0.030	0.033	0.038	0.037	0.029	0.035
Standard deviation	0.012	0.014	0.017	0.019	0.018	0.008
No. of observations	26	9	8	8	7	4

Analysis of variance table

Source of variation	Sum of squares	df	Mean square	F	prob
Between Traces	6.514374×10^{-4}	5	1.3162875×10^{-4}	0.6421	0.669
Within Traces	1.1480082×10^{-2}	56	2.0500147×10^{-4}		
Total	1.2138226×10^{-2}	61			

df = degrees of freedom.

F = F value.

prob = the probability of obtaining such an F value by chance.

Sequential Q (a) = the number of means in the comparison.

(f) = the number of degrees of freedom for the standard error.

The difference required for 5% significance is $Q_{0.05}$ times the standard error for the difference between the means.

measure the distance moved by the tube tip after the first propulsive stroke of a swimming burst. The picture often became blurred and confused during successive strokes, hence only the tip movement caused by the first stroke of a swimming burst was measured. The blurring of the picture and the rebound of the tube tip on the recovery stroke made observation of the force produced on the recovery stroke impossible. The tube tip was then calibrated in terms of the applied force required to move the tip small distances. The tube was held horizontally in a flat sided glass vessel containing seawater. The tip was viewed end on with a stereomicroscope. Very small v-shaped pieces of copper wire were balanced on the end of the tube tip and the distance moved by the tip measured with a micrometer eyepiece. As far as was possible the tube was held such that the weight of the wire was applied in roughly the same direction as the force produced by the swimming cyprids. Each piece of wire was then weighed on the suspension wire of a Chan Electrobalance.

The result of the calibration is shown in Fig 7.10 where the tube tip displacement (D) in μm has been plotted against applied force (F) in Newtons. A regression line fitted to the data by a least squares method gave the following calibration equation

$$F = (D + 1.3746)/11090258.5 \text{ Newtons} \quad \ldots\ldots\ldots(1)$$

with a product moment correlation coefficient of 0.994 for 15 degrees of freedom. The tube tip displacements caused by cyprid swimming were substituted for D in equation (1) to estimate the force produced by the swimming stroke. The results from 111 observations of the force produced by the propulsive strokes of four cyprids are shown in Table 7.4. Estimates of the force produced were very variable, the highest observed force being nearly ten times the lowest (Table 7.4). The range of observed forces was

Fig 7.10 Calibration curve for suction tube displacement against applied force

Regression fitted to :

$$\text{Force} = \frac{\text{displacement} + 1.3746}{11090258.5} \text{ Newtons}$$

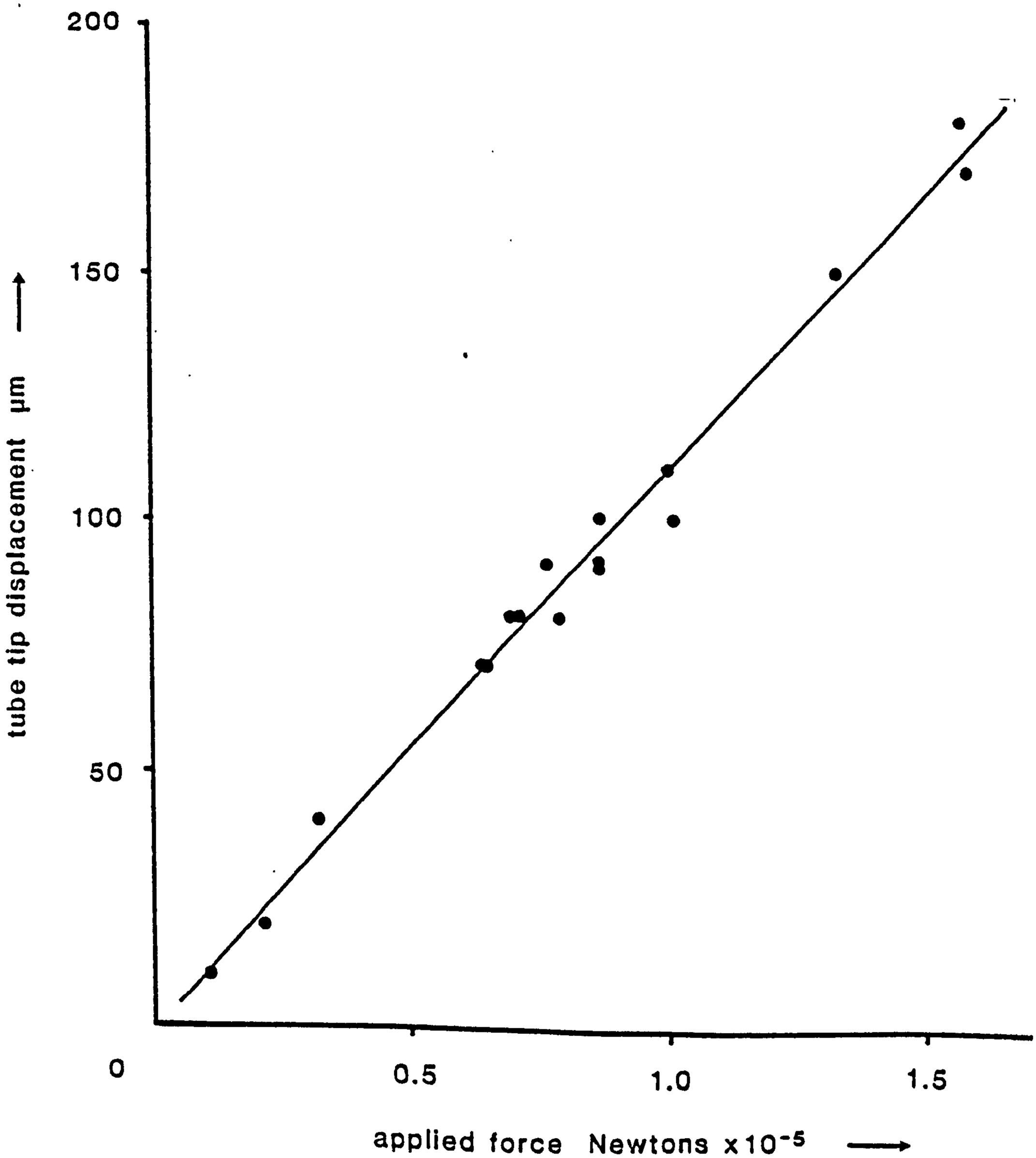


Table 7.4

The force produced by the swimming stroke of B.balanoides cypris larvae.

Force in Newtons $x10^{-6}$

	Observations	Minimum	Maximum	Mean ± standard error
Cyprid 1	30	1.332	3.866	2.236 ± 0.139
Cyprid 2	25	0.927	3.334	1.822 ± 0.145
Cyprid 3	30	0.412	3.677	2.025 ± 0.183
Cyprid 4	26	0.557	3.217	1.801 ± 0.137

Combined Data

Mean ± standard error = 1.9276 ± 0.076 $x10^{-6}$ Newtons.

Range of observations = 0.412 - 3.866 $x10^{-6}$ Newtons.

very similar for all four cyprids, suggesting that the variability shown by the forces produced by a single cyprid was almost as great as that within a group of cyprids.

(d) The direction of thrust from the propulsive phase of the stroke

During the initial observations of the movements of the cyprid limbs it was noticed that the position of the limbs prior to a stroke was quite variable. The posterior part of the cyprid body could be extended beyond the edge of the open carapace or be held within the carapace. The limbs always moved in an arc about the limb bases yet the observed variation in position of the limb bases at the beginning of a swimming stroke would lead to a variation in the average direction of the thrust produced by the propulsive stroke of the limbs relative to the cyprid carapace. The direction of the thrust produced from the swimming stroke of B.balanoides cyprids was therefore measured from video-recordings of several restrained cyprids. Again only the initial propulsive stroke of a swimming burst was used for measurement for the reasons given above. The measurements were made by marking the long axis of a cyprid on a perspex sheet covering the T.V. screen. The position of the limb bases and the tips of the limb setae were also marked at the beginning of the stroke. At the end of the propulsive phase the marked long axis was adjusted, if necessary, to coincide with the image of the cyprid on the screen and the new position of the limb bases and tips of the setae marked.

Fig 7.11 is a diagrammatic representation of the limb movements of a cyprid during a swimming stroke and the positions of the limb bases and setal tips, as marked from the T.V. monitor, at the beginning and end of

Fig 7.11

A diagram showing the limb movements of B.balanoides cyprids as marked from video-recordings.

(a)..shows the positions of the limbs at the start and end of the propulsive stroke, with the direction of movement denoted by arrows.

● = tips of the setae at the start of the stroke.

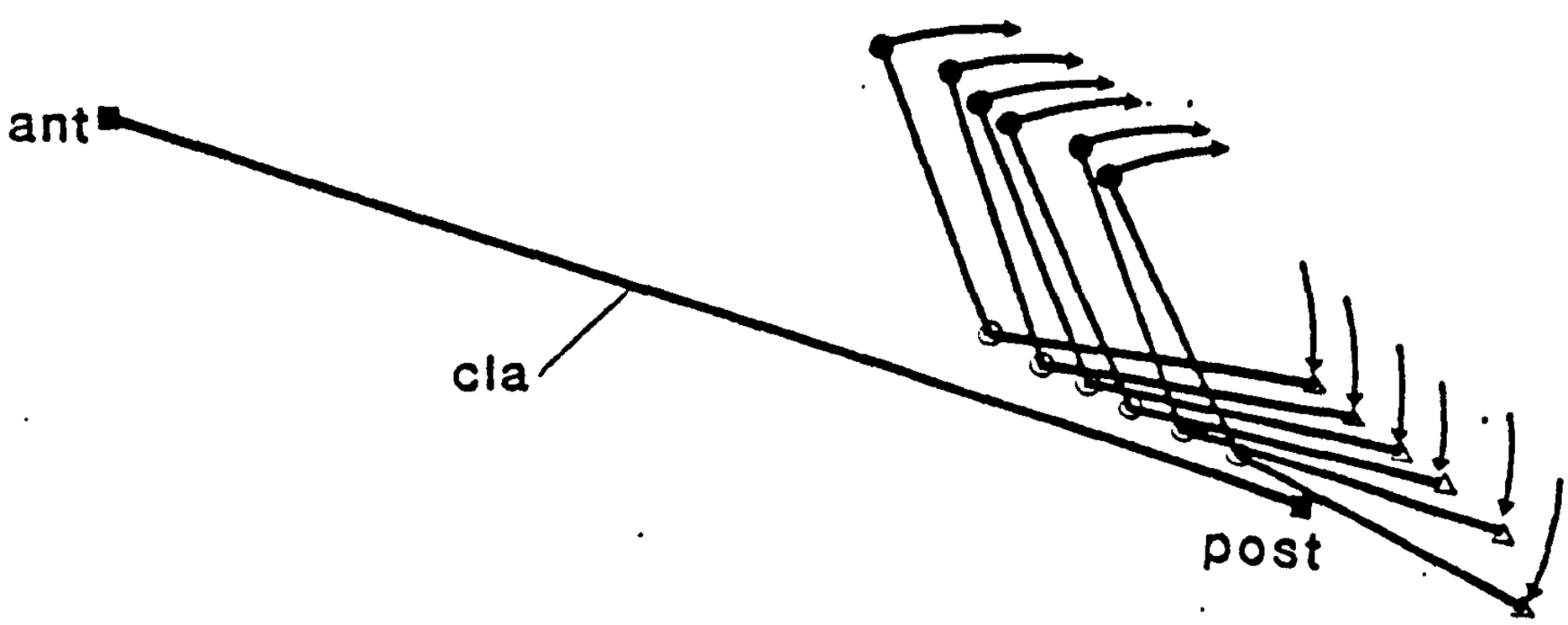
○ = limb bases.

△ = tips of the setae at the end of the stroke.

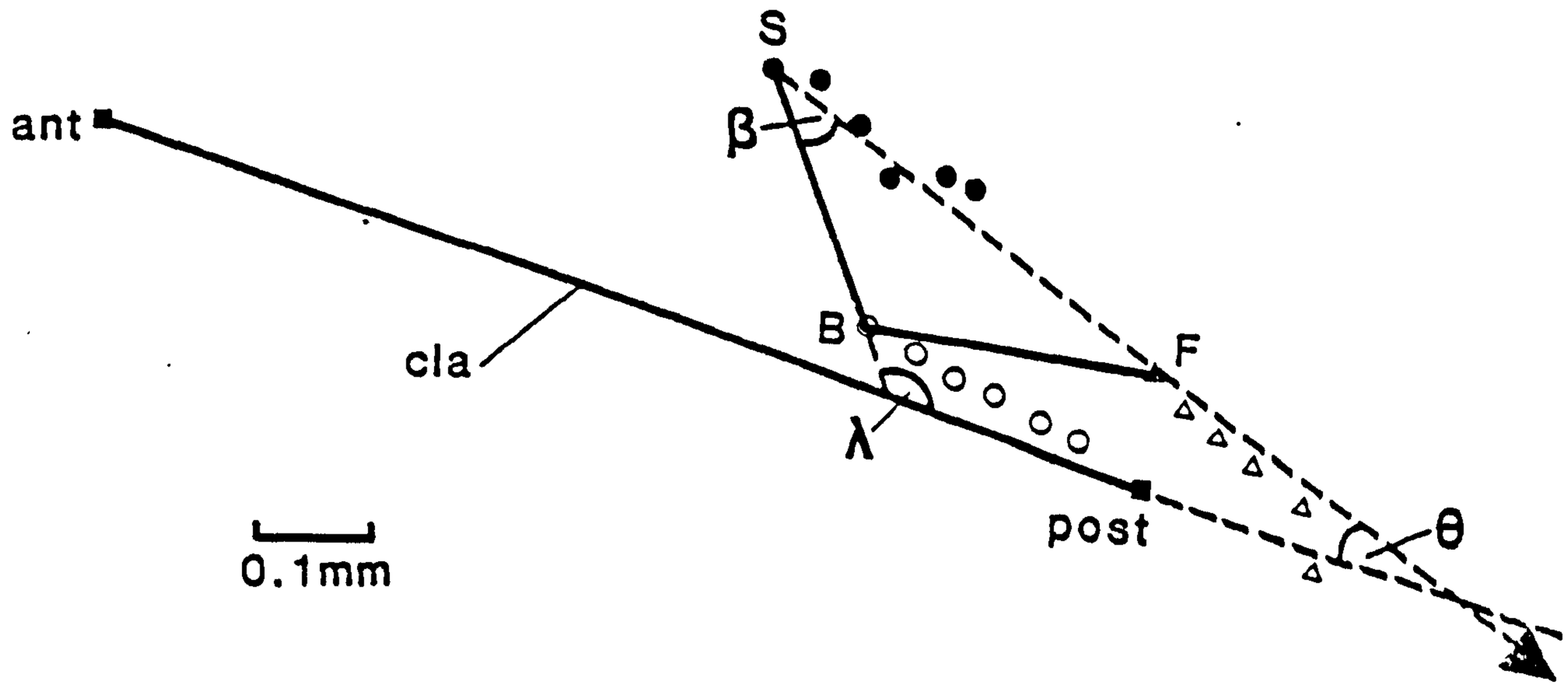
(b)..shows the construction used to find the angle θ . between the trust vector (SF) and the long axis of the cyprid (cla). Only the anterior limb is shown, with the limb base marked at B.

Fig 7.11 Diagram of cyprid swimming stroke as marked from video-recordings

(a) Limb positions, start and end of stroke



(b) Construction for angle of thrust θ



the propulsive stroke. Fig 7.11 (a) demonstrates the construction used to calculate the average direction of thrust produced by each limb in propelling the cyprid forwards. The construction shown in Fig 7.11 (b) is that for the first pair of thoracic limbs. The intention of the construction was to calculate the angle (θ) made between the displacement vector of the setal tips from the beginning to the end of the stroke (SF Fig 7.11(b)) relative to the long axis of the cyprid (ant - post, Fig 7.11(b)). θ was calculated by measuring the angles between the position of the limb at the start of the stroke (BS Fig 7.11(b)) and (i) the long axis of the cyprid (λ Fig 7.11 (b)), and (ii) the limb displacement vector SF (β Fig 7.11 (b)). Since the angles of a triangle sum to 180°

$$\theta = 180 - (\beta + \lambda)^{\circ}$$

The direction of thrust produced by all six limbs during one propulsive stroke will therefore be the resultant of the addition of the thrust vectors provided by each limb pair. As the limbs are more or less uniform in size and shape, the magnitude of the thrust vector provided by each limb pair, i.e. the amount of water moved, relative to the other pairs will largely depend on how far the limbs move during the stroke. The limb displacement vector (SF Fig 7.11 (b)) can thus give a reasonable estimate of the contribution of each limb pair to the total thrust for one propulsive stroke.

26 observations were made from 5 cyprids where the length of SF and θ were measured for all six limbs during the propulsive phase of a swimming stroke. The results are tabulated in Table 7.5 where the resultant average angle of thrust to the long axis of the cyprid ($R\theta$) is shown for each propulsive stroke analysed. There was considerable variation in the

Table 7.5

The resultant angle of thrust relative to the long axis of the cyprid of B.balanoides produced by the swimming stroke.

An.	Linear displacement (SF) µm						θ°						$R\theta^\circ$
	Limb						Limb						
	1	2	3	4	5	6	1	2	3	4	5	6	
1	585	575	575	565	534	411	3.0	5.5	7.5	8.5	9.5	12.0	7.408
1	687	683	675	659	591	501	2.0	0.0	0.0	1.0	3.5	5.0	1.740
2	520	581	577	553	524	472	2.0	2.0	4.0	4.0	1.0	-2.0	1.953
2	664	644	616	592	560	528	5.0	6.0	5.0	5.0	5.0	3.0	4.886
2	542	545	539	526	472	400	9.5	8.0	10.0	9.0	12.0	16.0	10.481
2	543	538	520	520	516	477	15.0	15.0	12.5	10.5	4.5	-2.5	9.421
2	686	664	636	636	598	550	-5.5	0.0	0.5	-1.0	-1.5	-5.0	-2.052
2	762	750	728	713	678	639	5.0	7.5	7.5	6.5	4.0	0.5	5.040
3	621	629	636	572	577	563	5.0	6.0	6.5	7.5	7.0	1.5	5.611
3	703	705	716	716	713	623	12.5	15.0	17.0	17.5	17.0	13.0	15.394
3	636	649	668	633	629	576	11.0	12.0	13.0	14.0	13.0	10.0	12.205
3	683	679	699	710	702	602	19.5	22.0	19.5	18.0	19.0	19.0	19.495
3	704	704	712	712	701	635	13.0	14.0	14.0	14.0	13.0	8.5	12.826
3	590	594	603	576	585	485	0.0	1.0	2.5	2.5	3.0	0.0	1.543
4	662	634	642	644	644	608	13.0	9.0	10.5	12.0	15.0	18.0	12.880
4	526	473	480	441	451	484	8.0	11.0	22.5	28.5	30.0	27.0	20.813
4	564	564	561	573	581	593	18.0	19.0	18.0	21.0	22.0	24.5	20.462
4	495	562	603	597	591	572	10.0	11.0	12.0	10.0	13.0	14.0	11.704
4	567	556	573	593	546	546	1.5	6.0	7.0	11.0	18.0	20.5	10.567
4	622	608	603	615	587	581	5.5	9.5	12.5	15.5	18.0	23.0	13.881
5	464	470	492	504	510	467	34.5	29.5	26.0	22.5	20.0	14.0	24.336
5	635	611	595	563	535	523	0.0	1.5	2.0	2.5	1.5	-1.5	1.020
5	532	523	526	507	472	469	2.0	4.5	6.0	8.0	6.0	4.0	5.064
5	619	646	679	649	643	612	13.0	12.5	11.5	12.0	8.0	7.0	10.693
5	735	735	732	747	750	738	3.0	4.5	5.5	5.5	6.5	6.0	5.175
5	514	514	514	508	508	487	13.0	13.0	13.5	11.0	7.5	3.0	10.236

The limb displacement vector (SF Fig 7.11(b)) is given as the linear displacement of the setal tips in µm and the direction of movement relative to the long axis (θ Fig 7.11(b)). The resultant, $R\theta$, was found by vector addition of the dispalcement vectors for each limb. A positive θ or $R\theta$ indicates a vector directed towards the long axis and a negative θ or $R\theta$ a vector directed away from the long axis. The limbs are numbered from anterior (1) to posterior (6). Five animals (An.) in all were used.

Fig 7.12

A diagram showing the range of resultant directions from the thrust vector of the swimming stroke of B.balanoides cyprids.

ant = anterior carapace.

cla = cyprid long axis.

dlm = direction of limb movement.

epl = end position of posterior thoracic limb (after power stroke).

lb = limb bases.

post = posterior carapace.

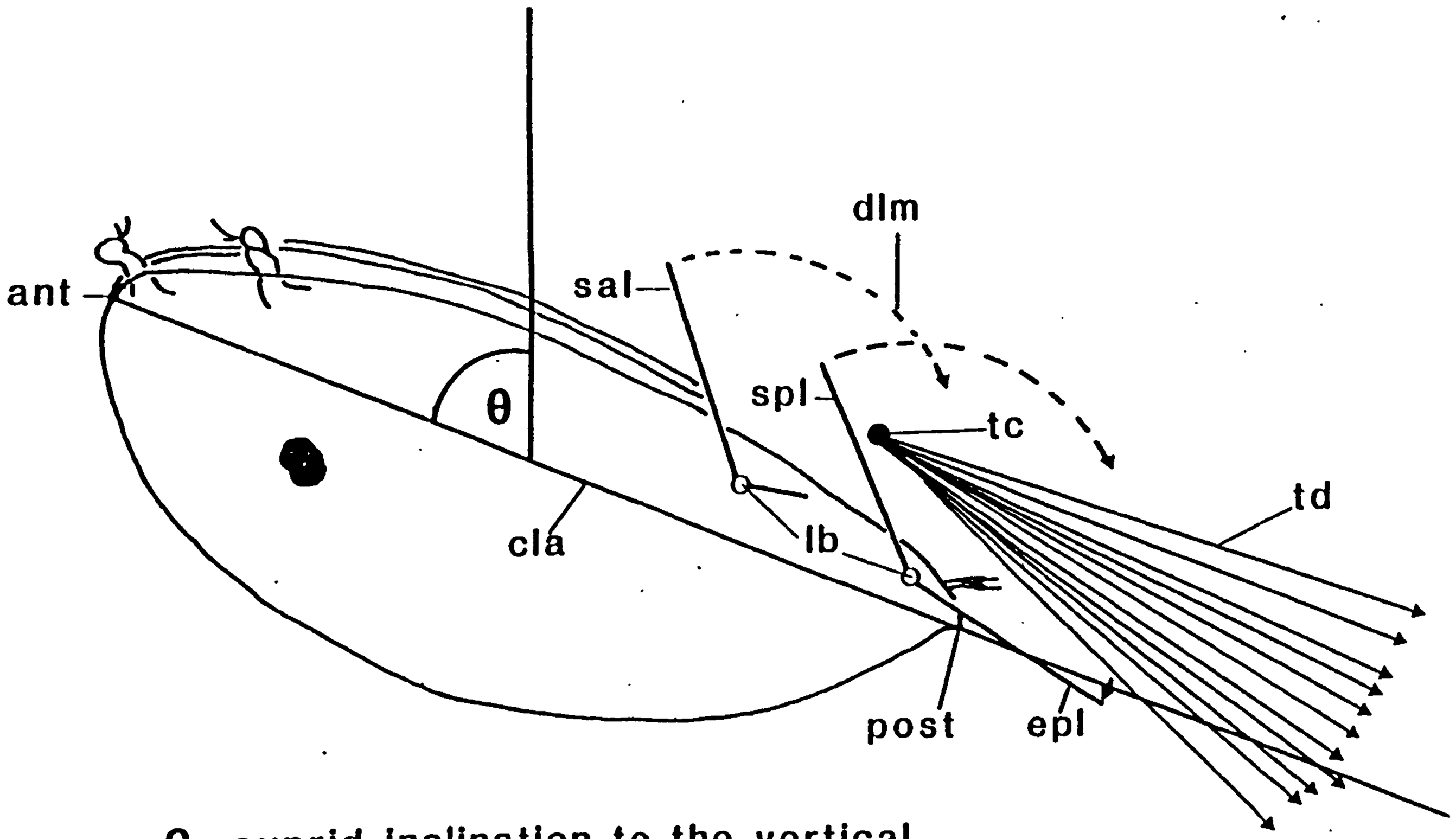
sal = starting position of anterior thoracic limbs.

spl = starting position of posterior thoracic limbs.

tc = thrust centre; defined, for this diagram, as the mid point of a line from the intersection of the paths of the setal tips of the anterior and posterior limbs during a power stroke, and the mid point of a line connecting the limb bases of those limbs.

td = direction of thrust, relative to the cyprid long axis, representitive of the range from 26 observations.

Fig. 7.12 Resultant direction of thrust from the cyprid swimming stroke



θ = cyprid inclination to the vertical

resultant angle of thrust, the range varied from -2.052^{o} directed away from the cyprid long axis to $+24.336^{o}$ directed towards the long axis. Again it may be noted from Table 7.5 that the range of resultant angles varies almost as much for a single cyprid as it does for all 5 cyprids. The majority of propulsive strokes were directed towards the long axis of the cyprid and Fig 7.12 is a diagram of a cyprid indicating the range of directions observed. The direction of thrust (td Fig 7.12) has been drawn from an arbitrary thrust centre (tc Fig 7.12) merely to show the relationship between the cyprid and the direction of the thrust produced by the limbs.

Conclusions and Discussion

Passively sinking cyprids

The cyprids observed in this study sank in the water column with a spiral path and the long axis of the cyprid inclined at an angle to the vertical. The attitude of the cyprids in the water column may be determined by uneven drag forces acting on the carapace and by the fact that the centre of gravity of the cyprid lies towards the posterior end. The chitinous limbs lie at the posterior end while the bouyant lipid reserves are concentrated towards the anterior end. The angle of inclination was shallower when the limbs protruded from the carapace than when the carapace was closed. The increased surface area of the protruding limbs undoubtedly increased drag at the posterior end causing a tilt of the cyprid long axis about the centre of gravity.

The effect of increased drag caused by the protruding limbs was clearly demonstrated by the differences shown in the rate of cyprid descent. Larvae with limbs protruding sank significantly more slowly (0.44 cm s^{-1}) than

those with limbs withdrawn (0.54 cm s^{-1}). There is no published data with which to compare the sinking rates of B.balanoides cypris larvae, although Forward (1977) gives figures for 6 species of Brachyuran stage 1 zoea (2 mm + in length) sinking in the water column ranging from 0.29 to 0.49 cm s^{-1} which are of the same order as those obtained here for B.balanoides cyprids. Walker and Rainbow (1975, unpublished) have kindly supplied some unpublished data on the sinking rates of B.balanoides cyprids. They also noticed that the path of the cyprid tended to be spiral during the descent. From 70 observations their mean sinking rate was 0.604±0.058 cm s^{-1} (±standard error). These measurements were made with animals that had been killed in formalin and at temperatures between 17.5 - 20.0°C, whereas all the observations in the present study were made with live animals at 15°C.

Cyprid swimming speeds

Table 7.6 shows recorded swimming speeds for various aquatic creatures including marine and freshwater arthropods and fishes. The results for the present study are shown at the top giving the observed maximum speed for B.balanoides cyprids. There is agreement with the results of Crisp (1955) even though his measurements were average speeds measured over longer time periods. The measurements of Hardy and Bainbridge (1954) were also made over much longer time periods and represent sustained swimming speeds, however, their figures show how much more slowly the barnacle nauplii swim in comparison with the cyprid. The swimming performance of an organism can be compared relative to it's length. At constant velocity, however, the propulsive force of the organism equals the hydrodynamic drag on the animal, which will be proportional to the square of velocity and a characteristic

area (L^2) at Reynolds numbers beyond 1.0, although much uncertainty exists concerning the nature of motion through fluids between Reynolds numbers 1 - 100 (see Lamb 1932, Vlymen 1970). The propulsive force produced by the organism will be proportional to the muscular volume, hence to the volume of the organism (L^3). Larger animals will tend to lose out in comparisons of swimming speed based purely on their body length, since the velocity of the animal will be proportional to the square root of the body length, i.e.

$$\text{if propulsive force} = k_1 L^3 \qquad \text{(volume x a constant)}$$

and at constant velocity V the opposing drag force is given by

$$\text{Drag} = 0.5 \rho C_d V^2 L^2$$

where ρ = the density of water and C_d the drag coefficient. At a constant velocity $0.5 \rho C_d$ will be constant thus

$$k_1 L^3 = k_2 V^2 L^2$$

$$\text{hence} \quad V^2 = (k_1 L)/k_2$$

$$\text{i.e.} \quad K = V/L^{0.5}$$

where K = a constant. A dimensionally fair comparison between organisms of widely differing sizes could thus be postulated as velocity divided by the square root of body length.

Table 7.6 shows the size, maximum velocity, Reynolds number, for a variety of aquatic organisms including a comparison of their swimming performance based on body lengths per second (V/L) and $V/L^{0.5}$. The smaller organisms, even clumsy looking swimmers like the barnacle nauplius and the crab zoea, appear better swimmers than the larger organisms, when the comparison was made in terms of V/L. The comparison of $V/L^{0.5}$ seems a more reasonable comparison, showing the swimming capabilities of the barnacle cyprid to be exceptional, for organisms moving at Reynolds numbers below 1000, and on a par with the acknowledged swimming capabilities of fishes

Table 7.6

The swimming performance of some aquatic organisms. Most figures represent V_{peak} velocities measured over short time periods, those marked with * represent sustained swimming speeds (average speeds over 1 - 3 minutes). The number of body lengths per second (V/L) is also shown along with a dimensionally fair assessment of the organisms' swimming capability given by $V/L^{0.5}$.

Species	Body length L(cm)	Velocity ($cm\ s^{-1}$)	Reynolds Number	V/L	$V/L^{0.5}$	Reference
Balanus balanoides (cyprid)	0.1	9.5	97	95	30	This Study
Balanus balanoides (cyprid)*	0.1	6.0	61	60	19	Crisp (1955)
Balanus sp (nauplius)*	0.06	0.7	4	12	2.8	Hardy and Bainbridge (1954)
Brachyuran zoea *	0.2	2.0	40	10	4.4	Hardy and Bainbridge (1954)
Callinectes sapidus (zoea)*	0.2	2.4	48	12	5.4	Forward (1977)
Labidocera trispinosa (copepod)	0.2	10.0	205	50	22	Vlymen (1970)
Bidessus geminus (water beetle)	0.2	5.5	110	28	12	Nachtigall (1960)
Hyphydrus ferrugieus (water beetle)	0.4	12.0	516	28	18	Nachtigall (1960)
Abylopsis tetragona (siphonophore)	2.0	8.0	1.60×10^3	4	7	Bone and Trueman (1982)
Chelophyes appendiculata (siphonophore)	1.0	30.0	3.00×10^3	30	30	Bone and Trueman (1982)
Dytiscus margina (water beetle)	3.5	50.0	1.75×10^4	14	27	Nachtigall (1960)
Carassus auratus (gold fish)	12.5	159.0	1.90×10^5	13	45	Blaxter and Dickson (1959)
Pleuronectes platessa (plaice)	23.5	120.0	2.80×10^5	5	25	Blaxter and Dickson (1959)
Scomber scomber (mackerel)	35.0	300.0	1.05×10^6	9	51	Blaxter and Dickson (1959)

like the mackerel. The rather poor swimming capabilities of the barnacle nauplius, crab zoea, and a slow moving siphonophore are reflected in the low values obtained for $V/L^{0.5}$, as shown in Table 7.6.

The cyprid swimming stroke

The mean force produced by the propulsive phase of the cyprid swimming stroke was found to be 1.97×10^{-6} Newtons. The mass of a body times it's acceleration is equal to the net applied force. The maximum observed acceleration for a B.balanoides cyprid was about 2 m s^{-2} (see Table 7.1). As the dry weight of a B.balanoides cyprid is about 34 ug (Holland and Walker 1977, Lucas et al 1979) and animals without calcareous skeletons contain 70 - 80% water (Crisp 1971), the wet weight of a cyprid would be 136 ug, assuming the dry weight to be 25% of the wet weight. The force required to accelerate a body of mass 1.36×10^{-7} Kg at 2 m s^{-2} would thus be 2.72×10^{-7} Newtons, some seven times less than the force produced by the cyprid. When a body moves through a fluid, however, an amount of fluid, similar in volume to that of the body, must be moved from in front of the body to behind it as it passes through the fluid. A force is required to move this volume of water, in addition to that required to move the mass of the body. The volume of a cyprid is about 1×10^{-4} cm^3 (Rainbow and Walker unpublished data) and the density of seawater is 1.024 g cm^{-3}, thus up to 1.024×10^{-7} Kg of water (often referred to as the induced-mass tensor, e.g. Vlymen 1970) must be accelerated by the cyprid in addition to its own mass. The force required to accelerate the cyprid at 2 m s^{-2} could thus be as great as 5×10^{-7} Newtons.

However, the measured acceleration of the cyprid is the net result of the forward force produced by the propulsive swimming stroke and the resistance of the surrounding medium opposing forward motion, in addition to the reverse thrust produced on the recovery phase of the swimming stroke. The difference between the calculated force to accelerate a cyprid and that measured from the propulsive stroke will be accounted for by drag and the reverse recovery stroke thrust. For example, the drag acting on a body moving through a fluid at Reynolds numbers close to 100 could be assessed as

$$\text{Drag} = 0.5\,\rho C_d V^2 A \quad \ldots\ldots\ldots (2)$$ (e.g. Lamb 1932)

where A is a characteristic area, usually taken as the greatest projected area in the direction of motion, and C_d the drag coefficient, which is the same for all bodies of the same shape moving at the same Reynolds number. The characteristic area of a cyprid will correspond to the area of an ellipse of major axis the greatest height (500 μm) and minor axis the greatest width (380 μm); i.e. $A = 1.4923 \times 10^{-7}$ m^2. For Reynolds numbers below 100 the drag coefficient varies considerably with Reynolds number and experimental investigations have shown that for cylinders and spheres, and even well streamlined bodies, $C_d > 1$ (Prandtl and Tietjens 1934). The cyprid has some of the qualities of a streamlined body, but the projection of the limbs and their action during swimming are likely to disrupt the streamlining. C_d for spheres and cylinders at Reynolds numbers close to 100 are similar and of the order 1.5 to 3.5. Using equation (2) and the values for a cyprid moving with constant velocity 0.09 m s^{-1}, the force opposing movement for a C_d of 2.0 would be 1.4×10^{-6} Newtons.

The thrust produced on the propulsive phase of the cyprid swimming stroke (observed mean 1.97×10^{-6} Newtons, see Table 7.4) is, as expected, in excess of that required to overcome drag at the highest observed velocity.

The thrust produced by the limbs beyond that accounted for by the acceleration or resistive drag will be offset by the reverse thrust produced on the recovery stroke. From Table 7.2 it can be seen that the recovery stroke was always faster than the propulsive stroke, suggesting that, even though the limbs were feathered on the recovery stroke, the complete swimming stroke may not be very efficient, as the thrust produced by the limbs, on either phase of the stroke, will be a function of limb velocity as well as surface area. If we accept that the drag on a cyprid travelling at $0.09\ m\ s^{-1}$ is $1.4x10^{-6}$ Newtons, and that the cyprid produces, on average, $1.97x10^{-6}$ Newtons of propulsive thrust, for the cyprid to move without acceleration, over several limb beat cycles, the negative thrust from the recovery stroke would be $0.57x10^{-6}$ Newtons, some 29% of the propulsive thrust, thus implying a lack of mechanical efficiency in the swimming stroke. The cyprid, which may have to spend some considerable time in the plankton without feeding before finding a suitable settlement site, could thus be expected to expend energy in swimming only when pressed to do so. Such energy conservation might explain the cyprids' reluctance to swim in still and slow moving water (Crisp 1955), where minimal stimulation is presented to the larvae.

In addition to the variability in the magnitude of the thrust provided by the thoracic limbs, which would suggest a considerable degree of control exerted by the cyprid over it's swimming speed, the angle of thrust produced, relative to the long axis of the cyprid, was also found to be variable. Generally the thrust generated by the thoracic limbs was directed, to a greater or lesser degree, towards the dorsal side of the carapace. As the cyprid swims on it's back, such an inclination of thrust will produce a lift component, as well as a forward component, to the cyprid

motion. The alteration in the direction of thrust will, to a great degree, dictate the angle at which the cyprid rises in the water column. Cyprids carry no obvious rudder device, except perhaps the antennules, and their limbs do not appear to twist and deviate from the mid line during a swimming stroke. Yet cypris larvae are capable of directed motion towards a light source (e.g. Crisp and Ritz 1973). A possible steering mechanism that could be employed by cypris larvae may, however, be postulated from consideration of the kinematic data given above. As the cyprid sinks in the water column it tends to spiral downwards. The cyprid could, therefore, turn through 360° simply by sinking a short distance. The angle of rise in the water column could then be determined by the direction of thrust relative to the axis of the cyprid, and the direction of forward motion maintained by balancing the magnitude of the thrust produced with the direction of the thrust.

Section 8

Responses of Cypris Larvae of

B.balanoides to Mechanical Stimulation

Responses of cypris larvae of B.balanoides to mechanical stimulation

Introduction

The morphology and anatomy of the cypris larvae of B.balanoides has been particularly well studied from the early monograph of Darwin (1854) to the detailed electron microscope studies such as that of Nott and Foster (1969) or Walker and Lee (1976). The cyprid ,which has two compound eyes in addition to the single nauplius eye, was found to be very sensitive to light (e.g. Visscher 1928, Crisp and Ritz 1973) and the larva orientates to light during settlement (Barnes,Crisp and Powell 1951) with equal sensitivity (Forbes, Seward and Crisp 1971).

B.balanoides cyprids have been shown to be particularly discriminating in their choice of settlement site. The water current over the site must be within specified limits (Crisp 1955, Crisp and Stubbings 1957), the contour of the surface must be appropriate (Crisp and Barnes 1954), and perhaps, most importantly, the chemical nature of the surface, or one nearby, must stimulate the cyprid to begin exploring prior to settlement (Crisp and Meadows 1962, 1963, Larman and Gabbot 1975). The cyprid walks around on a surface prior to settlement using its antennules. The fine structure of the antennular discs has been studied in great detail by Nott (1969) and Nott and Foster (1969), as has the structure of the fourth segment of the antennule by Gibson and Nott (1971). Both these structures of the antennule are bristling with sense organs, some of which are thought to be chemosensory and some mechanosensory, through homology with similar structures known to serve those functions in other organisms.

The cyprid cuticle and other external structures such as the cirri and caudal appendages have been described, using both scanning and transmission electron microscopy, by Walker and Lee (1976), and they discovered numerous sensory setae dotted over the cyprid carapace. Walker and Lee (1976) further showed that the caudal appendages consisted of two stout basal segments bearing five setae with differing structures. Only the single large seta is obvious when live cyprids are viewed with a binocular microscope. Walker (1974) demonstrated the complexity of yet another sensory structure common to barnacle larvae, the frontal filaments. He discussed the structure and possible sensory function of the frontal filaments in relation to homologous structures in other organisms.

The purpose of the present study was to attempt to directly stimulate some of the sensory structures of tethered cyprids and to analyse the results in terms of the behavioural response of this larva.

Materials, Methods and Results

Cypris larvae of the barnacle B.balanoides were collected by plankton net hauls from the Menai Strait during late April, early May. The larvae were thoroughly washed and maintained in 3 litre glass jars at 15°C until required.

All the observations described below were obtained from larvae which had been restrained on the end of a fine suction tube as described in 'Methodology' (Section 1 above). The larvae were held, facing into a seawater flow of approximately 1 cm s^{-1} in the apparatus described in Fig 1.2 ('Methodology', Section 1 above). The temperature was maintained at

$15^{o}C$ throughout the experiments.

Response of cypris larvae to mechanical stimulation with a solid object

Method

Fig 8.1 shows a diagram of a cyprid and the notation used throughout the experiment to define specific areas of the body to which a stimulus was applied. The suction tube was consistently positioned within region 7 (Fig 8.1) on the side of the cyprid away from that being tested. The stimulus was applied to the cyprid carapace by means of a very fine hair which was rigidly mounted in a thin plastic tube attached to a micromanipulator. The hair was fine enough to touch very small areas, such as the short sub-terminal setae of the fourth antennular segment, without interfering with other parts of the cyprid.

Once a cyprid had been attached to the suction tip for about 45 minutes the experiment commenced. When the larva stopped swimming, yet the carapace remained open and the limbs and antennules were protruding, the hair was manipulated towards and touched up against one of the body regions shown in Fig 8.1. The behaviour of the animal in the two or three seconds immediately following the stimulus was then noted. The larvae responded in one of four ways, viz. (a) no change, the cyprid remained open (b) swimming (c) closing the carapace and withdrawing the limbs (d) waving the antennules in large arcs around the ventral anterior area of the carapace. Antennular movements quite often accompanied either a swimming response or a closing response. In all a total of 15 cyprids were used.

Fig 8.1

Definition of the regions of the cypris larvae of B.balanoides touched by a small hair. An indication of the most likely behavioural change shown to mechanical stimulation from each region is also given below.

1..Basal region, caudal appendages; No Response.

2..Distal region, caudal appendages; Closing.

3..Posterior tip of carapace; Closing.

4..Posterior carapace; Closing.

5..Posterior ventral carapace; Closing.

6..Posterior dorsal carapace; Closing

7..Median dorsal carapace; Closing.

8..Median ventral carapace; Closing.

9..Anterior dorsal carapace; Swimming + Antennule waving.

10..Anterior ventral carapace; Swimming + Antennule waving.

11..Anterior tip of carapace; Swimming + Antennule waving.

12..Limbs; Closing.

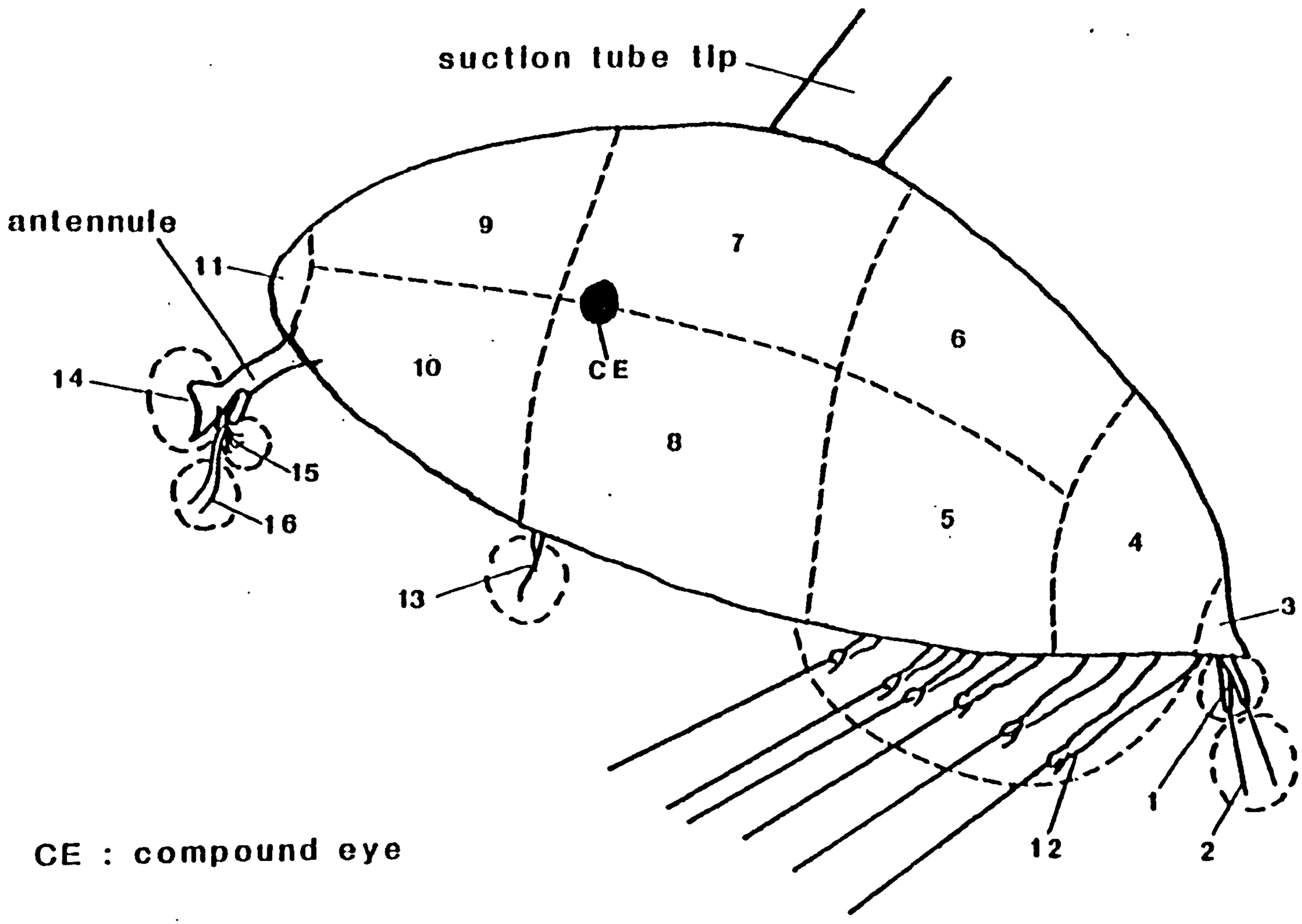
13..Frontal filaments; No response.

14..Antennular disc; Swimming + Antennule waving.

15..Sub-terminal setae (4^{th} segment); Swimming + Antennule waving.

16..Terminal setae (4^{th} segment); No Response.

Fig 8.1. Diagram of a cypris larva of B.balanoides defining the regions touched by a small hair



CE : compound eye

As a measure of control for the above observations, the behaviour of restrained cyprids was noted in a similar way except that no stimulus was applied. Larvae were left until they stopped swimming as above. After 5 - 10 seconds, the approximate time required to position the stimulating hair, the behaviour of the cyprid was noted as above. 100 control observations were made on 8 cyprids which had previously been tested with the hair.

Results

The results have been tabulated in Table·8.1. The first two columns of Table 8.1 show the regions stimulated, columns 3 and 4 show the numbers of observations showing a change in behaviour and those showing no change. Columns 5 to 9 detail the type of response made by the larvae. The figures in brackets show the numbers of observations of antennular movement accompanied by either swimming or closing and have been included in the totals for antennular movements and either swimming or closing. The next five columns list the observed response from the larvae as a proportion of the numbers of observations for each region. Column 14 shows the proportion of antennular movements, inclusive of observations of antennular movements accompaning either swimming and closing behaviour. The control observations show that when no stimulus was presented to the larvae almost 90% of the observations resulted in no change in behaviour. The basal region of the caudal appendages (Region 1 Fig 8.1), the frontal filaments (Region 13 Fig 8.1) and the long terminal setae of the fourth antennular segment (Region 16 Fig 8.1) gave results very close to those of the control and can therefore be considered to be regions which do not respond to mechanical stimulation by a solid object.

Table 8.1

Response of the cypris larvae of B.balanoides to mechanical stimulation.

		Number of observations							Behaviour after stimulation as a proportion of the No of observ.					
		Behaviour		Behaviour after stimulus										
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
Region	Code	Change	no Change	Swim	Close	Ant.	(S/A)	(C/A)	no Change	Swim	Close	Ant. Only	Total Ant.	Most likely result of stimulation
Control		11	89	3	7	1	(0)	(0)	0.89	0.07	0.03	0.01	0.01	
Caudal appendage	1	3	27	0	3	0	(0)	(0)	0.90	0.00	0.10	0.00	0.00	No response
	2	27	3	8	19	0	(0)	(0)	0.10	0.27	0.63	0.00	0.00	Closing
Posterior Carapace	3	46	1	9	34	8	(1)	(4)	0.02	0.19	0.72	0.06	0.17	Closing
	4	22	38	5	16	7	(1)	(5)	0.63	0.08	0.26	0.02	0.12	No response
	5	14	28	5	9	2	(1)	(1)	0.67	0.12	0.21	0.00	0.05	No response
	6	19	28	1	15	4	(0)	(1)	0.60	0.02	0.32	0.06	0.09	No response
	7	12	35	0	10	4	(0)	(2)	0.74	0.00	0.21	0.04	0.08	No response
	8	9	29	2	7	3	(1)	(2)	0.76	0.05	0.18	0.00	0.08	No response
	9	35	12	22	13	18	(14)	(4)	0.26	0.47	0.28	0.00	0.38	Swimming + Ant.
	10	32	9	18	10	18	(5)	(9)	0.22	0.44	0.24	0.10	0.44	Swimming + Ant.
Anterior	11	45	0	27	17	31	(17)	(14)	0.00	0.60	0.38	0.02	0.69	Swimming + Ant.
Limbs	12	20	10	5	12	5	(0)	(2)	0.33	0.17	0.40	0.10	0.17	Closing
Front.Fils.	13	1	29	0	1	0	(0)	(0)	0.96	0.00	0.04	0.00	0.00	No response
Antennule	14	30	0	19	7	29	(18)	(7)	0.00	0.63	0.23	0.13	0.97	Swimming + Ant.
	15	30	0	14	11	29	(13)	(11)	0.00	0.47	0.37	0.17	0.97	Swimming + Ant.
	16	2	28	1	1	0	(0)	(0)	0.93	0.03	0.03	0.00	0.00	No response

Code = the region label for Fig 8.1. Ant. = waving of antennules in long arcs. (S/A) = antennular movements and swimming. (C/A) = antennular movements and closing. Ant. only = observations of antennular movements only. Total Ant. = the total number of observations of antennular movements including those occurring with other movements. Front.Fils = Frontal filaments.

Table 8.2

Chi-squared analysis for the difference from control in the proportions of change to no change in behaviour of cypris larvae of B.balanoides stimulated by the touch of a hair on various regions of the carapace.

	Change	No change	Total
Control	11	89	100
Region 8	9	29	38
Totals	20	118	138
Chi-squared cor. = 2.6247 p = 0.105			
Control	11	89	100
Region 7	12	35	47
Totals	23	124	147
Chi-squared cor. = 4.0739 p = 0.044			
Control	11	89	100
Region 6	19	28	47
Totals	30	117	147
Chi-squared cor. = 4.8854 p = 0.027			
Control	11	89	100
Region 5	14	28	42
Totals	25	117	142
Chi-squared cor. = 8.6886 p = 0.003			
Control	11	89	100
Region 4	22	38	60
Totals	33	127	160
Chi-squared cor. = 13.563 p = 0.001			

Chi-squared cor. = Chi-squared corrected for continuity i.e.

$$\text{Chi-squared cor.} = \text{Sum of}(|O - E| - 0.5)^2/E.$$

where $|O - E|$ = the positive difference between observed (O) and expected values (E). All the tests have 1 degree of freedom and p = the probability of obtaining such a high chi-squared value by chance.

There are other regions where the proportion of observations resulting in no change in behaviour was very much lower than for the control observations. The anterior and posterior regions of the carapace (Regions 3,9,10,11), the distal portion of the caudal appendages (Region 2), the limbs (Region 12), the antennular disc (Region 14) and the short sub-terminal setae of the fourth antennular segment (Region 15), all show a clear response to the stimulus. The nature of the response appears to be complex; both swimming and closing were increased over control levels. There was, however, a trend; the most likely response to stimulation of the posterior region was for the animals to close, whereas the response to stimulation of the anterior carapace and antennules was for the animals to swim and wave their antennnules.

The remaining regions of the carapace (Regions 4 - 8) gave a result which was intermediate between control results and those regions showing a clear result. In particular the two median regions of the carapace (Regions 7 and 8) show particularly high proportions of no change results. Table 8.2 details a Chi-squared analysis performed between the numbers of observations for change and no change of behaviour from control observations and for those for regions 4 - 8. The low Chi-squared value for region 8 shows that the result does not differ significantly from the control and could have been obtained by chance. The significance level for the remaining Chi-squared values was less than the 5% level, thus these regions (4 - 7) also show a response to mechanical stimulation more often for the cyprid to close than to swim (Table 8.1)

Response of cyprids to a small water jet

Methods

A thin plastic tube, similar to the one used to restrain the cyprids, was used to direct jets of water at different regions of B.balanoides cyprids. The plastic tube was attached to an agla syringe and the free end held in a micromanipulator for positioning close to the cyprid. Several tests were made prior to the experiments, shooting jets of Methylene blue dye from the syringe tube, firstly to determine which areas of the cyprid carapace could be stimulated by the jet without seriously affecting others, and secondly to determine the velocity of a jet of water produced by one standard half-turn of the agla syringe barrel. The dye tests were performed in flowing seawater, at right angles to the flow, and recorded on video-tape. The recordings were analysed at slow playback speeds, and the time taken for the leading edge of the water jet to pass two marks 0.3 mm apart was noted. The mid point of the two marks coincided with the position of the cyprids relative to the syringe tube during an experimental run. The average velocity of the jet between the two marks was then calculated as distance divided by time. A total of 13 dye tests were performed and the mean jet velocity found to be 0.582 ± 0.058 cm s^{-1}.

The dye tests further showed that the standard water jet could be applied to five regions of the cyprid with minimal overlap :- (a) the posterior carapace and caudal appendages (Regions 1 - 4 Fig 8.1), (b) the posterior median carapace (Regions 5,6 Fig 8.1), (c) the anterior median carapace (Regions 7,8 Fig 8.1), (d) the anterior carapace (Regions 9 - 11 Fig 8.1) and (e) the antenuules alone (Regions 14 - 16 Fig 8.1). It was not

possible to watch the behaviour of the cyprids and operate the water jet at the same time, hence all the experiments were recorded on video-tape and analysed later. At least 5 to 10 minutes were allowed between applications of the water jet to different regions of the carapace.

The video-recordings were analysed by using an event recorder consisting of a Kymograph fitted with four pens, each pen connected by a pulley to a sprung switch. Depressing the switch caused the pen to rise, showing the onset of a particular behaviour pattern, thus releasing the switch caused the pen to fall, denoting the end of the behaviour pattern. The paper speed around the kymograph was calibrated in minutes, thus the duration and the occurrence of the behaviour pattern could be determined from the trace. Three event recorders were used corresponding to swimming, closing and antennular movements as described above. Therefore, by watching the video-recording and operating the event recorder, an accurate record of cyprid behaviour could be obtained. The fourth event recorder channel was used to indicate the onset of the stimulus.

The duration of each behaviour pattern was measured for 30 seconds preceding the application of the water jet and again for the 30 seconds following the stimulus. Four animals were used for the tests and several of the tests on specific regions of the carapace were repeated. Control observations were made on five animals which were restrained in flowing seawater, and their behaviour recorded on video-tape and analysed using the event recorder. The control observations were run for approximately one hour and for each animal two points were chosen at random. The duration of the behaviour patterns exhibited for 30 seconds either side of the points was determined. The results for the experimental run were analysed by

paired sample t-tests of the difference in duration of swimming, closing or antennular movements, before and after the stimulus. The control results were analysed in the same way, but before and after a randomly chosen point in time.

Results

The results have been tabulated in Table 8.3 where the duration, in seconds, of each behaviour pattern is shown. Table 8.4 shows the results of a paired sample t-Test performed on the difference between the duration of behaviour before the stimulus minus the duration after the stimulus. The control results show that there were no significant differences in duration of either swimming, closing or antennular movements either side of the observation point. When the posterior carapace or the antennules were subjected to a water jet the cyprid likewise showed no significant changes in behaviour after the stimulus. Application of a water jet to the other areas of the carapace, however, resulted in a significant difference for closing behaviour between that measured before, and that after the stimulus. The negative sign of the mean difference (Table 8.3) shows that the animal spent more time closed after the stimulus than before. No significant differences were demonstrable between the duration of either swimming or antennular movements before and after the stimulus.

Discussion and Conclusions

All the previous studies of the fine structure of cyprid sense organs have tended to interpret the function of the structures observed in relation to the well documented behaviour of the cypris larvae at settlement. The cyprid, however, can spend some considerable time as a free-swimming

Table 8.3

The Response of cypris larvae of B.balanoides to a water jet directed at various regions of the carapace. Tabulation of the duration in seconds for various behaviour patterns before and after stimulation.

	Swimming		Closing		Antenular movements	
	Before	After	Before	After	Before	After
Control	5.6	6.4	0.0	0.0	0.0	0.4
	8.3	6.0	0.0	9.8	0.0	0.0
	4.5	3.0	0.0	0.0	0.0	0.0
	3.0	3.8	4.9	0.0	0.4	0.4
	5.6	4.5	7.5	6.4	0.4	0.8
	5.3	1.5	0.0	1.9	0.0	0.0
	7.5	7.9	0.4	0.0	0.8	0.8
	3.4	1.1	1.5	1.1	0.0	0.0
	0.4	2.8	7.2	10.3	0.0	0.0
	3.0	3.0	2.1	0.4	0.0	0.0
Posterior Carapace	1.5	10.5	8.3	1.5	0.0	0.4
	2.6	1.5	0.0	8.3	0.0	0.0
	13.9	15.4	10.9	12.8	0.4	0.0
	15.0	12.8	7.9	1.1	0.0	0.0
	3.0	0.0	19.5	28.9	0.0	0.0
Posterior Median Carapace	4.5	13.9	0.4	6.4	0.0	0.0
	9.8	4.5	0.4	4.4	0.0	0.0
	11.3	0.0	0.8	15.0	0.0	0.4
	13.5	16.5	0.4	12.8	0.4	0.0
Anterior Median Carapace	2.6	2.3	0.8	6.8	0.0	0.0
	8.6	5.6	0.8	16.5	0.0	0.0
	5.3	0.0	0.8	28.5	0.0	0.0
	13.5	16.5	0.4	12.8	0.0	0.0
Anterior Carapace	3.0	1.1	0.0	5.3	0.0	0.0
	14.6	10.9	1.1	12.8	0.0	0.0
	10.5	0.0	0.0	30.0	0.0	0.0
	8.3	19.5	3.0	12.6	0.0	0.0
	13.1	14.3	0.4	2.6	0.4	0.0
	12.8	12.4	0.4	15.4	0.0	0.0
Antennules Only	7.5	4.5	0.4	1.1	0.4	0.4
	4.5	12.0	1.9	0.4	0.0	0.4
	9.0	7.5	0.0	1.1	0.4	0.0
	23.3	8.3	0.0	1.1	0.0	0.0
	5.6	4.9	1.1	0.4	0.0	0.4

Table 8.4

Paired sample t-Test analysis of the duration of behaviour patterns of the cypris larvae of B.balanoides before and after the application of a water jet to various regions of the carapace.

Region	Mean Difference	Standard Error	t Value	df	prob	Behaviour
Control	+0.66	0.59	+1.1187	9	0.292	
Post.Car	-0.84	2.18	-0.3859	4	0.719	
Pos.Med.Car	-1.60	4.50	-0.3556	3	0.746	
Ant.Med.Car	+1.40	1.79	+0.7832	3	0.491	Swimming
Ant.Car	+0.68	2.90	+0.2359	5	0.823	
Ant.Only	+2.54	3.61	+0.7035	4	0.521	
Control	-0.63	1.22	-0.5169	9	0.618	
Post.Car	-1.20	3.51	-0.3421	4	0.750	
Pos.Med.Car	-9.15	2.46	-3.7223	3	0.034	
Ant.Med.Car	-15.45	4.55	-3.3935	3	0.043	Closing
Ant.Car	-12.30	4.00	-3.0774	5	0.028	
Ant.Only	-0.14	0.53	-0.2657	4	0.804	
Control	0.00	0.07	0.0000	9	1.000	
Post.Car	0.00	0.13	0.0000	4	1.000	
Pos.Med.Car	+0.10	0.10	+1.0000	3	0.391	
Ant.Med.Car	0.00	0.00	+1.0000	5	0.391	Ant. Movements
Ant.Car	+0.07	0.07	+1.0000	5	0.363	
Ant.Only	-0.08	0.15	-0.5345	4	0.621	

The difference was calculated as the duration (in seconds) before application minus duration after application.
Post.Car = Posterior carapace; Pos.Med.Car = Posterior median carapace; Ant.Med.Car = Anterior median carapace; Ant.Car = Anterior carapace; Ant.Only = Antennules only.

planktonic organism. Holland and Walker (1975) were able to successfully maintain B.balanoides cyprids in the laboratory for eight weeks without settlement. However, De Wolf (1973) collated data from various sources to reveal that many barnacle cyprids, such as those of E.modestus or B.crenatus and even B.balanoides, are able to settle well within two weeks of their planktonic life as cyprids, although some 1 - 3 days in the plankton appeared necessary before the cyprids would begin to settle.

None of the cyprids observed during the present study were presented with a surface during the experiments, hence the response of the larvae to the stimuli presented must be interpreted from the point of view of a free swimming plankter. Furthermore, Crisp and Meadows (1963) found that the "urge to settle" appeared to increase with the age of the cyprids. The cyprids used for the present study were from wild populations and thus of indeterminate age, the strength of the "urge to settle" amongst the experimental animals could, therefore, have been quite variable.

The stimulation of cyprids by a solid object

Although the response of the cyprid to the touch of a fine hair was found to be variable, there was a tendency for the larvae to close when the posterior regions were touched and for the larvae to swim when the anterior regions were touched. No response was detectable when the frontal filaments, terminal setae of the fourth antennular segment, or the long setae of the caudal appendages, were touched. Walker (1974) considered that the frontal filaments could function as mechanoreceptors, either by sensing the orientation of the cyprid or the acceleration and deceleration of the larva. This now seems unlikely, as the filaments were bent relative to the

body of the cyprid when they were touched by the hair and no response from the larva was shown. Perhaps Walker's (1974) further suggestion that the frontal filaments could function as baroreceptors was more appropriate.

There are five terminal setae on the fourth segment of the antennule (Gibson and Nott 1971). The two largest are setulate and flattened at the distal end. One is very small and the other two are not setulate. All the terminal setae possess sensory tissue and Gibson and Nott (1971) suggested that they could be either mechano- or chemoreceptors. From the results above a mechanoreceptive function again seems unlikely for the terminal setae of the fourth antennular segment. When the four subterminal setae of the fourth antennular segment were touched, however, a clear response was noted which nearly always involved waving of the antennules, accompanied more often by swimming than closing. The fine structure of the subterminal setae led Gibson and Nott (1971) to conclude that these setae were mechanoreceptors that were probably used to determine surface contour during settlement.

The antennular discs also showed a strong reaction to touch, and here again several structures were found by Nott and Foster (1969) which could have been mechanoreceptive. Three large setae were clearly distinguished around the outside of the disc by Nott and Foster (1969) which, in their opinion, were mechanosensory.

When the long setae of the caudal appendages were touched, no response from the larva was obvious. Stimulation of the proximal segments of the appendages did, however, elicit a strong response from the larvae, more often to close than to swim. Walker and Lee (1976) showed that the proximal

segment of the caudal appendages bears the long setae and four smaller setae, all except the smallest of which bear spines. Crisp and Barnes (1954) have hinted that the caudal appendages were sensory as they were regularly brushed over the surface by the cyprid prior to settlement. Such brushing behaviour was thought to clear the debris from the surface to enhance the permanent adhesion of the cyprid, or to gain information about surface contour. Proprioreceptors within the muscles at the base of the caudal appendage were considered by Crisp and Barnes (1954) to function in sensing surface contour. The stimulus provided by a small hair on the tips of the setae, therefore, might not be expected to elicit a response from a sensory complex relying on the conscious movement by the cyprid of part of the sensory apparatus.

The carapace of the cyprid was shown to be sensitive to touch, particularly at the posterior and anterior regions. Walker and Lee (1976) found that the cyprid carapace was covered with aggregations of small sensory setae. These setae were considered by Walker and Lee (1976) to be either mechano- or chemosensory as suggested by Bullock and Horridge (1967) for similar organs in larger arthropods. The distribution of these setae (see Walker and Lee 1976 Fig 1) shows that they are fairly densely aggregated at the anterior and posterior regions of the carapace but more widespread along the flanks of the larvae. Such a distribution of possible mechanoreceptors would seem to agree with the results from the present study where more positive responses were obtained from the anterior and posterior regions than from the flanks of the cyprids. None of these small setae could be seen with a binocular microscope. Thus, when an area was touched, the chances of touching a sensory seta were dependant on the size of the area and the density of the sensory structures.

The techniques described above for restraining very small planktonic organisms have widened the horizons for investigation of larval physiology. The discussion above has compared the results from direct behavioural analysis of the stimulation of very small sensory structures with the inferences made from structural studies using the electron microscope. In some cases a mechanoreceptor has been correctly described from its morphology, e.g the subterminal setae of the fourth antennular segment, but in others, such as the function of the frontal filaments, at least one suggested function has been shown to be in error.

When the cyprids were touched with the hair they would have been falling in the water column, had they not been restrained. Should the posterior region brush past a solid object, the general response of the larva is to close, offering less resistance to water current, and thus to fall faster (see Section 7 above). This response could be expected to bring the cyprid onto the surface for further exploration. Were the anterior regions of the cyprid to brush against a surface, the more likely response would be to swim forwards and wave its antennules thus increasing the chances of alighting on the surface. The response of the larvae to touch was, however, shown to be variable in outcome. Swimming and antennular movements were sometimes elicited by application of the stimulus to the posterior regions and closing by application to the anterior. The cyprids were thus playing a probability game in order to find a suitable response which would increase their chances of attachment to a surface. Cypris larvae in the plankton will undoubtedly brush up against floating debris and other planktonic organisms. In many instances the larval response will tend to take the larvae away from such encounters in the open sea, yet occasional exploration of floating debris by the cyprids can be observed. Walker

(pers. comm.) has observed cypris larvae clinging to each other in very turbulent laboratory cultures.

The response of cyprids to water jets

When water jets are directed at most regions of the cyprid carapace the larvae respond by closing. This would lead to the cyprid sinking in the water column. The response was not shown by application of the stimulus to the antennules or the posterior carapace. In the natural environment, stimuli similar in nature to the water jets used in this study could be expected from the swimming movements of other plankters, particularly the stronger swimmers such as copepods and even small medusae. Water disturbances from the swimming of planktonic organisms would only be apparent very close to the organisms. The response of a cyprid to a water jet of closing, thus falling in the water column, may be a crude means of avoiding potential predators.

In the vicinity of a colonised substrate the exhalent siphons of bivalves and tunicates, and even the cirral activity of adult barnacles, could produce localised water currents similar to the water jet used above. Should a cyprid respond to such a jet, it would sink and be brought closer to the colonised substrate, although the risk run by the cyprid responding in such a manner is that it may be eaten as it falls.

Although the effect on the sense organs of a water jet and the touch of a hair would be basically similar i.e. to bend or otherwise deform the structure, the resultant changes in cyprid behaviour were not the same. The relative intensities of the two stimuli cannot be fully quantified, yet it

seems likely that the touch with the hair presented the stronger stimulus. The antennules were found to respond strongly to the touch of a hair but not to the water jet. Water jets, however, stimulated a much wider area of the cyprid at any one time than did the hair, thus a simpler, standard response from the more general stimulus was perhaps not unexpected.

Section 9

The Response of the Cypris Larvae of B.balanoides to Vibrational Stimuli

The Response of the Cypris Larvae of B.balanoides to Vibrational Stimuli

Introduction

The speed of sound propagation in water (1500 m s^{-1}) is nearly 5 times that in air (332 m s^{-1}). Sound waves thus offer a fast method of communication and detection of prey or predator for marine organisms. It is well known that marine mammals such as whales and dolphins use sound for communication and echolocation (e.g. Evans and Prescott 1962, Norris and Evans 1967). Fishes too produce and perceive vibrational stimuli (Tower 1908, Tavolga 1964, 1971, Hawkins 1973). Teleost fishes can use sound to attract mates or ward off rivals (Winn 1967) and some elasmobranchs can locate the low frequency vibrations produced by their prey (see Wisby et al 1964). All organisms moving through a fluid medium will produce disturbances in that medium giving rise to vibrational type stimuli. Where an organism swims with a regular limb beat the major frequency component of the sound produced will be that of the limb beat frequency. Not surprisingly, therefore, marine invertebrates have also been shown to respond to vibrational stimuli. Horridge and Boulton (1967) demonstrated that the chaetognath Spadella would attack a glass probe which was vibrating with a frequency between 11 and 20 Hz. Newbury (1972) added to this result by showing that the limb beat frequencies of various copepods, on which Spadella feeds, fell well within the perceptive range of the Spadella audiogram. Lobsters show a sensitivity to water borne vibrations (Laverack 1962) and the fiddler crab, Uca, detects vibrations of the substratum during it's courtship displays (Salmon and Atsaides 1970). In many cases where marine invertebrates have been shown to, or suspected of, being able to

perceive vibrational stimuli, no clear indication of the biological significance of such a sense has been proposed (see for example Frings 1964).

Sound waves in seawater consist of rapid fluctuations in hydrostatic pressure and water movements. The barnacle cyprid has been shown to respond to absolute, and fluctuations in, pressure (Knight-Jones and Qasim 1967) thus the cyprid could be expected to carry the necessary sensory apparatus to detect either component of a travelling sound wave. It was therefore decided to investigate the response of cypris larvae of B.balanoides to vibrational stimuli.

The nature of the stimulus

An erudite and concise discussion into the nature of sonic stimuli underwater, and the resultant problems presented to biologists involved with the study of animal behaviour towards such stimuli, is given by Hawkins (1973). The problems encountered when dealing with fish hearing (Hawkins 1973) are merely magnified when techniques are extrapolated for use with small invertebrates and tiny planktonic organisms (Parvulescu 1964, Frings and Frings 1967). Any object which vibrates in a fluid medium will transfer some of its motion to the surrounding medium. Motion of the fluid particles close to the object will likewise transfer some of that motion to more distant particles and so on, hence a pressure wave will be propagated away from the original vibrating source.

The fluid particles will oscillate with the frequency of vibration of the source. The amplitude or displacement of fluid particles and the frequency of the oscillations will determine the particle velocity accompanying the passage of the sound wave. For the ideal situation of a plane wave propagated through an homogenous medium, the effective root mean square (r.m.s.) value of the sound pressure (p) is related to the r.m.s. particle velocity (u) by :

$$p = \rho cu \quad \ldots\ldots\ldots (1)$$ (see Kinsler and Frey 1950)

where ρ is the density of the medium and c is the speed of sound propagation. The rate of energy flow, or intensity (I), of a sound wave is the product of the particle velocity and the sound pressure. Animals may possess sense organs which are capable of responding either to sound pressure or particle velocity, or indeed to both. The use of small tanks to investigate the response of marine invertebrates to sound leads to considerable problems of reflection from the walls. The walls of small tanks can usually be ignored when reflections are considered (Kinsler and Frey 1950, Parvulescu 1967) and the boundaries of the tank can be taken as air/water interfaces. As the acoustic impedance (ρc) for water is considerably higher than that for air only a small part of the incident energy will pass through the boundary. Thus, for a normally incident plane wave some 99.9% of the sound energy will be reflected from an air/water interface. In a small tank where a sound is operated continuously, the sound field produced will become diffuse and the sound intensity will quickly rise to a maximum value in excess of that produced at the sound source, due to the additive effects of random reflections.

The plane wave equation, (1) above, is only applicable to sounds generated from the simplest of sources and then only at large distances from the source. Unless the sound is restrained in some way, by a tube for example, spherical spreading of the sound takes place if generated from a sound source with dimensions less than a wavelength. [The wavelength (λ) of sound is defined as the distance between pressure or velocity maxima and is related to frequency by λ =c/frequency.] The equations relating sound pressure to particle velocity or displacement are more complicated for a spherical spreading wave than for a plane wave (e.g. Camp 1970). In particular the relationship between p and u depends on the distance from the source. Close to the source, termed the 'near-field' (see Siler 1969), particle velocities associated with a given sound pressure are higher than at greater distances from the source. In the near-field region of a simple source p decreases inversely with distance from the source, while particle velocity decreases with the square of the distance from the source.

Further from the sound source, in the 'far-field', the spherical wave closely resembles the plane wave. Equation (1) again relates p and u and both values decrease inversely with distance from the source. The transition from near- to far-field conditions is by no means abrupt (Siler 1969), yet it is generally agreed that the change-over occurs at a distance of approximately $\lambda/2\pi$ from the source. For a spherical wave frequency 100 Hz and hence wavelength 15 m., the change-over point would occur about 2.4 m. from the source, or at 240 m. for a spherical wave of frequency 1 Hz. Where no attempt is made to produce plane waves almost all work done with simple sources generating low frequency vibrations in small tanks will deal with near-field effects.

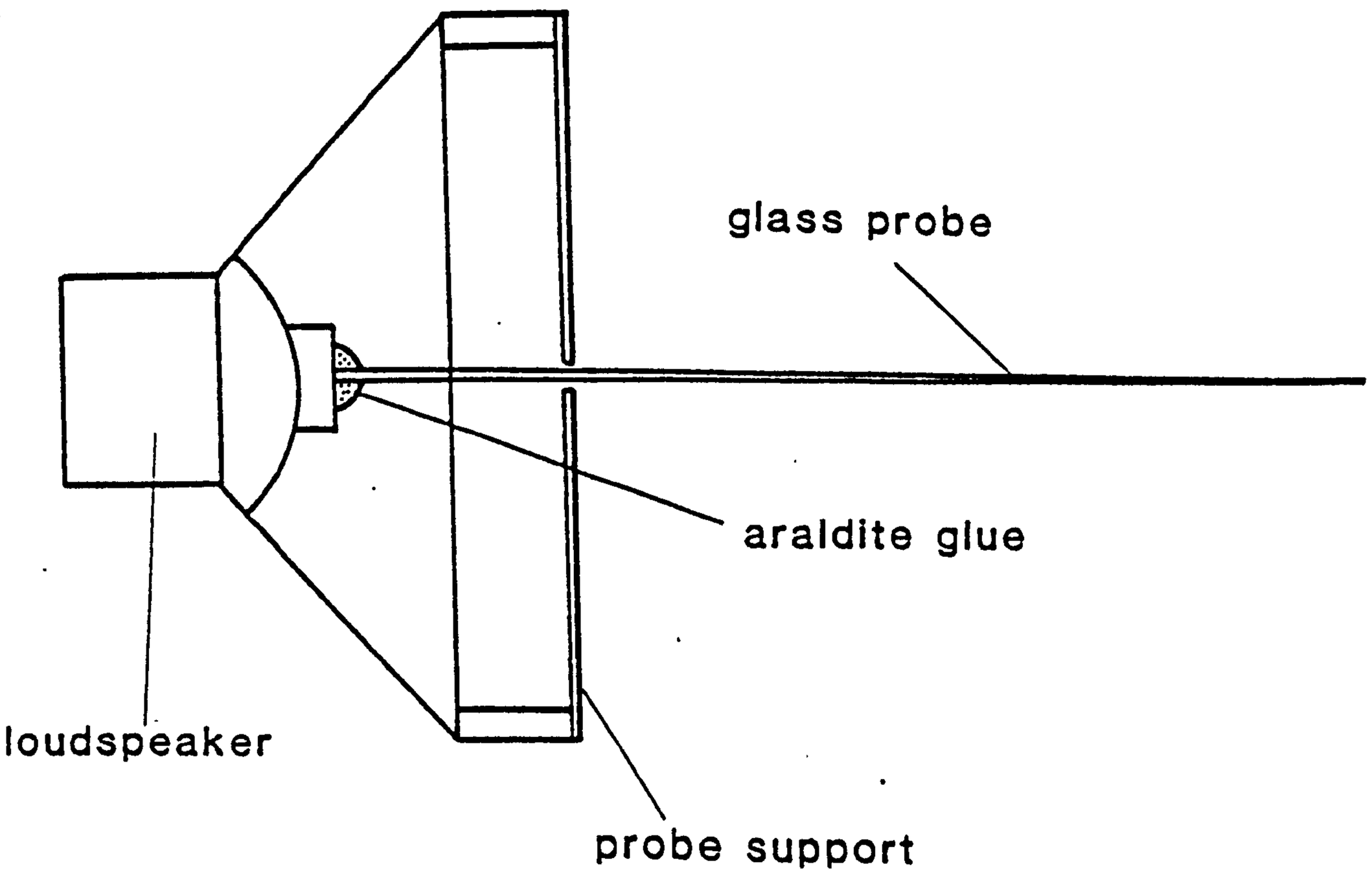
The experiments described below are of two kinds. Firstly, a simple source was used to generate low frequency sound waves in a small tank thus producing near-field effects in a closed system, resulting in fairly high displacements' accompanying sound pressures. The sound pressures generated would rapidly asymptote to a maximum value and the sound field become randomly distributed. Secondly an attempt was made to constrain sound waves within a tube, thus encouraging plane wave conditions. The characteristics of the free end of the tube could then be altered to allow sound production of predominantly pressure or displacement fluctuations.

Materials and Methods

Cyprids of the barnacle B.balanoides were collected from the Menai Strait as described in 'Methodology' Section 1 above, and maintained in 3 litre glass jars of vigorously aerated filtered seawater. Individual cyprids were attached to a suction tube in a seawater flow of 0.25 cm s^{-1} at 15°C as described above (see Fig 1.2 Section 1).

Water borne sound fields were produced by a small transducer very similar to that described by Frings and Frings (1967) and used by Fraser (1969). A glass probe was mounted on the central dome of a small loudspeaker from a transistor radio as shown in Fig 9.1. The probe was supported by a frame attached to the loudspeaker (Fig 9.1). A Levell oscillator was used to drive the loudspeaker and vibrate the probe over the frequency range 1 Hz to 300 kHz. The loudspeaker and probe assembly were fixed to a rigid stand fitted with a rubber compound base to reduce the transmission of vibrations through the bench. The probe tip was placed in the observation chamber with the longitudinal axis of the probe making a

Fig 9.1 Diagram showing the construction of the probe used to provide the vibrational stimuli



shallow angle with the horizontal. The probe tip was pointed towards the cyprid and was positioned 1.5 cm from the larva.

The cyprids were allowed at least 45 mins. attached to the suction tip before the experiment commenced, and the animals behaviour recorded on video tape throughout. The probe tip was vibrated at a range of frequencies between 10 and 1000 Hz at the highest intensity and a narrower bandwidth of 20 - 125 Hz at lower vibration intensities. The following experimental procedure was adhered to : the behaviour of the cyprid was recorded for 1 minute then the vibrating probe was switched on and the behaviour of the larva recorded for 1 minute. A further 1 minute recording was made after the probe had been switched off. The procedure was repeated at successively higher frequencies with a 10 minute period between changes in frequency. Each animal used experienced the full range of frequencies although it was not always possible for each animal to experience each change in intensity. If, for example, an animal fell off the suction tip halfway through the frequency range and could not successfully be reattached, the results from that animal at that vibration intensity were ignored and observations on another larva started afresh.

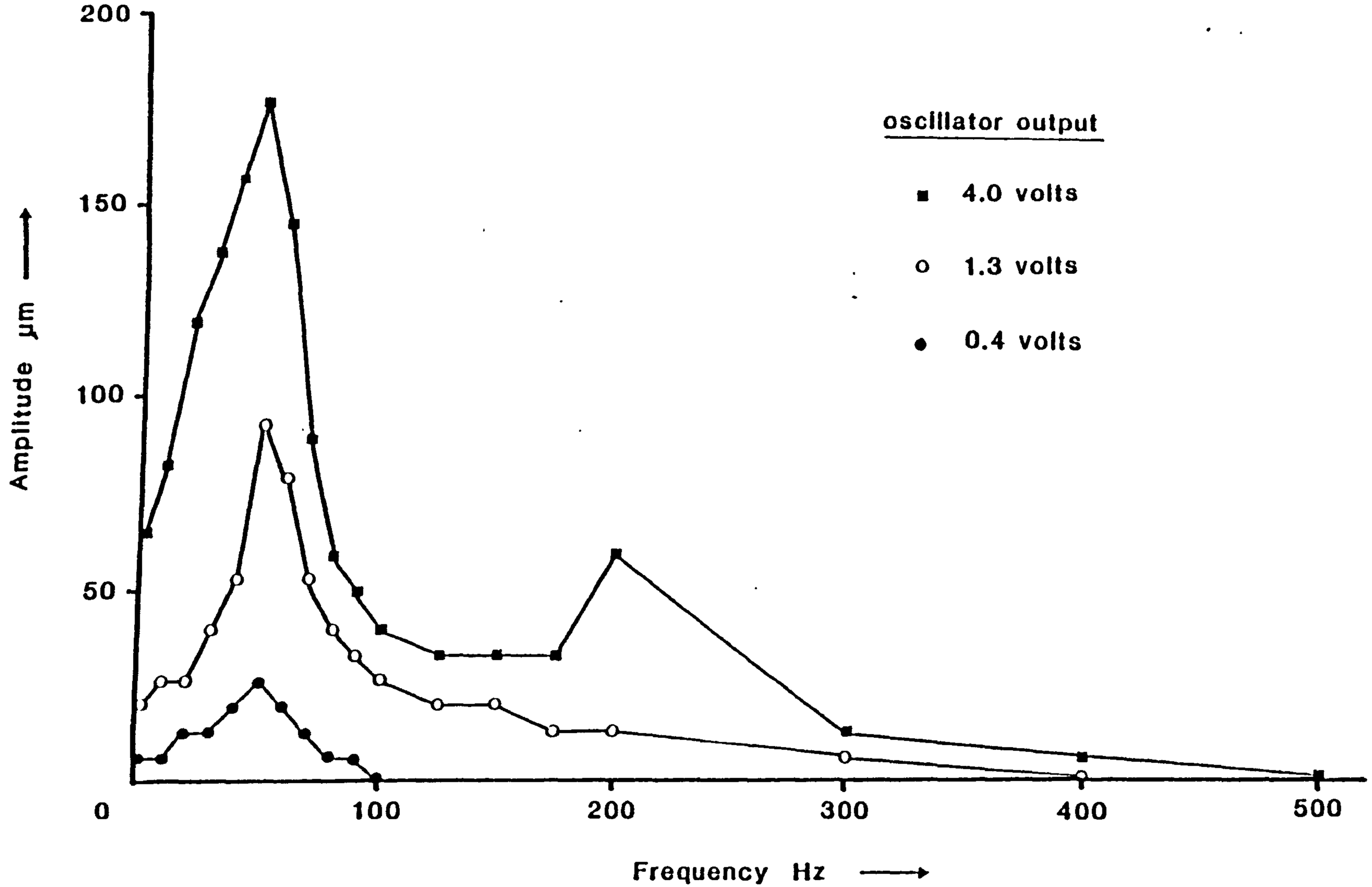
The information from the video-recordings was transferred to a kymograph event recorder for ease of analysis as described in Section 8 above. It was quickly seen that the cyprids did not wave their antennules in large sweeping arcs very often and the frequency of this antennular behaviour pattern did not change with stimulation, hence only the occurrence and duration of swimming and carapace closing by the cyprids was noted from the video-recordings. Several further video recordings of the behaviour of cyprids not exposed to vibrations, to serve as controls, were made over

periods exceeding one hour. The duration of both swimming and closing behaviour was analysed by paired sample t-test on the difference in duration before and during the vibration stimulus. For the control observations the difference in the duration of swimming and closing behaviour was analysed in the same way using the observed behaviour in alternate minutes to compare the duration of behaviour patterns when no stimulus was presented.

The character of the stimulus presented to the larvae could not be determined by recording the intensity and frequency of vibration with a hydrophone, since the volume of water available was too small to allow the use of commercially available hydrophones. The stimulus was therefore characterised, as far as possible, by using a stroboscope to measure the frequency and amplitude of the probe tip movement. The frequency of movement was obtained by matching the probe movement to the flash rate of the stroboscope in the manner described for the measurement of limb beat frequencies of barnacle nauplii in Section 2 above. All the measured frequencies agreed with those chosen by the settings on the oscillator. The amplitude of probe movement in seawater was measured by adjusting the flash rate of the stroboscope to twice that of the probe, thus producing two stationary images of the probe tip at either end of its travel. The distance between the two probe tip images was measured by micrometer eyepiece on a stereomicroscope.

The result of the calibration of the probe tip amplitude is shown in Fig 9.2. A peak amplitude was shown at each measurable intensity at 50 Hz and a secondary resonance peak at 200 Hz was demonstrated at the highest intensity setting. The movement of the probe tip at the lowest intensity (a setting of 130 mV on the oscillator) or at frequencies greater than 400 Hz

Fig 9.2 The amplitude of probe tip oscillation plotted against frequency for various oscillator voltage outputs



for any other intensity level, were not measureable by this technique.

Results

Table 9.1 shows the results obtained from the analysis of the duration of cyprid behaviour patterns under control conditions. Paired sample t-tests were performed on the difference in duration of both swimming and closing behaviour in succeeding minutes of the control observation. 12 samples were taken, each consisting of observations from each of 6 cyprids. 4 samples were taken at the beginning, middle, and end of the control period and all the control observations lasted at least one hour. The result shows that throughout the control period the difference in the duration of either swimming or closing behaviour in successive minutes did not differ significantly from zero. In all cases the t-value indicated a probability greater than 0.05 that the observed differences could have been obtained by chance.

The results shown in Table 9.2 represent a similar analysis, by paired sample sample t-test, of the mean difference between the duration in seconds of either cyprid swimming or closing behaviour in the minute before minus that during the minute's exposure to a vibrational stimulus. Values of t indicating a significant increase or decrease, at the 5% level, in the duration of a behaviour pattern during the stimulus presentation, compared with that before the presentation, have been underlined. Throughout the frequency range 10 - 1000 Hz no significant increase or decrease in the duration of cyprid closing behaviour, compared to that in the minute preceding stimulation, could be demonstrated at any of the probe amplitudes employed (Table 9.2).

Table 9.1

Paired sample t-test analysis of the control behaviour measurements for B.balanoides cyprids. The results are shown for the mean difference in the duration (in seconds) of swimming and closing behaviour in alternate minutes, at sample times from the beginning to the end of the control observation periods.

		Behaviour Pattern							
Control		Swimming				Closing			
Obser.	Sample	Mean	SE	df	t	Mean	SE	df	t
Start	1	1.75	1.39	5	1.2600	-2.08	1.99	5	-1.0495
Start	2	-0.59	0.99	5	-0.5862	0.75	1.44	5	0.5222
Start	3	-1.75	1.11	5	-1.5785	1.08	1.09	5	0.9930
Start	4	0.33	1.97	5	0.1689	-0.58	1.16	5	-0.5039
Middle	5	-0.75	1.86	5	-0.4040	0.92	0.52	5	1.7524
Middle	6	-1.67	0.89	5	-1.8699	0.50	1.55	5	0.3227
Middle	7	-2.50	1.79	5	-1.3939	0.08	0.42	5	0.2000
Middle	8	-0.25	2.89	5	-0.0865	0.08	2.46	5	0.0338
End	9	1.58	1.92	5	0.8242	-1.83	1.17	5	-1.5714
End	10	-0.75	3.03	5	-0.2475	-0.67	1.00	5	-0.6685
End	11	-0.17	0.94	5	-0.1779	0.17	2.06	5	0.0809
End	12	2.42	1.74	5	1.3860	-0.75	1.44	5	-0.5201

Mean = mean difference in duration (seconds) between alternate minutes.
SE = the standard error of the mean.
df = degrees of fredom.
t = paired sample t-value.
[Tabulated $t_{0.05}$ for 5 df = 2.571]

Table 9.2

Paired sample t-test analysis of the mean difference in duration of swimming and closing behaviour of the cyprids of B.balanoides before and during the presentation of vibrational stimuli. The mean difference was calculated as the duration in seconds of closing or swimming behaviour in the minute before presentation - the duration of closing or swimming behaviour in the minutes exposure to the stimulus.

Freq = frequency of probe vibration (Hz).

Ampl = amplitude of probe vibration (μm).

Mean = mean difference in duration of behaviour (seconds).

SE = standard error of the mean difference.

df = degrees of freedom for the t-test.

t = paired sample t-value.

Tabulated $t_{0.05}$ = 2.776 for 4 degrees of freedom.

Tabulated $t_{0.05}$ = 3.182 for 3 degrees of freedom.

Table 9.2

Analysis of behaviour of B.balanoides cyprids stimulated by a vibrating probe.

Freq	Ampl	Swimming				Closing			
Hz	µm	Mean	SE	df	t	Mean	SE	df	t
10	82.25	-0.7	3.57	4	-0.1961	7.5	6.41	4	1.1706
20	<5.00	3.1	1.30	4	2.3882	-0.3	0.20	4	-1.5000
20	13.16	-1.0	1.15	4	-0.8687	-0.7	2.60	4	-0.2696
20	26.32	0.1	1.83	4	0.0548	1.9	1.08	4	1.7641
20	118.44	4.6	1.91	4	2.4127	-1.8	3.67	4	-0.4910
30	<5.00	5.4	2.48	4	2.1757	0.2	3.00	4	0.0734
30	13.16	2.9	2.01	4	1.4437	-2.5	2.53	4	-0.9882
30	39.48	5.0	1.19	3	4.2008	-0.4	0.63	3	-0.6000
30	137.4	7.7	1.17	4	6.5906	0.9	3.33	4	0.2700
40	<5.0	3.0	2.02	4	1.4861	2.9	4.11	4	0.7057
40	19.74	7.6	1.65	4	4.5955	-0.4	1.56	4	-0.2563
40	52.64	9.1	1.56	4	5.8317	-0.6	1.21	4	-0.4966
40	156.40	10.5	1.64	4	6.4199	-6.0	3.28	4	-1.8300
50	<5.0	3.6	0.81	4	4.4313	-3.3	1.74	4	-1.8927
50	26.32	3.9	0.97	4	4.0333	1.3	3.03	4	0.4288
50	92.12	9.1	1.39	4	6.5419	1.5	1.63	4	0.9214
50	177.66	9.1	1.96	4	4.6469	0.1	3.10	4	0.0323
60	<5.0	3.1	0.66	4	4.7002	0.8	4.65	4	0.1721
60	19.74	8.2	1.93	4	4.2544	5.0	3.84	4	1.3008
60	78.96	9.7	0.72	4	13.5166	-3.7	2.72	4	-1.3588
60	144.76	9.4	1.18	4	7.9874	-6.9	4.43	4	-1.5582
70	<5.0	2.2	2.15	4	1.0241	-1.2	2.55	4	-0.4701
70	13.16	4.2	1.40	4	2.9962	-3.9	3.08	4	-1.2680
70	52.64	5.8	1.52	4	3.8120	-0.1	0.19	4	-0.5345
70	88.83	7.6	1.13	4	6.7044	-0.3	0.80	4	-0.3750
80	<5.0	1.4	0.81	4	1.7233	-2.9	2.30	4	-1.2585
80	6.58	2.6	1.50	4	1.7200	-1.5	3.21	4	-0.4668
80	39.48	4.0	1.23	4	3.2660	-12.6	8.13	4	-1.5491
80	59.22	4.4	1.54	4	2.8491	0.3	0.30	4	1.0000
90	<5.0	-0.7	1.36	4	-0.5161	-3.5	3.56	4	-0.9841
90	6.58	3.4	2.74	4	1.2407	-10.9	6.55	4	-1.6630
90	32.90	-0.8	2.73	4	-0.2928	-0.1	0.40	4	-0.2500
90	49.35	5.9	2.26	4	2.6100	-7.2	5.16	4	-1.3943
100	<5.0	1.0	2.22	4	0.4506	0.1	0.62	4	0.1612
100	<5.0	-0.9	1.28	4	-0.7039	-1.0	2.14	4	-0.4663
100	26.32	1.5	1.82	4	0.8226	2.5	1.61	4	1.5504
100	39.48	3.4	3.18	4	1.0680	-4.7	5.87	4	-0.8003
125	<5.0	-0.2	1.39	4	-0.1436	0.2	0.64	4	0.3105
125	<5.0	0.7	1.34	4	0.5232	-1.0	2.15	4	-0.4622
125	19.74	0.8	2.34	4	0.3422	2.8	3.26	4	0.8584
125	32.9	0.6	1.56	4	0.3845	0.2	0.49	4	0.4083
150	32.9	-3.0	3.39	3	-0.8863	-9.5	10.2	3	-0.9312
175	13.16	0.5	2.22	3	0.2255	-4.4	5.22	3	-0.8385
200	59.22	1.9	2.55	3	0.7346	0.8	4.71	3	0.1591
400	6.58	-1.5	1.17	3	-1.2792	1.3	1.81	3	0.7325
600	<5.0	-0.8	0.48	3	-1.5667	2.9	3.04	3	0.9445
800	<5.0	1.1	2.09	3	0.5395	-2.5	1.76	3	-1.4237
1000	<5.0	-0.5	2.65	3	-0.1884	0.5	0.65	3	0.7746

Significant decreases in the duration of cyprid swimming behaviour were, however, demonstrated over a narrow frequency range (Table 9.2, significant t-values underlined). The amplitude of the probe vibration was also shown to affect the magnitude of the cyprid response. Fig 9.3 shows a plot of the decrease in the duration of cyprid swimming behaviour (y axis Fig 9.3) against both the frequency (x axis Fig 9.3) and the probe tip amplitude (z axis Fig 9.3) of the stimulus. Points which showed no significant difference in swimming behaviour duration between the minute before, and that during, the stimulus presentation have all been assigned values of zero on the y axis so that the diagram would be less cluttered. Where a significant decrease in swimming duration was demonstrated (Table 9.2) the response has been shown on the y axis (Fig 9.3) as the mean difference in seconds between the duration of swimming burst in the minute before and that during the stimulus presentation. Points at the same frequency have been joined by thick lines and thinner lines join points with the same probe tip amplitude, whereas shaded areas denote edges of the surface.

Fig 9.3 demonstrates that the significant decreases in the duration of cyprid swimming behaviour were confined to the frequency range 30 - 80 Hz. Higher probe amplitudes produced the largest decreases in swimming behaviour duration. Following lines of equal amplitude (thin lines Fig 9.3) it can be seen that the frequency of vibration also affects the magnitude of the response such that the response at 40 and 60 Hz was nearly always greatest irrespective of amplitude. The result for a frequency of 50 Hz was somewhat anomolous because, although a significant response was demonstrated at the lowest amplitude, indicating a low threshold of sensitivity for the cyprid at that frequency, the magnitude of the response at higher amplitudes was

Fig 9.3

The response of B.balanoides cyprids to the presence of a vibrating glass probe.

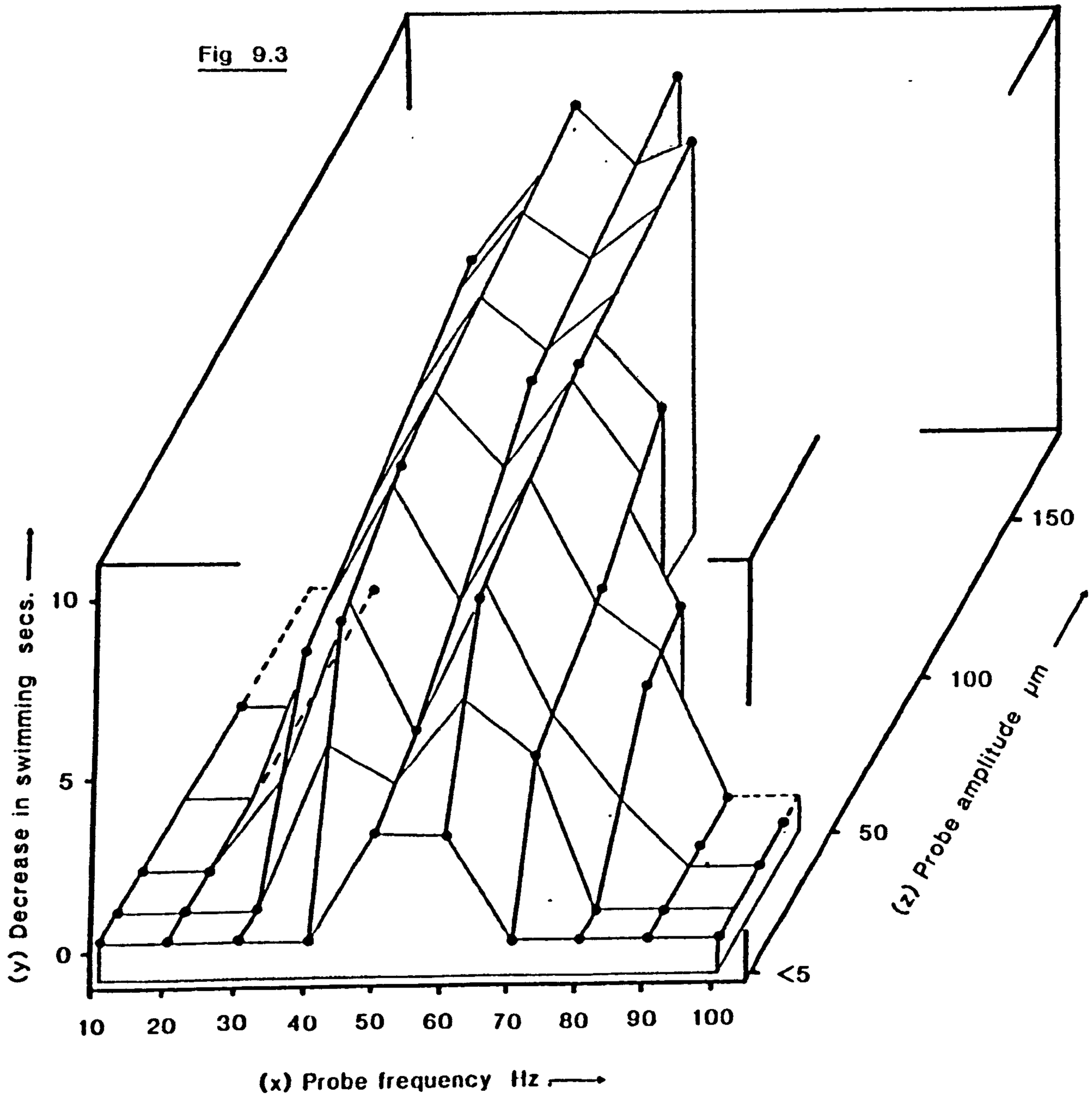
x axis = probe frequency (Hz).

y axis = decrease in cyprid swimming duration, below control levels, in the presence of the stimulus (seconds).

z axis = probe tip amplitude (μm).

Thick lines join points at the same frequency, whilst the thinner lines join points at the same probe amplitude. Shaded areas denote the edges of the surfaces.

Fig 9.3


(y) Decrease in swimming secs.
0
5
10
10 20 30 40 50 60 70 80 90 100
(x) Probe frequency Hz
<5
50
100
150
(z) Probe amplitude µm

consistently below that for either 40 or 60 Hz. The cyprids were then sensitive to periodic disturbances in the water over a narrow bandwidth of frequencies, showing peak sensitivity between 40 and 60 Hz, although the magnitude of the response at 50 Hz fell short of that of the response at 40 or 60 Hz at higher amplitudes.

Discussion

On the sound source

The source of the propagative periodic disturbances used to stimulate the cyprids during this investigation was a simple vibrating probe with a flat circular tip approximately 1 mm in diameter. The sound waves produced would tend to spread through the water as a spherical wave. The shape of the chamber dictates that the sound field produced by such a probe will not be simple. The chamber was very small in acoustical terms (9 cm diameter when the wavelength of sound at 50 Hz in seawater is 30 m.) and bounded on all sides, except for a small supporting region around the edge, by air. The perspex sides of the chamber were thin and would have no affect on the transmittance of sound from the seawater to air (see Kinsler and Frey 1950 page 152, or Blitz 1964 page 32). The acoustic coupling between air and water is so poor that 99.9% of the incident sound energy will be reflected at the boundaries. A sound wave travelling towards such an interface encounters a so-called soft or pressure release boundary. The phase of the reflected sound pressure wave is reversed while that of the reflected velocity wave remains unchanged. The net result in the water close to the boundary is that incident and reflected pressure waves at right angles to the boundary tend to cancel, whereas normally incident velocity waves tend to summate (e.g. Kinsler and Frey 1950).

The spherical spreading of the sound waves from the vibrating probe would have resulted in reflection from all the boundaries of the chamber. After a very short time the sound field would have been diffuse and the reflected sound waves would be randomly orientated. It may be assumed, therefore, that the phases and amplitudes of the reflected waves would also be randomly distributed, such that cancellation of reflected waves would have been negligible, except close to the walls. The frequency of the disturbances presented to the cyprids were defined by the probe frequency but the intensity of the stimulus could only be roughly estimated from the probe tip amplitude (see Kinsler and Frey 1950).

The response of the cyprids

It can be seen from Fig 9.3 that B.balanoides cyprids respond to pure tone sound waves over a limited frequency range for the amplitudes of vibration used. The significant response shown was neither dramatic nor immediately directed. The cyprids merely reduced the amount of time spent swimming and this reduction increased with increasing amplitude of vibration. The net result of a sufficiently intense sound field in the frequency range 30 - 80 Hz would be for the cyprids to be lower in the water column, relative to their position in the water column in the absence of the sound field. The outcome of the cyprids response to low frequency vibrations was similar to that obtained from cyprids by positive pressure pulses in excess of 10 mbar (Knight-Jones and Qasim 1967). Cyprids exposed to positive pressure pulses became immobile and sank during the decrease phase of the pulse. They were aroused to swimming activity when subjected to a pressure increase equal in magnitude to the preceding decrease. Cypris larvae showed increased swimming activity to sustained high pressure (0.5

(0.5 bar and above) although they swam up only slowly, at the appropriate pressure, after sinking in response to a pressure pulse. The net result of fluctuating pressures, with pulses lasting 1 min. (0.017 Hz), would be to take the cyprids deeper in the water column (Knight-Jones and Qasim 1967).

Cyprids, therefore, perceive pressure changes and respond to very low frequency pressure changes causing them to sink, a response similar in outcome to that shown by cyprids to sound fields at much higher frequencies. The sound pressures produced in the present study can be only roughly inferred from the acoustic power (W) radiated from the probe :

$$W = [(\rho_o ck^2)/4\ ].S^2.U_o^2 \ldots\ldots (2)$$ (Kinsler and Frey 1950)

where $\rho_o c$ = the characteristic impedance of seawater, k = the wave number (2π /wavelength), S = the surface area of the probe tip and U_o = the velocity amplitude of the probe tip (viz. displacement amplitude x frequency of vibration). The mean square pressure in the chamber will reach a maximum value of :

$$Pm^2 = (4W \rho_o c)/a \ldots\ldots (3)$$ (Kinsler and Frey 1950)

where Pm^2 is the maximum mean square pressure and a is the total absorptive power of the boundaries of the chamber. The coefficients of absorption are normally found experimentally for diffuse sound fields and no exact solution for the small observation chamber can be given. However, we know that the absorption coefficient will be small as air/water interfaces have a low transmission coefficient (0.00112 for a normally incident plane wave). For sound waves incident at angles approaching 0^o ("grazing incidence") the transmission coefficient will be zero, all the sound energy being reflected. A very rough estimate of the absorption coefficient could be considered as 0.5(0.00112 + 0) = 0.00056, the median transmission

coefficient for incident sound waves at angles from 0 - 90°. This estimate, of course, ignores the frequency related absorptive powers of the boundary. a in equation (3) would then be the total surface area of the chamber times 0.00056.

For a probe frequency of 50 Hz, amplitude of 1.8×10^{-4} m and a probe tip radius of 5×10^{-4} m, the acoustic power radiated from the source (W eq(2)) = 2.616×10^{-13} watts. The total surface area of the enclosure was 1.8373×10^{-2} m^2, thus a (eq(3)) = 1.0292×10^{-5}. The r.m.s. pressure within the enclosure could thus be approximated from equation (3) as $Pm^2 = 0.391$ N m^{-2} or 3.91 µbar, which is considerably below the 10 mbar pulses used by Knight-Jones and Qasim (1967). Using the same reasoning as above, the maximum pressure produced in the chamber for a probe frequency of 200 Hz and an amplitude of 6×10^{-5} m. would be 43.4 µbar. The sound pressure within the small chamber will tend to be higher at higher frequencies than at lower frequencies with the same source displacement amplitude.

The response of the cypris larvae to sink in the water column in response to a sufficiently intense sound field of low frequency vibration is perhaps a difficult phenomenon to interpret. It is, however, clear that sound levels in the sea increase inshore and in windy, turbulent conditions. In particular the intensity of low frequency sound (1 - 1000 Hz) under these conditions predominates over the higher frequency ranges (see Wenz 1962, 1964). Cypris larvae could thus be expected to be found deeper in the water column inshore and in storms, dependant on the effects of other, stronger stimuli prevailing at the time.

It is known that adult barnacles can produce noises as they feed (Busnel and Dziedzic 1962, Fish 1964). These noises range from rasping sounds as the opercular plates open, to clicks as they shut, and even to very weak sound fields produced by the beating of the cirral net. Busnel and Dziedzic (1962) analysed the sounds produced by B.balanoides in a small aquarium tank and found that the frequency range of the higher intensity sounds were well beyond the limited 'hearing' range demonstrated above for cypris larvae. It is, therefore, highly improbable that the cyprids could utilise sonic clues from adults of their own species in order to assist in the location of a suitable settlement site, or to avoid being eaten by the adult barnacles.

Organisms can respond to either the sound pressure or the particle velocities and displacements associated with a propagated sound wave. Close to the sound source near-field effects predominate and given sound pressures are associated with much larger particle displacements than would be found further from the source (in the far-field). The use of a sonic probe of the type described above, and used by Horridge and Boulton (1967) and Fraser (1969), in enclosed volumes of water will create complex sound fields where no clear distinction can be made between the two possible stimuli presented by the sound wave, i.e. particle displacement or sound pressure. It has been shown that fishes with swim bladders are particularly sensitive to sound pressures whereas those with no swim bladder can best respond to the particle displacements generated within a sound field (see Tavolga 1964 and Hawkins 1973).

Barnacle cyprids can respond to pressure fluctuations although the pressure levels required to evoke such a response are probably well in excess of the pressures associated with sound fields in seawater. For example, the r.m.s. sound pressure hearing threshold for the cod is 32 µbar at 400 Hz (Beurkle 1968), whereas at 35 Hz the threshold is between 0.3 and 3 µbar depending on the ambient noise level. The cyprid has also been shown to respond to unidirectional water currents (Crisp 1955, Section 8 above). The oscillatory displacements of the medium during the propagation of a sound wave would be capable of stimulating the type of receptor organs which respond to water currents (see Laverack 1963).

Since the investigation described above did not distinguish between the pressure and displacement components of the sound field, it was decided to build a chamber capable of restraining sound waves in such a manner that the two components could be distinguished. The intention was to establish whether the cyprid might respond to oscillating pressures or displacements separately.

A dual mode sound chamber

Frings and Frings (1967) discussed various methods of producing periodic disturbances for the investigation of the behaviour of marine organisms exposed to sound fields. They had progressed little beyond the probe mounted on the loudspeaker coil. Parvulescu (1964, 1967) forcefully pointed out the problems associated with the production and measurement of sound fields in small tanks of dimensions considerably less than the wave length of the generated sound waves. Olson and May (1953) and Parvulescu (1961, 1964) suggested how the reflections from sound waves at water

interfaces with different materials could be employed to produce clearly defined sound fields. The idea was to propagate sound waves from one end of a tube, and to alter the characteristics of the other end of the tube in such a way as to affect the phase of the components of the reflected waves. By constraining low frequency sounds in a tube plane wave conditions will prevail (Parvulescu 1964). If the end of the tube is made very soft such that the fluid boundary can oscillate easily (open end), then the phase of the reflected pressure wave will be opposite to that of the incident wave and the waves will cancel, i.e. only the displacement component of the wave will be apparent. On the other hand, if the end of the tube is made sufficiently rigid (closed end) to allow negligible fluid movement, then the phase of the reflected displacement wave will be reversed to cancel the incident wave, i.e. only the pressure component will be apparent.

In practice such devices have been constructed from large tubular steel tanks with a sonic projector at each end. The same sound signal can be generated from each projector, yet by adjusting the phase of the signal from one projector relative to that of the other, the ends of the tube can be made to appear as soft or rigid boundaries. Alternatively, the projectors could be driven with such phase differences as to generate pressure and displacement maxima at any point along the length of the tube. Bobber (1962) tested the effectiveness of such an acoustic tank for a fluid medium and Cahn, Siler and Wodinsky (1969) used the method to investigate the hearing sensitivities of grunts (Pisces).

Methods

The scaling down of the type of tank used by Cahn et al (1969), for use with very small planktonic organisms, was attempted with the help and advice of Dr.A.D.Hawkins (M.A.F.F. Laboratory Aberdeen). The chamber employed is shown in Fig 9.4. A rigid perspex tube was constructed with square cross section and mounted on a perspex base which was bolted to a heavy, 5 cm thick slate and stood on a sand tray to reduce transmitted vibrations. It was found impractical to use a sonic projector at either end of the small tank shown in Fig 9.4, hence only one projector (Derritron, V2 vibrator, V Fig 9.4) was bolted underneath the tank. The cone of the vibrator was linked to two solid, plastic coated, steel washers (sw Fig 9.4) which sandwiched a rubber diaphragm (d Fig 9.4) thus forming the projection end of the tank. A 25 watt amplifier was used to drive the vibrator from a Levell oscillator over the range 1 Hz - 300 kHz.

The other end of the tank could either be left open allowing the water to move freely under the forced vibrations of the projector, resulting in the soft termination of the tube required to provide a pressure minimum within the tank. Alternatively, a lid (l Fig 9.4) of 3.5 cm thick perspex, corresponding to the section of the tank, could be fitted and tightly clamped in place with a brass clamp (c Fig 9.4). The free end of the tank was machined to a funnel shape so that the lid could be positioned without the introduction of air bubbles. With the end of the tank rigidly sealed, negligible water movement could occur in the tank and the sound field produced with a minimum displacement component.

Fig 9.4

The apparatus used to produce sound fields of either predominantly pressure or displacement fluctuations in seawater.

c = brass clamp.

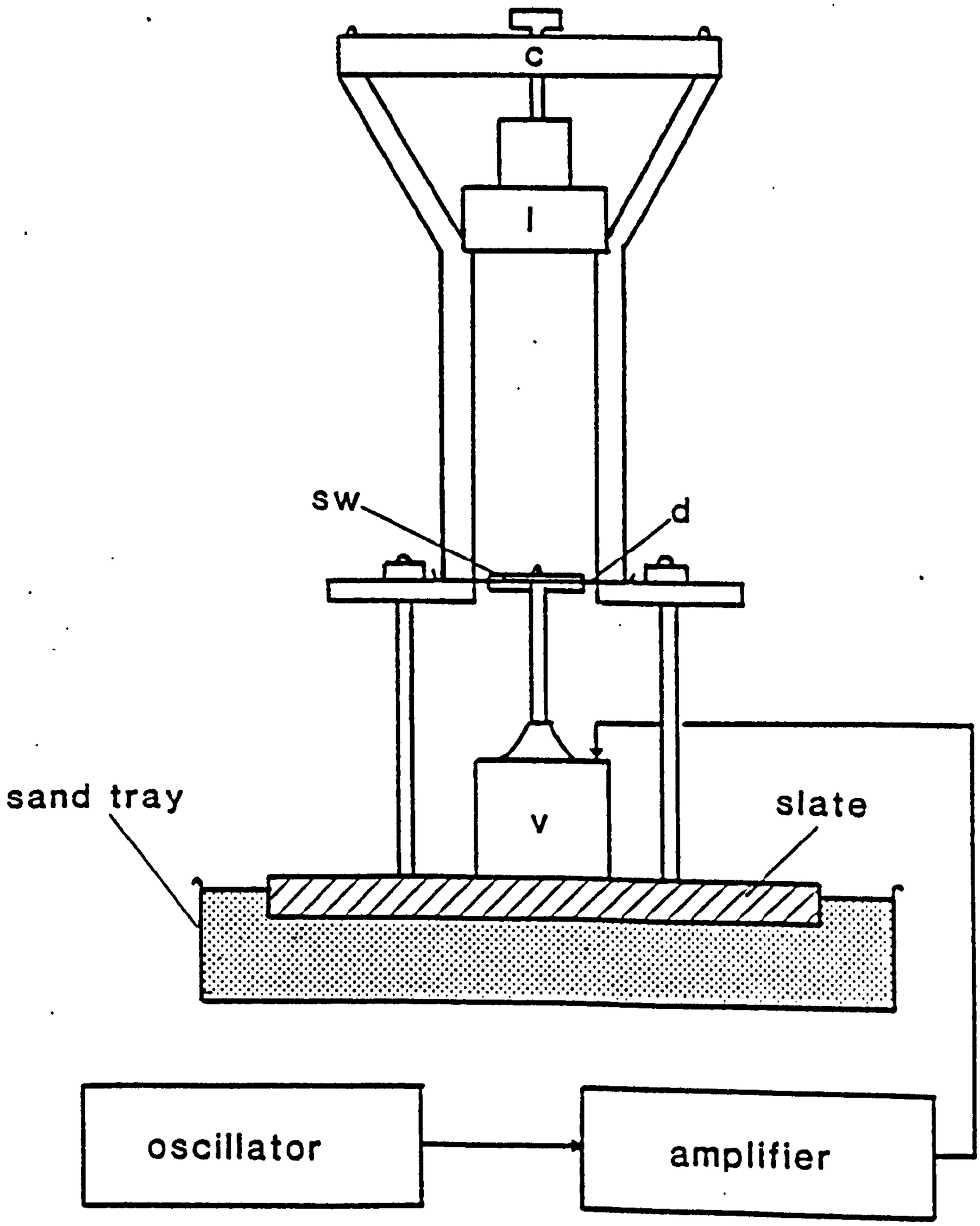
d = rubber diaphragm.

l = perspex lid.

sw = steel washer.

v = Derritron vibrator.

Fig 9.4 A dual mode sound chamber



The tank was filled with seawater and allowed to equilibrate to room temperature at between 18 and 20°C. Batches of cyprids were then added to the seawater and the tank evenly illuminated from two sides by 12" strip lights. The behaviour of the cyprids was recorded on video-tape throughout the experiments. A line was drawn across the front of the tank, 8 cm above the projector face and the number of cyprids rising and falling across the line was counted for one minute. The vibrator was then switched on for a further minute and a second count made. The frequency of the sound stimulus was varied over the range 1 - 1000 Hz leaving a minimum period of 10 minutes between changes in stimulus frequency. The stimulus range was repeated on several batches of cyprids. A further count was made of cypris larvae which moved off the bottom (actually the face of the projector diaphragm) and swam past a line 1 cm above the projector, both before and during the presentation of the sound stimulus. The observations were made either with the lid off the chamber, presenting the cyprids with a sound field with negligible pressure component, or with the lid clamped on, presenting the cyprids with a sound field possessing negligible displacement component. Experiments performed to count the numbers of cypris larvae rising from the bottom of the chamber were performed with the lid off the chamber, as the nature of the stimulus close to the projector face would have been essentially the same regardless of the mode of operation.

Results

The cypris larvae were not at all active in the still water of the sound chamber (see Crisp 1955) and increasingly large numbers of cyprids had to be introduced before sufficient numbers could be observed swimming up and down in the water column. Most of the cyprids merely sank to the bottom of

the tank and lay on the projector face, showing very little movement. Early observations at low sound intensities failed to show a response from the cyprids and were therefore discontinued, thus most of the results shown below refer to the highest intensity possible from the equipment (-45 dB re 1mW).

The results obtained were very variable and were analysed in two ways. Firstly the total numbers of cypris larvae moving up and down in the water column across the fixed reference point in the presence of the sound stimulus were compared to the total numbers moving during a control period, which was normally the one minute period before the presentation of the stimulus. The numbers of cyprids in the tank were quite variable between experiments hence the results were calculated as the proportional difference between the numbers of cyprids moving during the control period and the numbers moving during an experimental period, to maintain comparability between experiments. Thus the difference in total numbers moving between control and experimental periods was calculated as : (the sum of the numbers moving up and down in the control period - the sum of the numbers moving up and down in the experimental period) divided by the sum of all the cyprids moving up and down in both periods. Table 9.3 shows the results for the two operant modes of the sound chamber. The mean difference in the proportion of the total numbers moving up and down between control and experimental periods was compared to zero by dividing the mean difference by the standard error of the mean. The t-values and associated probability of obtaining the observed differences by chance are given in Table 9.3. A positive mean difference indicates greater cyprid activity in the control period, a negative mean difference indicating greater activity in the presence of the sound stimulus. The results for sound fields of

Table 9.3

An analysis of the relative numbers of active cyprids moving at sound frequencies between 1 and 1000 Hz in a tank producing sounds in either displacement or pressure modes at an intensity of -45 dB (re 1 mW). The results show mean values for numbers of B.balanoides cyprids moving past a line on the tank in a control period minus those moving past during the stimulus presentation, divided by the sum of the numbers of larvae moving in both periods.

(a) Tank operated in the pressure mode

Frequency(Hz)	Mean	St error	df	t	p
1	+0.089	0.064	7	1.3748	0.212
10	-0.041	0.054	8	0.7688	0.464
20	+0.061	0.086	8	0.7111	0.497
30	-0.026	0.101	6	0.2570	0.806
40	-0.059	0.072	7	0.8147	0.442
50	-0.047	0.120	7	0.3890	0.709
60	-0.089	0.144	5	0.6156	0.565
70	-0.056	0.173	4	0.3239	0.762
80	-0.092	0.063	4	1.4518	0.220
90	+0.043	0.101	2	0.4219	0.714
100	+0.008	0.069	5	0.1210	0.908
200	-0.054	0.095	2	0.5703	0.626
300	+0.003	0.080	3	0.0408	0.970
500	+0.028	0.096	2	0.2892	0.800
1000	+0.036	0.086	2	0.4227	0.714

(b) Tank operated in displacement mode

Frequency(Hz)	Mean	St error	df	t	p
1	-0.124	0.052	8	2.3830**	0.044
10	-0.032	0.089	9	0.3613	0.726
20	-0.175	0.081	8	2.1749*	0.061
30	-0.020	0.141	5	0.1382	0.896
40	+0.032	0.088	6	0.3645	0.728
50	+0.010	0.055	9	0.1739	0.868
60	-0.160	0.069	7	2.3256*	0.053
70	-0.038	0.081	8	0.4689	0.652
80	-0.145	0.085	7	1.7069	0.132
90	-0.111	0.086	7	1.2843	0.240
100	-0.160	0.054	12	2.9673**	0.012
150	+0.047	0.081	5	0.5869	0.583
200	-0.052	0.077	7	0.6741	0.522
300	-0.064	0.064	5	1.0000	0.363
500	-0.162	0.201	3	0.8040	0.480
1000	-0.077	0.107	2	0.7224	0.545

df = degrees of freedom. t = t-value.
p = probability of obtaining difference by chance.
** = significant difference. * = difference approaching significance.

predominantly pressure fluctuations (Table 9.3 (a)), show very small mean differences and none could be considered significantly different from zero. No apparent trend in the sign of the difference with frequency was readily apparent, nearly equal numbers of positive and negative signs were obtained.

The results for sound fields of predominantly displacement fluctuations, (Table 9.3 (b)), were similar, with the majority of mean differences showing no significant difference from zero. However, the mean differences for 1 and 100 Hz were shown to differ significantly from zero and those for 20 and 60 Hz approached the 5% significance level. Furthermore, negative mean differences predominated over positive differences (Table 9.3). Within the variability of the results obtained, cyprids appeared to be more active when the tank was operated in the displacement mode, than they were during control periods. The results do not, however, reflect the influence of frequency, as the increase in total numbers moving during experimental periods, as compared to control periods, were noted at 1 and 100 Hz and large mean differences between control and experimental periods shown at 500 Hz (Table 9.3 (b)).

The analysis was carried a stage further to determine the direction of cyprid movement when they were exposed to sound fields. As before, the results were expressed as a proportion of the total numbers moving, for comparability between experiments. Tables 9.4 and 9.5 show the results obtained for the two operant modes of the sonic tank. The mean difference was calculated as the numbers of cyprids moving up over the reference line minus the numbers moving down past the line, divided by the sum of the numbers moving up and down during the observation period. A positive mean difference, therefore, indicates more cyprids moving up than down and vice

Table 9.4

Results for the relative difference in the numbers of B.balanoides cyprids moving up minus those moving down in a tank producing sound fields in the frequency range 1 - 1000 Hz predominantly of pressure fluctuations at an intensity of -45dB (re 1 mW). Differences for equivalent periods in the absence of the sound stimulus (controls) are also shown.

	(a) Stimulus					(b) Controls				
Freq	Mean	SE	df	t	p	Mean	SE	df	t	p
1	−0.061	0.056	7	1.0974	0.309	+0.037	0.039	7	0.9507	0.370
10	−0.025	0.049	8	0.5112	0.623	−0.082	0.086	8	0.9522	0.369
20	+0.031	0.032	8	0.9529	0.369	−0.010	0.028	8	0.3507	0.735
30	−0.026	0.047	6	0.5476	0.604	+0.005	0.070	6	0.0678	0.948
40	+0.107	0.087	7	1.2369	0.256	−0.075	0.049	7	1.5370	0.168
50	−0.199	0.131	7	1.5169	0.173	+0.025	0.071	7	0.3528	0.735
60	−0.081	0.191	5	0.4219	0.691	−0.145	0.176	5	0.8243	0.447
70	+0.17	0.099	4	0.1747	0.870	+0.041	0.072	4	0.5715	0.600
80	+0.047	0.025	4	1.8544	0.137	+0.059	0.026	4	2.2515	0.088
90	−0.191	0.126	2	1.5119	0.270	+0.145	0.158	2	0.9178	0.456
100	+0.054	0.026	5	2.2891	0.071	−0.032	0.014	5	2.3281	0.067
200	−0.116	0.077	2	1.1949	0.355	+0.028	0.031	2	0.9239	0.453
300	+0.017	0.088	3	0.1881	0.863	+0.043	0.081	3	0.5306	0.633
500	−0.081	0.144	2	0.5611	0.631	−0.048	0.048	2	1.0000	0.423
1000	+0.087	0.208	2	0.4269	0.711	−0.078	0.049	2	1.6075	0.249

Freq = frequency of vibration.
Mean = mean value for observations of (Numbers of larvae swimming upwards - Numbers of larvae sinking downwards)/(Sum of the numbers moving up and down.
df = degrees of freedom, t = the t-value for comparison of the mean difference with zero, i.e. mean divided by standard error of the mean (SE).
p = probability of obtaining observed difference by chance.

Table 9.5

Results for the relative difference in the numbers of B.balanoides cyprids moving up minus those moving down in a tank producing sound fields in the frequency range 1 - 1000 Hz predominantly of displacement fluctuations at an intensity of -45dB (re 1 mW). Differences for equivalent periods in the absence of the sound stimulus (controls) are also shown.

	(a) Stimulus					(b) Controls				
Freq	Mean	SE	df	t	p	Mean	SE	df	t	p
1	+0.026	0.035	8	0.7305	0.486	+0.006	0.051	8	0.1252	0.904
10	-0.032	0.048	9	0.6708	0.519	+0.035	0.022	9	1.6090	0.142
20	-0.068	0.031	8	2.2132	0.059	+0.018	0.042	8	0.4262	0.681
30	-0.038	0.050	5	0.7615	0.481	-0.021	0.017	5	1.1822	0.290
40	+0.163	0.109	6	1.4949	0.186	+0.002	0.037	6	0.0430	0.967
50	-0.029	0.044	9	0.6575	0.527	+0.039	0.033	9	1.1878	0.265
60	-0.103	0.142	7	0.7269	0.491	+0.135	0.128	7	1.0529	0.327
70	+0.076	0.092	8	0.8221	0.435	-0.011	0.037	8	0.2903	0.779
80	+0.010	0.033	7	0.1941	0.852	+0.003	0.029	7	0.0873	0.933
90	-0.027	0.061	7	0.4369	0.675	+0.012	0.041	7	0.2978	0.775
100	+0.012	0.036	12	0.3240	0.752	+0.026	0.029	12	0.8905	0.391
150	-0.001	0.054	5	0.0092	0.993	+0.045	0.027	5	1.6578	0.158
200	-0.049	0.060	7	0.8180	0.440	-0.037	0.056	7	0.7350	0.486
300	-0.086	0.126	5	0.6839	0.524	+0.042	0.035	5	1.1947	0.286
500	+0.107	0.086	3	1.2449	0.302	-0.054	0.071	3	0.7607	0.502
1000	-0.045	0.051	2	0.8808	0.471	+0.094	0.124	2	0.7565	0.528

Freq = frequency of vibration.
Mean = mean value for observations of (Numbers of larvae swimming upwards - Numbers of larvae sinking downwards)/(Sum of the numbers moving up and down.
df = degrees of freedom, t = the t-value for comparison of the mean difference with zero, i.e. mean divided by standard error of the mean (SE).
p = probability of obtaining observed difference by chance.

versa for a negative mean difference. Table 9.4 (a) shows the results for sound fields of predominantly pressure fluctuations from 1 - 1000 Hz, while Table 9.4 (b) shows the results for control periods taken from the same batches of cyprids. Again the mean differences were small and none were significantly different from zero. The signs of the differences were also evenly distributed with frequency and control batch. Movement of the barnacle cyprids within the chamber, thus appeared to be random in direction whether in the control or experimental situation.

Table 9.5 (a) and (b) exhibits the results for sound fields of predominantly displacement fluctuations and control periods respectively. The results were the same as those for the pressure mode; no significant differences could be observed. The sign of the differences shows that, in general, the cyprids tended to fall in the water column in the presence of the sound field whereas the majority of the control observations showed a positive difference, indicating that the cyprids were tending to rise during the control periods. The overall result for the mean difference in the experimental periods minus the mean difference in the control periods over the frequency range 1 - 1000 Hz gave a mean of -0.027 ± 0.026 ($\pm$ standard error). Comparing this mean to zero, as above, gave a t-value of 1.0403 for 30 degrees of freedom, corresponding to a probability of 0.31 that the result could have been obtained by chance. Thus, as for the results of the pressure mode experiments, the movement of cyprids within the sound fields of predominantly displacement fluctuations were random in direction.

Numbers of cyprids moving off the bottom

Towards the end of the 1976 B.balanoides settlement season a very limited number of observations were made on the numbers of cyprids moving off the bottom of the sound tank to cross a line 1 cm above the projector face. The tank was always operated with the lid off. The larvae would have experienced vibrations of the substratum rather than sound fields in the water column. A series of control observations were made, for one minute, of the numbers of larvae rising from the bottom of the tank and swimming above a line 1 cm above the bottom. Similar one minute observations were made at a range of frequencies. The procedure was repeated at progressively lower sound intensity settings on the oscillator. The results are shown in Table 9.6 where the mean ratio of experimental numbers/mean control numbers is shown for frequencies up to 200 Hz and vibration intensities between -45 and -59 dB (re 1mW). Where possible, standard errors for the mean ratio have been included. The results have been plotted in Fig 9.5, where the ratio of the numbers of cyprids swimming up during an experimental period to the numbers of larvae swimming up during a control period has been plotted against frequency at each intensity level measured. Although few observations were made, the highest intensity level used (-45 dB) caused an increase in the numbers of larvae moving off the bottom over the range 1 - 100 Hz. The response decreased steadily with vibration intensity as did the bandwidth of frequencies eliciting the response. The peak sensitivity of the cyprid appeared to be between 50 and 60 Hz.

Table 9.6

The mean values ± standard errors (where possible) for the numbers of B.balanoides cypris larvae moving off the bottom at various sound frequencies and intensities divided by the numbers of larvae moving off the bottom in the absence of the stimulus.

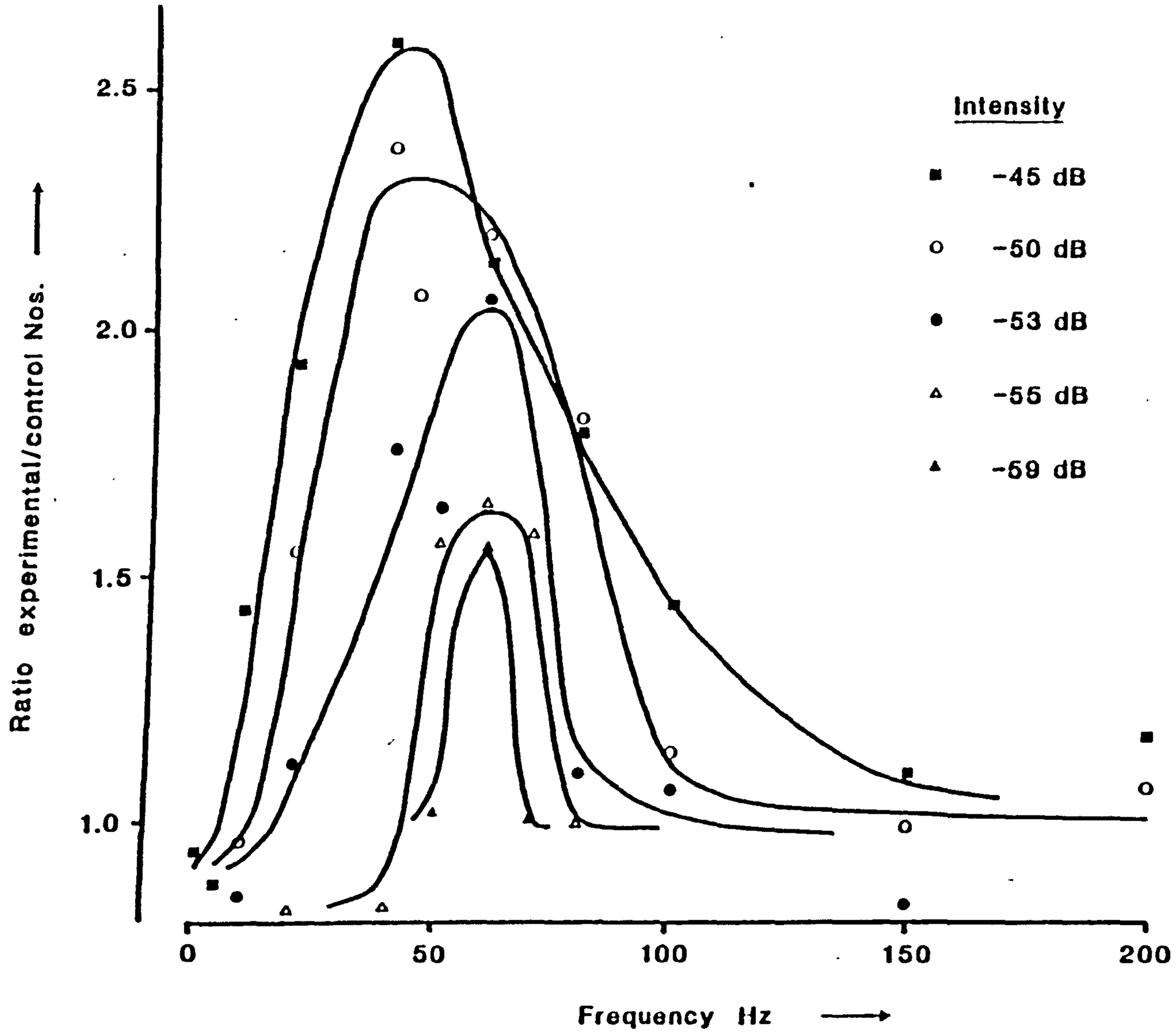
I	Frequency Hz											
dB	1	5	10	20	40	50	60	70	80	100	150	200
-45	0.94	0.87	1.43	1.93 ±0.03	2.62 ±0.59		2.14 ±0.22		1.79 ±0.06	1.44 ±0.19	1.10 ±0.02	1.18
-50			0.96	1.55 ±0.21	2.38	2.07	2.20		1.82	1.14 ±0.17	0.99 ±0.06	1.07
-53			0.85	1.12 ±0.16	1.76 ±0.35	1.64	2.07 ±0.57		1.10 ±0.38	1.07 ±0.10	0.83	
-55				0.82	0.82 ±0.10	1.57 ±0.30	1.65 ±0.47	1.59 ±1.16	0.99 ±0.06			
-59						1.02 ±0.15	1.56 ±0.37	1.00 ±0.08				

I = vibration intensity in dB (re 1 mW).

Fig 9.5

The response of B.balanoides cyprids to substrate vibrations. The ratio of experimental Nos. / control numbers moving off the bottom to a line 1 cm above the projector face, plotted against vibration frequency for intensities from -45 to -59 dB (re 1mW).

Fig 9.5 B.balanoides cyprids moving off the bottom in response to substrate vibration



Discussion

For the most part the results described above were not conclusive. Although it was intended to carry the experimental work further, particularly that relating to vibrations of the substratum, several factors conspired to defeat such an intention. The equipment was not suitable for operation in the pressure mode above -45 dB and the strain on the vibrator, caused by operating the tank in the pressure mode at this intensity, produced a malfunction in the amplifier as the experimental work progressed. Although the amplifier was repaired, the problem recurred. Eventually the vibrator broke down completely and damaged the amplifier beyond repair. Since the early results had proved disappointing, the cost of providing replacement equipment and also further equipment to characterise the sound fields produced in a more satisfactory manner was beyond that which could be justified from possible later use of the equipment. No further experimental work was carried out with the dual mode sound chamber.

The results above offer no firm conclusions, yet several points may be considered. Cyprids showed no detectable differences in behaviour from control larvae when presented with sound fields predominating in the pressure component. In similar circumstances, when the displacement component of the sound field predominated, the cyprids showed a tendency to move around more than controls irrespective of the sound frequency, i.e. cyprids were more active in moving than in still water, as has been already been noted by Crisp (1955).

When cyprids were presented with a vibrating substrate they responded by swimming off and rising a short distance in the water column. The bandwidth of frequencies over which the cyprids responded was very similar to that shown by cyprids tethered close to a vibrating probe (see above). The cyprids did not continue to swim upward in the tank but stayed close to the bottom, often falling back again onto the face of the projector. Knight-Jones and Moyse (pers. comm.) have demonstrated that the settlement of barnacle nauplii was not affected by high frequency vibrations (Ultra sonic) which was perhaps not surprising considering the very narrow bandwidth, at low frequencies, to which cyprids have been shown to be sensitive during the present study. It may be advantageous for cyprids to fall in the water column in stormy conditions and shallow water, yet no obvious advantage could be postulated for cyprid movement off surfaces vibrating at very low amplitude. The possibility remains that the behaviour is an artefact due to the unusual conditions of stillness and even lighting prevailing in the sound chamber, presenting the cyprids with minimal stimulation. The presentation of a vibrating surface caused the animals to move off the surface although, in the natural situation, other stimuli, such as light, water currents and organic molecules, would normally override such a response. Rocks on the shore are exposed to wave and current action which would produce low amplitude vibrations in the rocks, augmented by vibrations transmitted from the land through the rocks, within the frequency range perceived by the cyprids. The larvae ignore such vibrations when they settle because other stimuli are more important.

The methods of the later part of this section have tried to employ the movements of groups of larvae to evaluate the response to sound stimuli. In the control situation the numbers of larvae moving in the water column will

be determined by factors largely outside the control of the investigator. When the stimulus was presented the hope was that most of the larvae would react in a certain way and thus the difference in behaviour readily assessed. Where a strong stimulus is applied, such as light (Crisp and Ritz 1973) or pressure (Knight-Jones and Qasim 1967), almost all the organisms react by moving in the same direction. However, problems with this technique could be envisaged whereby the reaction to some stimuli may be affected by the behaviour of the organism prior to the stimulus presentation. For example, the stimulus may produce a reaction in the larvae which causes them to sink in the water column, if a larva was already sinking it would show no change in behaviour. On the other hand, the stimulus may simply produce a general response from the larvae, for example, the larvae may just reverse their behaviour pattern in the manner of an escape response by swimming up if they were already sinking or on the bottom, or by sinking if they were already swimming up. The outcome of such behaviour on a group of individuals may not be detectable by simply counting the numbers moving in one direction or another, as no directed response would be shown. Singarajah, Moyse and Knight-Jones (1967) used the phototaxic response of barnacle nauplii to demonstrate the effect of feeding on the nauplius' response to light. The results were fairly clear for starved larvae where they were nearly always photopositive. However, when fed larvae were used, the results were not so clear, the majority were photonegative, many remained photopositive and others seemed indifferent to the lighting conditions. Clear differences were shown between starved and fed individuals but the results indicated greater variability in response when previously fed animals were studied in pasteurised seawater. The time of day at which the experiments took place further complicated the results as more positive larvae were found in the late afternoon than in the morning

even when artificial light sources were used. The interaction of the various factors demonstrated by the results of Singarajah et al (1967) shows how important is the knowledge of ongoing behaviour and of the past experience of the larvae when the interpretation of the results of directed movement measurements from groups of animals is attempted.

The above considerations apply equally well to studies of the behaviour of single, restrained larvae, a topic which occupies much of the preceding sections of this Thesis. However, the uncertainties associated with group movement studies such as physical defects, general condition, whether or not the organism has been feeding etc. can be ascertained over a period of observation, and even preconditioning, prior to the experiment, when a single animal is restrained. Thus, apart from the obvious advantages of close observation of detailed behaviour and the mechanisms underlying specific behaviour patterns afforded by the use of restrained organisms, the performance of the individual can be gauged relative to prior observation of that individual. Experiments may extend over time periods beyond a week without apparent harm to the larva, were such lengthy periods deemed necessary. The unusual world of the minute planktonic organism has never been closer to inspection. Observations of these organisms in a situation more closely resembling the natural environment than in a small volume of seawater in a round bottomed watch glass will undoubtedly lead to a better understanding of the behaviour patterns which play an important role in the ecology of the oceans.

Section 10

A Study of Feeding Behaviour in *Temora longicornis* (Crustacea :Copepoda)

The contents of this section are intended for publication to detail the methods developed during the period of study. A description of the methodology has, therefore, been repeated, in a summarised form, and improvements to the seawater flow system, made during the course of the project, are given.

A Study of Feeding Behaviour in Temora longicornis (Crustacea :Copepoda)

Introduction

The study of zooplanktonic organisms has always presented problems. Small and delicate animals are difficult to handle and observe, even in the laboratory, and excessively so in the sea. The study of copepod feeding mechanisms clearly illustrates some of the problems involved. Esterly (1916) first suggested that water currents produced by the cephalic appendages of copepods were implicated in the feeding mechanism. Storch and Pfisterer (1925) gave a preliminary account of currents produced by swimming Diaptomus which was hotly contested by Cannon (1928). Cannon objected to the strong anterior to posterior swimming current postulated by Storch and Pfisterer (1925) on the grounds that copepods seemed to move too slowly for such a strong swimming current to be produced. From observations on Calanus and Diaptomus Cannon (1928) produced a model of copepod feeding based on the swirling vortices observed at the side of a swimming copepod. A large vortex was seen at each side of the copepods with the water currents moving in a clockwise direction on the left and anticlockwise on the right when viewed from above and behind the copepod. This large vortex was termed the swimming vortex and was shown to stretch in a continuous U-shape under the copepod from one side to the other. Seawater was drawn into the swimming vortex from the front of the animal and excess water pushed posteriorly from the vortex thus moving the copepod forwards. The vortex was created mainly by the motion of the antennae, mandibular palps and the maxillipeds. A second smaller vortex was also observed closer to the body of the copepods, swirling in the direction opposite to that of the swimming vortex. Cannon (1928) called this smaller vortex the feeding current. The feeding current

was created from a back eddy of the swimming vortex engendered by the rotary motion of the maxillipeds. Particulate matter carried in the feeding current was directed from the posterior of the animal towards a filtering chamber delineated by the maxillae and the thoracic limbs. Material collected on the maxillae was then scraped off and transferred to the mouth by the mandibles. Cannon's observations led to a widely held belief that copepod feeding was an uncontrolled filtration process, and an automatic consequence of slow swimming.

Cannon's view of copepod feeding was criticised by Lowndes (1935). He produced a wealth of evidence from observations on the behaviour of many copepod species and the study of copepod gut contents (Lebour 1922, Marshall 1924) to show that copepod feeding need not be automatic and that the mechanisms probably varied from species to species.

Despite Lowndes criticism, Cannon's view of copepod feeding persisted. Gauld (1966) repeated much of Cannon's and Lowndes' work and described an eddying current feeding mechanism in some copepods e.g. Calanus and Temora but not in others e.g. Acartia. Gauld described the morphological differences between the maxillae of various copepod species and related these differences to the mechanisms used for feeding. The differences in the feeding habits of copepods, some of which were predaceous (e.g. Anamalocera), were emphasised by Gauld (1966). Indeed most copepods were considered by Gauld (1966) to be capable of grasping large algal cells or other small plankters in addition to filtering particles less than 40 - 50 µm. either in the manner described for Calanus by Cannon, or in the manner described by Conover (1956) for Acartia, which use their maxillae as scoop nets. Gauld (1966) concurs with Cannon (1928) in his description of the

feeding currents produced by some copepods, yet agrees with Lowndes (1935) that it would be a mistake to assume that all calanoid copepods feed in the same manner.

Much of the later work on copepods has concentrated on the quantitative aspects of feeding rather than the mechanisms (Fuller 1937, Gauld 1951, Marshall and Orr 1955 (b), Adams and Steele 1966, Paffenhöfer 1971). The results of feeding rates so obtained differ widely and often conflict (see Conover 1968). Copepods have, however, consistently been shown to select large, as opposed to smaller, particles when presented with mixtures (Frost 1972 Calanus, Wilson 1973 Acartia, Poulet 1973 Pseudocalanus). The mechanism of size selection has given rise to some disagreement (see Boyd 1976). Particles in the size range from 5 - 7 µm. up to 150 µm. can be caught by copepods (Poulet 1973). However particles over 40 - 50 µm., would be excluded from the filter chambers of many copepods by "screening setae" on the maxillae, were the particles to enter the chamber from behind (Gauld 1966). Copepods have been observed to pursue and grasp larger sized particles (Petipa 1965). On this basis Cushing (1959,1968) developed the theory of "encounter grazing" by copepods along similar lines to those already developed for planktivorous fishes (Ivlev 1955).

Recently high speed cinématography (Koehl and Strickler 1981) has thrown considerable doubt on the classical view of the mechanisms of copepod filter feeding. Koehl and Strickler suggested that the copepods Eucalanus and Centropages, which feed and swim intermittently, capture food particles brought into the feeding area from in front, and not behind, the copepods. They made their observations in 120 ml of seawater and considered the feeding swirls reported from earlier works to be artefactual. The

experiments establishing the existance of swimming and anteriorly flowing feeding currents in copepods were performed in very small volumes of water, often with the animal wedged between two rigid boundaries (Cannon 1928, coverslip and slide) or with the animal at an air\water interface, swimming against the edge of a cavity slide or watch glass (Gauld 1966). Such situations are obviously not encountered by zooplankton in the sea and the hydrodynamic conditions must differ profoundly from those in nature. Larger volumes of water would be preferable for an adequate analysis of zooplankton movement as was long ago realised by Lowndes (1935) and by Hardy and Bainbridge (1954). However, these workers found it difficult to observe free swimming copepods closely, and accurate detailing of swimming and feeding currents was impossible.

From a quantitative study of feeding by planktonic copepods Adams and Steele (1966) suggested that some of their results could best be explained if the copepods adjusted their feeding speed to the food availability. They suggested feeding speed was low at low algal concentrations, increased at moderate concentrations, yet declined again at very high algal concentrations. Lam and Frost (1976) developed a wide ranging model of copepod feeding behaviour based on the extensive data of Frost (1972). They also suggested that the feeding speed was adjusted to maximise the net energy gain to the organism, particularly at low algal concentrations. Neither Adams and Steele (1966) nor Lam and Frost (1976), however, suggested how the copepod feeding speed might be altered.

The present investigation describes a technique for tethering small zooplankters in large volumes of water, making possible detailed observation of the behaviour under conditions approaching the natural situation. It

seemed pertinent to reinvestigate the feeding mechanism of a calanoid copepod and to use tethered animals to investigate the response of the cephalic appendages of Temora longicornis to the presence of algal cells in the seawater.

Materials and Methods

Adult females of the inshore copepod Temora longicornis were collected by a medium mesh plankton net held stationary in the current flowing through the Menai Strait, Gwynedd. Temora were isolated from the other captured planktonic organisms and maintained in 3 litre glass jars of fine-filtered, ultra-violet irradiated seawater at 14°C. The animals were fed a mixed diet of flagellates and diatoms, such as Tetraselmis, Pavlova and Skeletonema. Mortality was low and the animals appeared to feed well.

Initial observations of the copepods were made in small watch glasses or cavity slides. However, critical observations were made in a perspex flow chamber (D Fig 10.1) which was supplied with fine-filtered, u-v irradiated seawater from a constant head (tank H Fig 10.1). The constant head overflowed to a resevoir tank (RT1 Fig 10.1) and was filled from a second resevoir tank (RT2 Fig 10.1) by siphon. Seawater was pumped (TP Fig 10.1) from RT1 to RT2. The separation of the constant head tank from the main resevoir (RT1) minimised the transmission of disturbances to the observation chamber (D) while the resevoirs were refilled.

The seawater was passed through a heat exchanger (HE Fig 10.1) immersed in a constant temperature bath (CT Fig 10.1) to regulate the water temperature in the flow chamber. Temperature was continuously monitored by

Fig 10.1

Diagram of the apparatus used to tether and observe copepods in flowing seawater at a controlled temperature.

CT = constant temperature bath.

D = observation chamber.

F = flow meter.

H = constant head tank.

HE = heat exchanger.

M = micromanipulator.

MS = stereomicroscope.

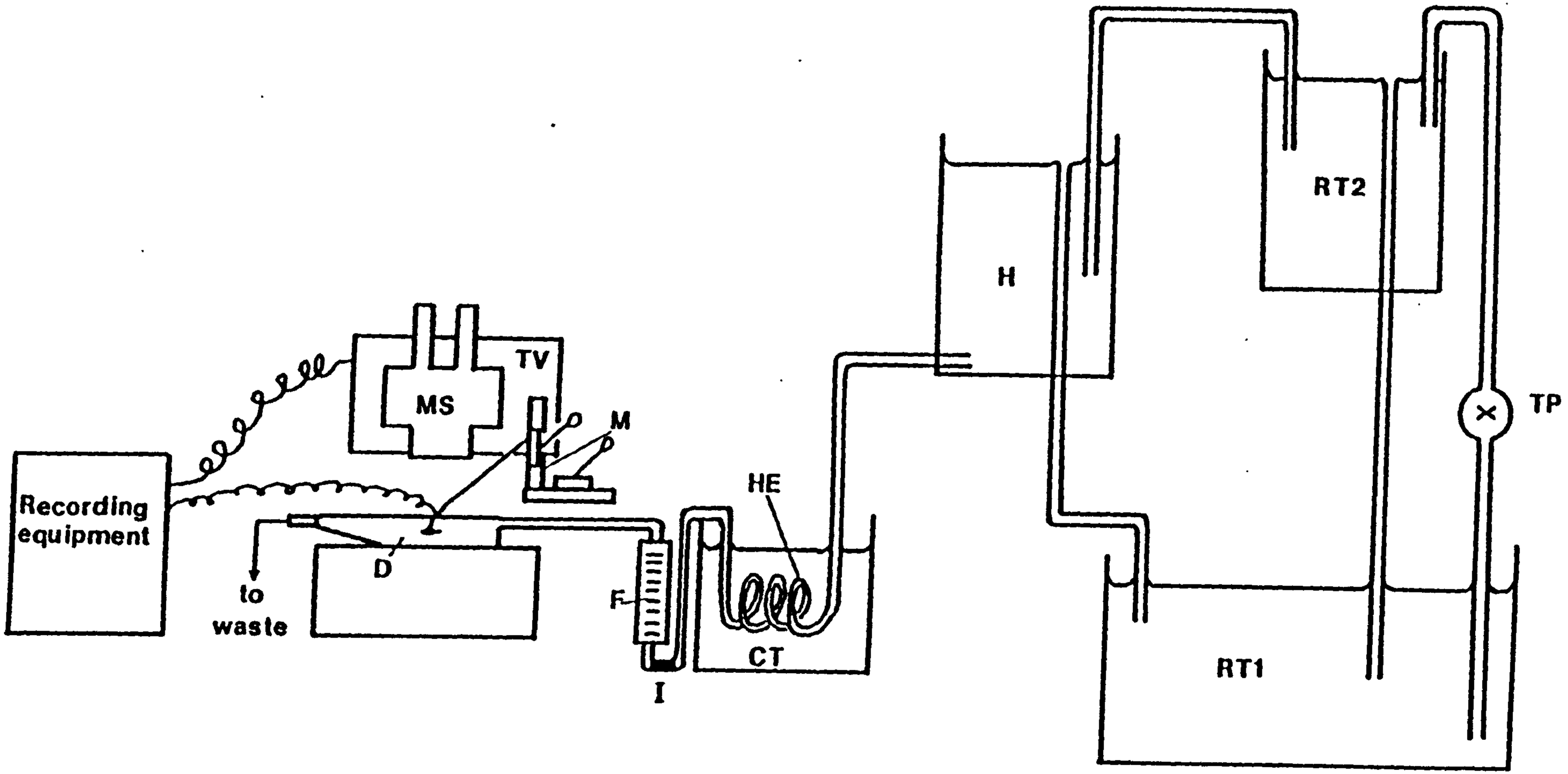
RT1 = resevoir tank (main).

RT2 = resevoir tank (secondary).

TP = T.E.P. pump.

TV = television camera.

Fig 10.1 Diagram illustrating the experimental apparatus



thermistor establishing control within the observation chamber at ± 0.25°C. A bead flow meter (F Fig 10.1) was fitted between the constant head tank and the flow chamber and calibrated to give controlled water flow in the centre of the chamber over the range 0 to 6.5 cm s^{-1}. Copepods were tethered in the centre of the flow chamber using an adaptation of the suction electrode used in electrophysiology. The device consisted of 50 cm. of 1 mm. diameter catheter tubing drawn out to a very fine tip at one end. The other end of the tube was fitted to a syringe, usually of 5 or 10 ml. capacity. The suction tip end of the tube was mounted in a micromanipulator (M Fig 10.1) and placed in the centre of the flow chamber under a Wild M5 stereomicroscope. Each copepod studied was anaesthetised in a weak solution of MS 222 Sandoz in seawater and placed near the suction tip. The animal was carefully positioned and sucked onto the tube tip by the syringe. The tip of the suction pipette was applied to the domed cephalothorax and in no way interfered with the swimming appendages. The copepods recover from the anaesthetic in approximately 30 seconds, so they must be attached speedily. All animals were left for at least 45 minutes on the suction tip, in a seawater flow of 0.75 cm s^{-1}, before any observations were attempted.

The suction tube can be bent to an appropriate angle before use, and the animal can be viewed from almost any angle by rotating the holding tube in the manipulator and repositioning within the microscope field of view. This handling technique is applicable to any planktonic organism which has a reasonably tough exoskeleton. Observations on six species of barnacle nauplii, two species of bivalve veliger, and several copepod species have been made using this technique and the results will be reported elsewhere. The success rate for the technique is very high, the animals always survived the attachment procedure.

In addition to direct observation and experiment with a microscope it was often desirable to record and store the information obtained for analysis at a later date. Two recording techniques were employed. A television camera (TV Fig 10.1) was mounted behind a photo-tube fixed to the microscope and the resultant image recorded on a time-lapse video-tape recorder (V.T.R.) (Hitachi). The behaviour of the organisms thus recorded could be played back through a Sony T.V. monitor at a range of tape speeds from about 14 cm s^{-1} to 0.6 cm s^{-1}. The second method of recording animal behaviour was based on the impedance pneumograph technique pioneered by Trueman (1967) for recording heart beat activity in bivalve molluscs and used by Davenport (1976) for recording the cirral activity of adult barnacles. Swimming and feeding in copepods are mainly effected by the cephalic appendages (Cannon 1928, Lowndes 1935, Gauld 1966), hence an impedance pneumograph record of the activity of the cephalic appendages should record the animal's swimming and feeding activity.

Records of the limb movements were obtained by using insulated, double stranded, fine copper wire thinned electrolitically until the two ends of the wire could be positioned on either side of one of the cephalic appendages without interfering with the movement of the limb. These electrodes were connected to an impedance pneumograph coupler on a George Washington MD2 chart recorder, on which changes in impedance caused by movement of the limb between the wires were recorded.

Fig 10.2a shows part of an impedance pneumograph trace, recording the movements of the mandibular palp of Temora longicornis. Fig 10.2b shows a portion of the trace in Fig 10.2a magnified several times. The distance a is proportional to the amplitude of limb beat while f is proportional to the

Fig 10.2

Specimen pneumograph trace

Temora longicornis : mandibular palp

(a)

one second

(b)

Enlargement of a section of trace (a)

a : represents amplitude of limb beat

f : proportional to the time for one limb beat

length of time for one complete beat. The undulating nature of the early part of the trace (Fig 10.2a) was caused by movement of the animal in the water flow. The electrode position was important in obtaining consistent amplitude records, hence throughout an experiment the output trace and electrode position were monitored to ensure that the amplitude record was comparable throughout. (Development of a single wire electrode technique to eliminate the unreliability of the limb beat amplitude record over long periods of time is now in progress.)

Experimental Procedures

Initially the experiments of Cannon (1928) and Gauld (1966) were repeated in small watch glasses and cavity slides. Particles of colloidal graphite and the flagellate alga Pavlova in seawater were introduced near the animals to observe the water currents produced. Observations were then made on tethered copepods in larger volumes of water. Some observations were performed with animals in still water and others in a seawater flow of 0.75 cm s^{-1}. The swimming speeds of free swimming T.longicornis were established from video-recordings of the gliding type of swimming at between 0.5 and 1.25 cm s^{-1}. Particles of colloidal graphite or suspensions of Pavlova were introduced near the animals through a small bore plastic tube and the particle flow controlled by a Desaga microdoser. The animals were viewed from various angles and the experiments recorded on video-tape. Finally the movements of the mandibular palp of T.longicornis were recorded using the pneumograph technique. The animal was tethered so that it faced into a seawater flow of 0.75 cm s^{-1}. At intervals, 1 ml. samples of stock Skeletonema culture were introduced to the seawater flow through a rubber section of the flow system (I Fig 10.1). All the seawater passing the

animal was run to waste. The observation chamber was observed to be free of algal cells approximately 5 minutes after an injection of algae.

Each pneumograph trace was analysed to give the frequency of limb beat in cycles per second (Hz.) from a measure of f (Fig 10.2b) and the rate of progress of the recording paper. The limb beat amplitude, in mm. of pneumograph trace deflection (a Fig 10.2b), was obtained from the trace. The proportion of time the limb showed continuous beating was also measured. The amount of water moved per unit time by the limb is proportional to the multiplicand of all three above measures. Five consecutive amplitude measurements were made at 10 second intervals and the beat frequency in each alternate second counted, so that an average measurement of frequency, amplitude and water movement over a minute could be recorded from the trace.

Results

Observations in small dishes

The present observations of the water currents created by free swimming specimens of T.longicornis in small dishes agree with those of Gauld (1966) and are very similar to those described for Calanus and Diaptomus by Cannon (1928). Large vortices could be seen at the side of the copepods (as in Cannon (1928) Fig 3) although it was not possible to follow these vortices in a U-shaped swirl below the copepods. These vortices were approximately 0.9 - 1.0 mm in diameter when measured in a cavity slide and slightly larger when measured in a small watch glass. A central stream of particles could also be seen starting posteriorly to the maxillipedes and moving towards the labrum. Difficulty was experienced in observing the animals for long

periods as they continually moved out of the field of view, and as the water in the dish became warm and stale the animals died.

Observations on tethered animals

When graphite particles were introduced near the animals in a large shallow chamber of still water no compact vortices at the side of the animals were noticed. After a period of a few minutes a large swirl was formed around the animal (Fig 10.3a) extending over the whole of the observation chamber. These vortices were oval shaped with major and minor axes of roughly 9 and 3 cm. respectively. Seawater was pushed backwards and slightly downwards from the animal (Fig 3b) but no form of tight U-shaped "swimming swirl" was obvious beneath the animal, in the fashion described for Calanus by Gauld (1966 Fig 2).

When a seawater flow of 0.75 cm s^{-1} was passed through the chamber to mimic forward movement of the animals, particles were pushed down behind the copepods in the main swimming stream and thence carried out of the chamber in the flow. No tight circular water currents were noted, though occasional, transient, vortices were produced close to the animal. All major currents were directed posteriorly and slightly downwards. The main propulsive current itself was turbulent.

Fig 10.4a shows the movement of particles around the anterior region of T.longicornis as seen from the under-side of the copepod. The arrows give the directions taken by particles of graphite. Particles further away from the mid-line of the copepod were not brought round in a circular manner to form a vortex. The main flow was in a direct anterior to posterior

Fig 10.3

A diagram showing a T.longicornis copepod tethered in the observation dish in still water. The arrows represent the direction of current flow around the copepod as shown by particles moving in the water.

(a).....Plan view.

(b).....Side view.

Fig 10.3 Water circulation around T.longicornis in still water

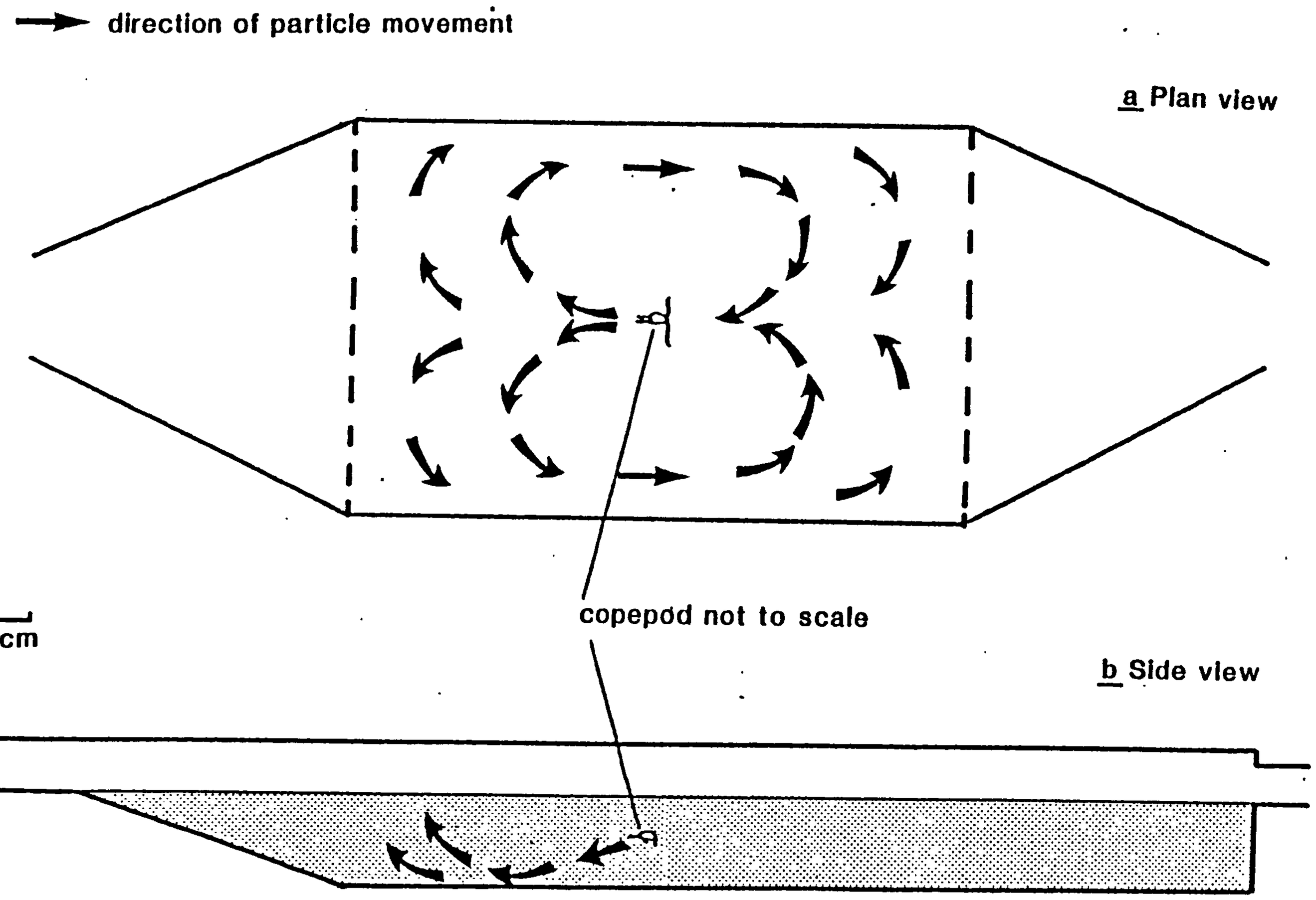


Fig 10.4

A diagram showing the direction of particle movement around the cephalic appendages of Temora longicornis.

(a).....ventral view.

(b).....side view.

a1 = antennule.

a2 = antenna.

L = labrum.

m = mandible.

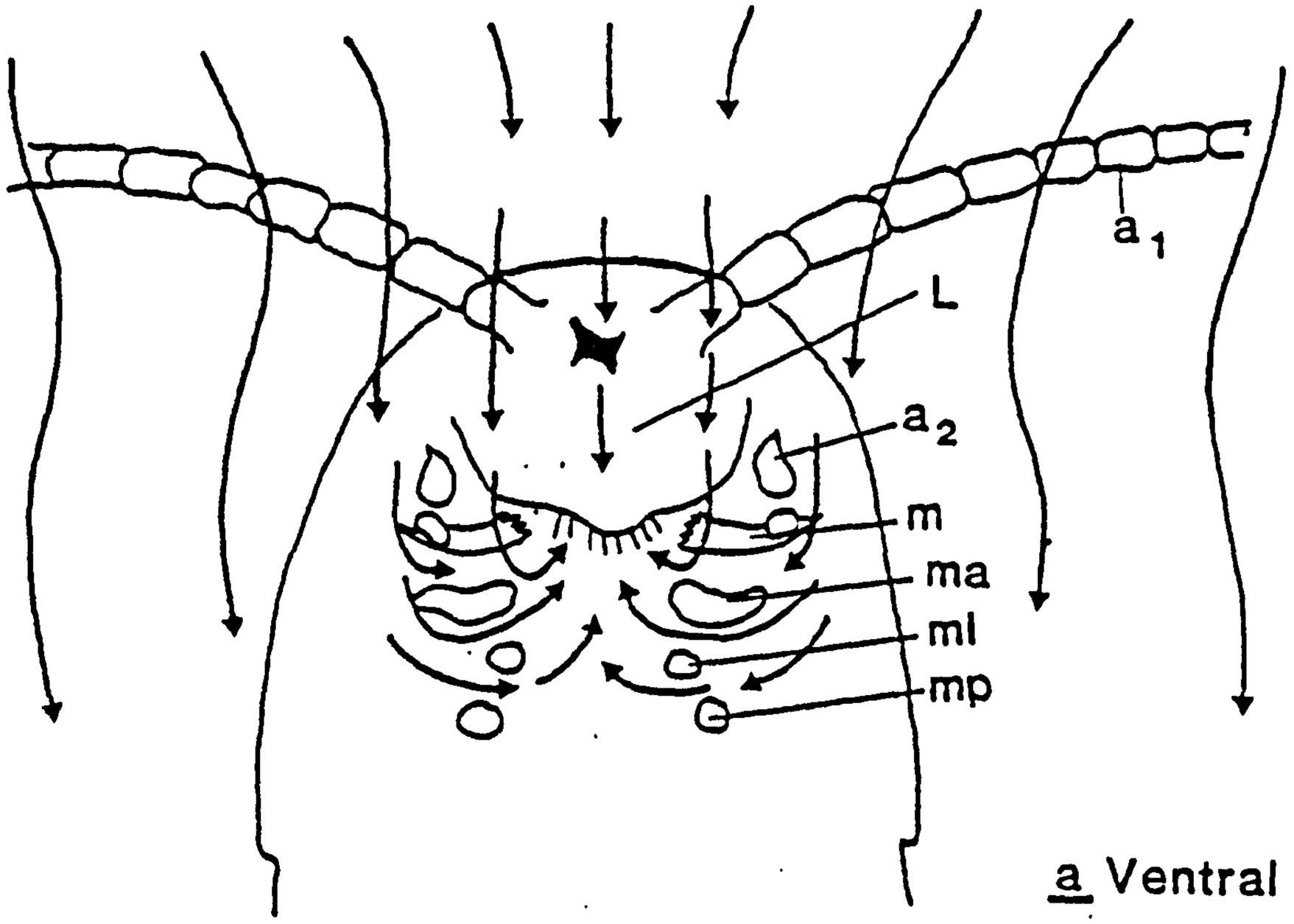
ma = maxilla.

ml = maxillule.

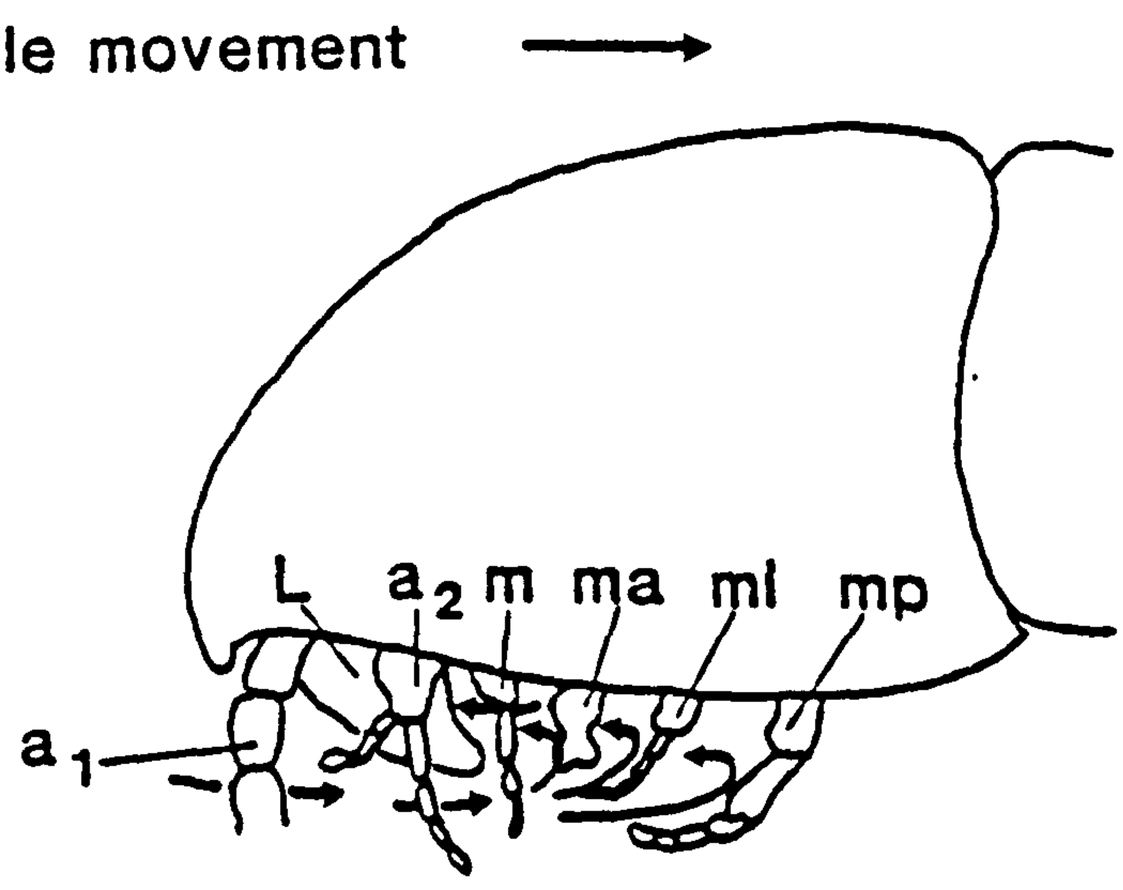
mp = maxillipede.

The limb setae have not been shown, and only the position of the limb bases is shown in (a) (ventral view).

Fig 10.4 Particle movements around T.longicornis



a Ventral view



b Side view

direction.

The setae of the maxillae bend forwards under the animal, forming the basket-like cup shape of the main collecting surfaces for particles. The setae of the maxillules and the maxillipedes also help to guide particles from the main stream flow towards the ventral surface, where the particles appear to be deflected by the vibrations of the limbs and swept by the action of the gnathobases up under the labrum to the mouth. Fig 10.4b shows in side view how particles avoiding the maxillary filtering basket can be restrained by the maxillipedes. No particulate matter could be observed moving towards the labrum from behind the maxillipedes.

The graphite particles varied in size from 10 μm. up to aggregates of over 200 μm. A very wide size range of particles could be seen to be collected on the filtering surfaces and gathered under the labrum. Smaller particles were accumulated until a bolus of particles was formed at the mouth. This bolus was partly pushed into the mouth by the bases of the mandibles and partly sucked in by peristaltic movements of the oesophagus. Single, larger particles arriving under the labrum, if small enough to enter the mouth whole, were immediately ingested in the above manner. Aggregates of particles too large to enter the mouth were broken up by the mandibles and although much was ingested, a modicum was lost. Particles too large to enter the mouth whole yet too tough for the animal to break up, were quickly rejected by the copepods and flicked out from under the labrum by the mandibles to be carried away in the current.

All the cephalic appendages, except the antennules, beat vigorously, but the main motive power appeared to come from the antennae, mandibular palps and to some extent the maxillules. The amplitude of beat of the three posterior cephalic appendages was considerably less than that of the antennae or the mandibular palps.

Limb beat in relation to the presence of algae

Fig 10.5 shows the analysis of the beat of the mandibular palps of a female T.longicornis recorded over a 140 minute period at 15°C. The beat frequency (Fig 10.5a) varied from about 23.5 Hz to 27.5 Hz. The thick bars along the bottom of the graph represent the periods when Skeletonema was present in the observation chamber. There was a noticeable increase in the beat frequency after the addition of the algae, followed by a decrease as the seawater flow carried the algae out of the chamber. The beat amplitude (Fig 10.5b), as represented by mm. of pneumograph scale deflection, shows a similar, but more pronounced, increase in the presence of algae. The amplitude record, however, was quite variable, the largest mean amplitude being recorded at 69 minutes, at a time when no Skeletonema was present in the water. However, when the effect of food particles was related to the calculated measure proportional to the amount of water moved by the animal (average frquency times amplitude, Fig 10.5c), the measure of water passed by the animal was almost doubled in the presence of Skeletonema, whereas the amplitude peak at 69 minutes was less evident. It was noticed also that immediately after a short break in swimming the limb beat amplitude tended to be greater until the normal beat frequency was resumed. The copepod's behaviour during the 69th minute followed this pattern.

Fig 10.5

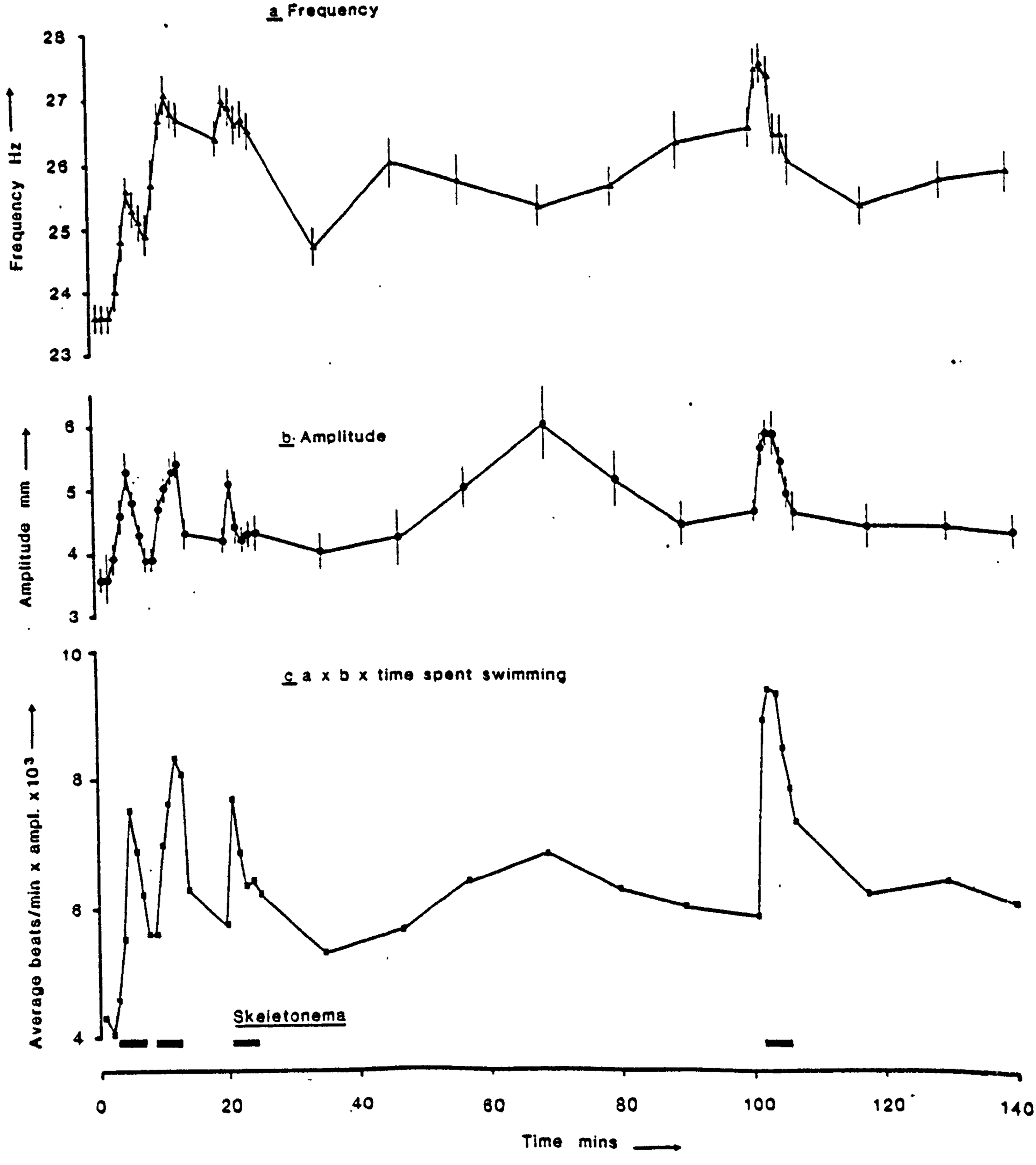
The analysis of the movement of the mandibular palp of T.longicornis over a 140 minute period. Solid bars near the horizontal axis denote the presence of S.costatum in the chamber.

(a) Mean limb beat frequency ± standard error (Hz).

(b) Mean limb beat amplitude ± standard error (mm trace deflection).

(c) Measure of the amount of water handled by the copepod; the beat frequency x amplitude x the proportion of time spent swimming by the copepod during that minute.

Fig 10.5 Analysis of the movement of T.longicornis mandibular palp



Conclusions

In a restricted volume of water my results demonstrated tight swirls, in agreement with those of Gauld (1966) for the same species T.longicornis, and those of Cannon(1928) for Calanus and Diaptomus. However, these tight vortices appear to be caused by the limitation of the container. In larger vessels (Fig 10.3a) the vortices increased in size. When a tethered copepod was restrained in a large volume of water which moved past the animal at a speed equivalent to that of slow swimming no such vortices, with accompanying back eddy feeding currents, were to be observed. The only current noted was an anterior to posterior turbulent flow. Cannon (1928) himself pointed out that fluid streams inherently create turbulence. Thus, like Eucalanus and Centropages (Koehl and Strickler 1981), copepods which swim more intermittently, the almost constant swimmer, T.longicornis, displays no complex current vortices for feeding and swimming. Kelvin's theorem (e.g. Lamb 1932) states that a standing vortex can only begin and end on a surface boundary. The water movements around a copepod in a small dish could set up a U-shaped standing vortex under and around the animal with the air/water interface as the starting and ending boundaries. In the open sea, at a depth where no bounding surface could exist, a completely circular vortex, like a smoke ring, would be necessary. It is difficult to imagine how such a complete circular vortex could possibly be produced and remain indefinitely surrounding the copepod. Hence the vortices or swimming and feeding swirls seen by Cannon (1928) and Gauld (1966), and which were also observed in small dishes in this study, were artefacts caused by the necessity for hydrodynamic continuity. They arise only as a result of the unnatural restrictions imposed by the sides of the small vessels containing the animals, which force a return flow compensating for the backward thrust

of fluid by the cephalic appendages.

The need to postulate a filtering chamber with transport of particulate matter from behind the maxillipedes does not seem necessary in the case of Temora. The major swimming current brings particles towards the collecting surfaces, primarily the maxillae, from the front of the animal. Some water passes through the setae posteriorly and some will be deflected towards the mouth ventrally because of the curved nature of the appendages. Particulate matter can be moved towards the mouth in this ventral current, but most food particles will be moved forwards by the reciprocating action of the limbs and in particular the scraping action of the gnathobases. The maxillule also bears setulose setae which collect particles and in addition the maxillipedes can collect further material beyond the scope of the anterior filtering surfaces. No circular back eddies were seen produced by the movements of the maxillipedes as was suggested by Cannon (1928). Apart from acting as supplementary collecting surfaces in dense patches of small algae, the maxillipedes may be used to grasp large algal cells or other plankters. Temora has been reported as commonly feeding on other copepods (Lebour 1922, Marshall 1924, Lowndes 1935) and the length and stout form of the maxillipedes would suit their use for capturing larger prey as was suggested by Gauld (1966).

In the presence of food algae an increase both in the amplitude of limb movement and to a lesser degree the beat frequency, resulting in an increase in the amount of water moved by the cephalic appendages of Temora, can evidently increase the amount of food bearing water handled by the organism. Response to suspended food particles is not unusual. Burns (1968) used a photoelectric recording device (McMahon and Rigler 1963, McMahon and

MacDonald 1966) to show that the beat rate of the limbs involved in Daphnia feeding similarly increase in the presence of algae above certain threshold algal concentrations.

It seems reasonable to expect that an organism's expenditure of energy on food capture be geared to the concentration of food available. The additional energy needed to increase the amount of water moved can be offset by the acquisition of energy present as food in the water. The organism which moves water across the filtering surfaces, regardless of the presence or absence of food, expends more energy than is necessary when food is absent, hence the need to adjust the amount of water moved to the available food density. Lam and Frost (1976) arrived at similar conclusions from an analysis of the feeding rates of copepods at different algal densities.

Since Temora increases its rate of food gathering when the algal concentration increases, it must be capable of assessing the concentration of suspended food particles. Mechanical and chemical cues would be the obvious candidates to enable the animal to respond. Unpublished work with barnacle nauplii has shown that an increase in the amount of water moved by the larvae in response to algal cells can be elicited by certain dissolved organic substances (Section 5 above). Further work is neccessary with Temora, to establish the mechanisms by which the animal senses the algal concentration in it's vicinity.

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Appendix I

A Computer Programme, Developed from Allen (1966), for the Fitting of Asymptotic Regressions using a Least Squares Iterative Technique

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