Locomotory behaviour and functional morphology of *Nematostella vectensis* (Anthozoa: Actiniaria: Edwardsiidae): a contribution to a comparative study of burrowing behaviour in athenarian sea anemones

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Key words: Behaviour; burrowing; climbing; creeping; England; functional morphology; locomotion; musculature; *Nematostella vectensis*; rugae; sea anemones.
The locomotory behaviour and functional morphology of English populations of a small (<2 cm long), burrowing athenarian sea anemone, *Nematostella vectensis* Stephenson, 1935 (= *N. pellucida* Crowell, 1946), which lives in soft mud in salt marshes and creeks, are described. Objectives were to ascertain the specific stimuli and environmental conditions determining when and how locomotion is employed, and to assess survival values. Experimentation and anatomical examination revealed how anemones move, and their functional morphology was interpreted in a physiological context. Finally, locomotion, morphology and habitat were compared for several athenarian anemones, to ascertain what correlations there are, if any. Essential environmental conditions for *N. vectensis* are: thixotropic mud of median particle size about 30 µm; a well defined oxidizing layer at the mud surface; and well oxygenated water overlying the mud. *N. vectensis* cannot burrow in sand. The ability to burrow in fine mud is the primary requirement for survival, providing a stable substrate for protection, feeding and reproduction. Anemones are forced to vacate their burrows if oxygen tension in the mud becomes lowered. Rarely, individuals adhere to underwater vegetation, which they reach by climbing. Locomotion in *N. vectensis* comprises burrowing of two types (penetrative and intrasubstrate), creeping and climbing. Penetrative burrowing, creeping or climbing (the animal progressing physa first), are all facilitated by the complementary deployment of direct peristaltic muscular waves passing down the column and the adhesive properties of the rugae on the column. If the anemone is lying on mud, penetrative burrowing ensues; if on an impenetrable surface, creeping or climbing ensues; these behaviours are not affected by light. At 21°C, in anemones 3-10 mm long, peristaltic waves are initiated about every 54 s at the mid-level of the actinopharynx. Waves progress to the tip of the physa at a mean speed of about 37 µm s^{-1}, with an observed range of 18-77 µm s^{-1}, unaffected by light intensity, and are directly correlated with anemone length, at least in the range 2-10 mm. Creeping is random in direction, whether in uniform light or dark conditions; daylight or darkness have no influence on speed of progression; and no phototactic response to unidirectional light is demonstrable. Climbing is not a geotaxis; initiation and passage of peristaltic waves are not responses to gravity or tactile stimuli; and flexion of the physa towards the substrate is neither a geotactic nor a tactile response. Physal flexion depends solely on ruga adhesion. If anemones established in their burrows become covered with a thin layer of mud, they extend the column until the tentacles reach the new surface. If, however, they are engulfed in deep mud, they employ intrasubstrate burrowing towards the mud surface, moving tentacles-first with alternate introversion and eversion of the scapus, sometimes aided by reversed peristaltic waves. Intrasubstrate burrowing may be a negative geotaxis, but chemotaxis was not tested for. During head-first movement below the mud surface, ciliary action on the oral disk and tentacles assists progress by increasing the fluidity of the thixotropic mud. *N. vectensis* is one of only two anemone species known to employ two methods of burrowing, and is unique in using them in the same kind of substrate, although in different circum-

stances. Burrowing behaviour in different species of athenarian anemone is extremely diverse, but there is no consistent association between modes of burrowing, types of substrate, musculature and familial relationships, at least when based upon the limited data available for only four athenarian families, the Edwardsiidae, Halcampidae, Halcampoididae and Haloclavidae. Hence, what these data on burrowing might tell us about adaptive evolution in athenarian sea anemones is still far from clear.

Contents

Introduction

Sea anemones (Anthozoa: Actiniaria) exhibit a great variety of co-ordinated muscular activities, including feeding (Shick, 1991); offence by means of catch tentacles or acrorhagi (Williams, 1975a, 1991); pedal locomotion (McLendon, 1906; Parker, 1917; Robson, 1976); avoidance behaviour (Williams, 1992); tentacle-walking (Robson, 1971); shell-climbing (Ross, 1974; McFarlane, 1982); swimming (Robson, 1966, 1971); creeping or climbing, using the sides of the column (Gosse, 1860; Portmann, 1926; Stephenson, 1928; Mackie, 1974); and burrowing (Faurot, 1895; Hargitt, 1907; Ansell & Trueman, 1968; Mangum, 1970; Pickens, 1988; Ansell & Peck, 2000). Feeding behaviour is, of course, common to all anemones, but the other activities may occur only in certain groups of species in which, moreover, particular details may differ.

With regard to burrowing, Shelton (1982) remarked that "Detailed descriptions of burrowing behaviour in anemones are rare … many unresolved problems in this area would benefit from further study … wide ranging comparative studies are needed and it may well prove that burrowing can be achieved using different mechanisms in different anthozoan species." Even now, some 20 years later, reasonably detailed descriptions of the burrowing behaviours of only three species have been published, that of the thenarian anemone *Phyllactis concinnata* by Mangum (1970) and Pickens (1988), and those of the athenarians *Peachia hastata* by Ansell & Trueman (1968) and a *Halcampoides* species by Ansell & Peck (2000). Several other brief notes on the locomotion of these and other burrowing species also appear in the literature (Gosse, 1853, 1860; Holdsworth, 1859; Haddon & Dixon, 1885; Faurot, 1895; Panikkar, 1936; Lindsay, 1975; Manuel, 1975, 1981; Ellehauge, 1978; D.N. Huxtable in Shelton, 1982), but quantitative data are lacking.

The lower end of the column of athenarian anemones consists of an ampullaceous physa, with which they may actively dig into mud, sand or gravel, but once buried they do not usually attach themselves to any solid object within the substratum. On the other hand, thenarian anemones possess a pedal disk, which enables them to adhere to any exposed impenetrable surfaces, such as boulders, massive rock or marine vegetation. Some thenarians, however, may habitually or occasionally fix themselves to stones or shells that are buried below the surface of mud or sand. Thus, athenarian anemones may be generally regarded as true "burrowing" forms, whereas those thenarians that adhere to objects under mud, etc. may be called "burying" forms (R.L. Manuel in Shelton, 1982). As might be expected from their different anatomies, the burying behaviour of the thenarian *Phyllactis concinnata* (Mangum, 1970; Pickens, 1988) is rather different from the burrowing behaviours of the athenarians *Peachia hastata* (Ansell & Trueman, 1968) and a *Halcampoides* species (Ansell & Peck, 2000), which happen to be fairly similar to each other.

The locomotory behaviour and functional morphology of another athenarian, *Nematostella vectensis* Stephenson, 1935 (= *N. pellucida* Crowell, 1946), are described herein. This study of English populations was completed in the 1970s, before *N. vectensis* was placed under the protection of Schedule 5 of the Wildlife and Countryside Act of 1981 in the UK. The objectives of this work were to discover and describe the various types of locomotion exhibited by *N. vectensis*, to ascertain the specific stimuli and environmental conditions that determine when each type of locomotion is employed and to assess survival values. An anatomical examination of the anemone was also carried out to ascertain how locomotion is achieved, and the functional morphology was interpreted in a physiological context. Finally, locomotion, morphology and habitat were compared for a range of athenarian anemones, in order to ascertain what correlations there are, if any.

Detailed quantitative analyses of penetrative burrowing, creeping and climbing exhibited by unburied *N. vectensis* showed that these activities are all achieved by the complementary effects of direct peristaltic muscular waves passing down the column and of adhesion by the column rugae. The particular locomotory effect that ensues depends on the kind of surface with which the anemone is in contact at the time, although the anemone always progresses physa first. However, individuals that accidentally become completely buried employ a quite different method of locomotion to regain the mud surface, progressing tentacles-first by intrasubstrate burrowing. *N. vectensis* is one of only two anemone species so far known to employ two different methods of burrowing, and is unique in using them in the same kind of substrate, although in different circumstances.

Materials and Methods

Terminology for locomotory behaviour

The three types of sea anemone locomotion studied here (burrowing, creeping and climbing) may be defined as follows. "Burrowing" is any form of locomotion directed into or within a loose substrate by a benthic animal that dwells in that habitat. "Penetrative burrowing" is the downward penetration into a substrate from the substrate-water interface. "Intrasubstrate burrowing" is progression in any direction by an animal that is already completely below the substrate surface. "Creeping" (= "*la reptation*" of Faurot, 1895) is the random progression of an anemone adhering by the side of its column to any impenetrable underwater surface. "Climbing" is creeping in any direction on an inclined or vertical surface. Athenarian anemones that live obligatorily within benthic substrates are termed "burrowing" forms; and thenarian anemones that adhere facultatively by the pedal disk to hard objects within substrates, with the tentacles expanded at the surface, are termed "burying" forms (R.L. Manuel in Shelton, 1982). A "*point d'appui*", or anchor, is any point where an animal's body is temporarily fixed to the substratum during locomotion (Elder, 1980).

Progression of a burrowing athenarian anemone may conveniently be described as "head-first" (with the oral end leading) or "tail-first" (with physa leading) (Shelton, 1982). The terminology used for descriptions of peristaltic muscular waves involved in locomotion follows that recommended by Elder (1980). Waves moving along the body in the direction of an animal's progression are termed "direct"; waves moving in the opposite direction from that of progression are termed "retrograde". This does not imply whether the "head" or "tail" leads in the direction of progression. A newly introduced term is a "reversed" wave, i.e., one that proceeds in the opposite direction from what is usual for a particular species, whether it is normally direct or retrograde. A reversed wave does not automatically change from direct to retrograde or *vice versa*. Thus, in a case described herein, where waves in an animal progressing tail-first are normally direct, the reversed waves change the progression to head-first, so the waves are still direct.

Collection, maintenance and handling of experimental animals

Nematostella vectensis is an edwardsiid sea anemone, usually <2 cm long, which normally burrows in the soft mud of poikilohaline pools or creeks in salt marshes and estuaries in England, Canada and the USA (Williams, 1973a, 1976; Lindsay, 1975). A detailed description of the species was provided by Williams (1975b). Animals used in the present experiments were collected during summer and winter, along with natural water and substrate, from Half-Moon Pond (Cley-next-the-Sea, Norfolk) and from Lagoon No. 1 (Shingle Street, Suffolk), both in England, UK. Field observations were made at other localities in England, noted where appropriate.

Animals were collected by wading into a lagoon known to contain *N. vectensis* and haphazardly scooping some surface mud down to about 6 cm, with overlying water, into a large (3 l) glass jar. When settled, the mud usually occupied about one-third of the jar, the rest of the jar's contents being water. At the time of each collection, a 500 ml bottle was filled with water and sealed without trapping any air, for determination of total salinity the next day. Jars of mud were transported to the laboratory at ambient temperature, and were left undisturbed overnight to allow any anemones present to make their way upwards to the mud-water interface. The next morning, having confirmed the presence of *N. vectensis* in the sample, the contents of each jar were poured into a large enamel tray, which provided a mud depth of about 2 cm with 4 cm water overlying it.

Anemones were kept in such trays at laboratory room temperature for up to one week before they were used in experiments. They were not fed, but the mud contained natural prey, such as copepod, amphipod and isopod crustaceans, and chironomid midge larvae. During May to October, the room temperature was close to the outdoor temperature at 16-26°C, whilst during November to April the room temperature was within the range 16-23°C. The total salinity of the natural water in which anemones were collected, maintained and experimented upon ranged between 29 p.p.t. and 33 p.p.t., and was not aerated at any time. Total salinity was determined by evaporating weighed water samples to constant dry weight at 105°C. During the day, natural light of a variable intensity shone through windows on one side of the laboratory. Additional light was provided during the daytime, if necessary according to the season, by overhead fluorescent tubes. No artificial lighting was provided at night. The months during which anemones were used for experiments are recorded in case any seasonal effects might be relevant.

Anemones remained undisturbed in their trays, buried in mud, until needed for experiments, but were examined several times daily and any unusual behaviour or asexual reproduction was recorded. When anemones were required for experimentation, they were removed from the mud with a wide-mouthed pipette and were always ejected under water, so that they were never exposed to air between the maintenance and experimental conditions. They were either ejected immediately into an experimental apparatus or, if intended for experiments on peristaltic waves or ruga adhesion, were placed temporarily in a glass dish to divest themselves of any mud. As soon as they were mud-free, they were transferred to a clean dish or other appropriate apparatus, and the experiment was begun immediately.

Observational methods

For laboratory analysis of locomotion, direct visual methods were used, since the anemones were too small and delicate for the use of techniques involving pressure transducers or myographs. Magnification was provided by a bench-magnifier, hand-lens, standard light microscope or dissecting microscope, as appropriate. Anemone sizes were expressed as relaxed column lengths to the nearest 0.5 mm, measured from the oral disk to the tip of the physa. All phases of behaviour were timed with a stop-watch to the nearest second; or for periods of >0.5 h, with a clock to the nearest minute. Water temperatures were measured with a standard bulb thermometer during each experiment; all readings were the same as those in the maintenance trays in the ambient room temperature annual range of 16-26°C.

Behaviour of anemones when burrowing was observed in shallow (5 cm deep) glass dishes, or in a chamber constructed from standard 76 × 26 mm glass microscope slides and "Plasticine" modelling clay (fig. 1a). Burrowing was studied in a control substrate of natural mud from Half-Moon Pond, and also in experimental non-natural substrates comprising graded samples of beach sand from Wells-next-the-Sea (Norfolk, England, UK). Water from the natural habitat was always used for maintenance and experimentation.

To examine the creeping behaviour of anemones on a glass surface, clean glass dishes, each containing one individual in well-oxygenated water, were placed directly (to avoid parallactic problems) on squared graph paper, which was visible through the bottoms of the dishes. Initially, each anemone was positioned over a starting point marked on the graph paper. The subsequent positions of the anemones were then recorded at timed intervals on a duplicate sheet of graph paper. To study some effects of light in these experiments on creeping, some dishes were left exposed to natural daylight with fluorescent lighting from above, and some were covered with a light-proof box. Others were covered with a slit-box to admit unidirectional artificial light, provided by a standard microscope lamp with the rheostat set for maximum light intensity. These experiments commenced and finished during daylight hours. When the positions of anemones under the light-proof box or slit-box were recorded, the box was replaced as quickly as possible to minimize any possible effects of exposure to daylight.

In order to observe possible effects of gravity on an anemone held in various positions relative to the vertical, it was first taken up with some water into a Pasteur pipette fitted with a rubber bulb on the wide end. Then after plugging the narrow end of the pipette with "Plasticine", the rubber bulb was removed and the pipette was completely filled with mud and water, or water only. The wide end also was then plugged with "Plasticine", avoiding the inclusion of any air bubbles. This "pipette apparatus" could then be held with a clamp, for observation in any position in three dimensions (fig. 1b).

To study further effects of gravity or tactile stimuli, anemones were placed on a 76 × 26 mm glass microscope slide on the bottom of a clean glass dish containing water about 4 cm deep. After they had adhered to the slide, it was then, without being lifted from the water, turned over and supported on blocks of "Perspex", so that the anemones were suspended out of contact with the bottom of the dish (fig. 1c). They could then be examined in this "suspension chamber" from above through the glass slide, or from the side through the dish wall.

Observations of the adherent rugae on the column of a live anemone were made by allowing the animal to adhere to a no. 1 glass cover slip, 22 mm square, lying on the bottom of a dish of clean water. A slip with an adhering anemone was inverted under water and was quickly removed from the dish, with the anemone contained in a hanging drop of water, and was placed over the water-filled depression on a cavity microscope-slide. The upper side of the cover slip was then wiped dry with a piece of filter paper, the slide was placed on a microscope stage, and the column wall of the anemone adhering to the slip was observed by transmitted light.

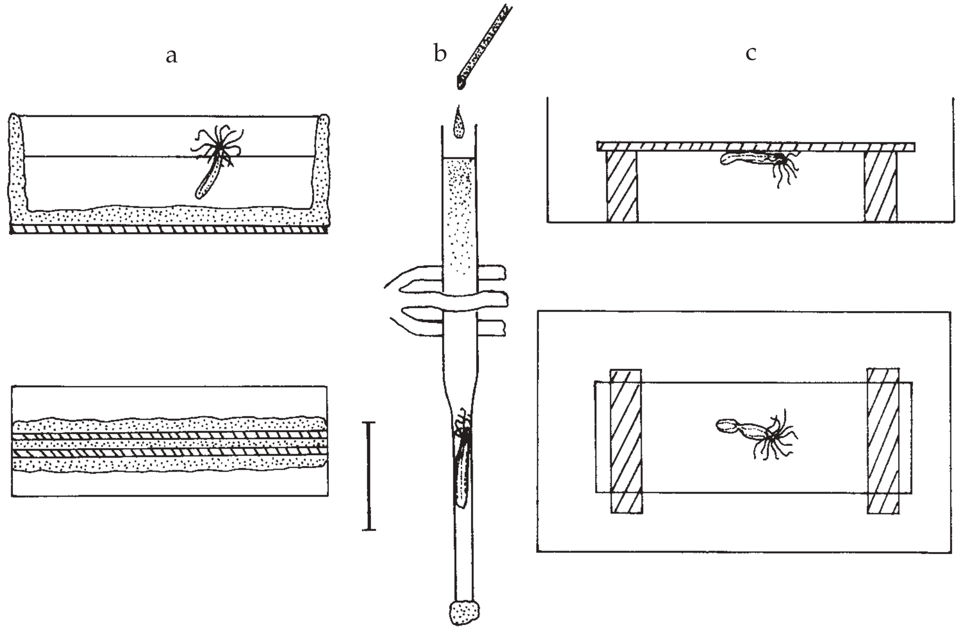

Fig. 1. Apparatus used for locomotion experiments. a) glass-"Plasticine" chamber from the side (with an anemone positioned in mud) and from above. b) pipette apparatus, containing an anemone, being filled with mud. c) suspension chamber from side and above, with an anemone adhering to the underside of the glass slide. Scale = 2.5 cm.

Physical analysis of natural and experimental substrates

Particle size analyses of substrates were carried out by sieving, following the principles of Morgans (1956). The mud from Half-Moon Pond was used as the control natural substrate. It was sieved wet, since preliminary experiments showed that drying the mud before sieving it caused aggregation, resulting in potentially inaccurate particle size assessments. It was, therefore, first sieved by washing it through screens with natural water collected with the mud. The fractions obtained were then individually washed with distilled water to remove the salts. After washing and drying at 105°C, the fractions of substrates that had passed through a stack of brass Endecott test sieves (British Standard 410) of mesh sizes 1180, 600, 300, 150, 75 and 38 µm were weighed, and graphical plots were made of the cumulative percentage weights to estimate the median particle size of the original sample (Morgans, 1956). Beach sand, used as a non-natural experimental substrate, was sieved when dry. The sea salt was first washed out with distilled water, then the ungraded washed sand was oven-dried at 105°C before sieving. The median particle size was estimated graphically as previously described (Morgans, 1956).

The interstitial water contents of samples of mud or sand were estimated by drying them to constant weight at 105°C and expressing the weight loss as a percentage of the original wet sample. To prepare the mud samples, each was allowed to settle by gravity for 24 h in natural water collected with the substrate and the supernatant water was then carefully removed, using a Pasteur pipette, to the level of the sediment surface. This water was weighed and its total salinity was determined by evaporating it to a constant dry weight at 105°C. A sample of the substrate, taken with a wide-mouthed pipette from about 1 cm below the surface, was then placed in a preweighed container, and the whole was weighed. The sample was oven dried at 105°C until reweighings were constant, and the container weight was subtracted from the whole. By using the previously determined salinity of the supernatant water sample, the weight of the substrate solids was calculated by subtracting the proportion accounted for by sea salts in the interstitial water. For the sand samples, previously graded sieve fractions were used. Excess distilled water was added to the dried sand fractions and the container was agitated to ensure that no fine sand remained floating. When the samples had settled, the supernatant water was removed and the remaining saturated sand was dried to constant weight, as previously described.

Anatomical studies

Anemones for sectionizing or for whole mounts were narcotized with menthol crystals and fixed in a 4% w/v solution of formaldehyde in brackish water from the natural habitat. Specimens were embedded in paraffin wax, and longitudinal or transverse sections, 8 µm thick, were prepared and stained with Mallory's Triple Stain (Pantin, 1964). The rugae on the column were demonstrated by staining whole, fixed specimens of anemones for 25 minutes with 10% mucicarmine solution (Southgate, 1927). Sections and whole specimens were mounted in Canada balsam on glass slides and examined by transmitted light with a standard microscope.

Live anemones were used to study the distribution of their ectodermal cilia. Specimens in the water-filled depressions on cavity slides were examined with a dissecting microscope. The beating cilia caused water currents, which were detectable by the flow of fine particles of detritus that were suspended in the water.

Live anemones were also used to study the effects of muscular contractions on body form. Some experiments involved perfusing the water with ammonia by adding a few drops of 0.880 sp. gr. solution. Others involved the effects on anemones of hydrogen sulphide in the water. Preparations were made by allowing natural mud to become reduced (deoxidized) in a small quantity of unaerated water, hydrogen sulphide being produced endogenously and accumulating in the overlying water.

Results

The natural posture of *N. vectensis*

Observed in a glass-"Plasticine" chamber (fig. 1a) or a glass dish, the natural posture of *N. vectensis* is upright, or nearly so, in the mud with its tentacles and at least the capitulum exposed above the mud-water interface. Individuals up to 1 cm long frequently had none of the scapus exposed, but larger ones revealed variable amounts of the upper scapus (fig. 2d). Hence the distance between the margin of the oral disk and the mud surface varied from <1 mm for small anemones, to 6 mm or more for larger individuals (≈4 cm long). The outer (exocoelic) tentacles usually pointed downwards to touch the mud whilst the inner (endocoelic) tentacles pointed upwards (fig. 2d). I have never seen anemones lying free on the mud surface in natural conditions in the field.

When *N. vectensis* is *in situ*, the mud surrounding the column is held together by

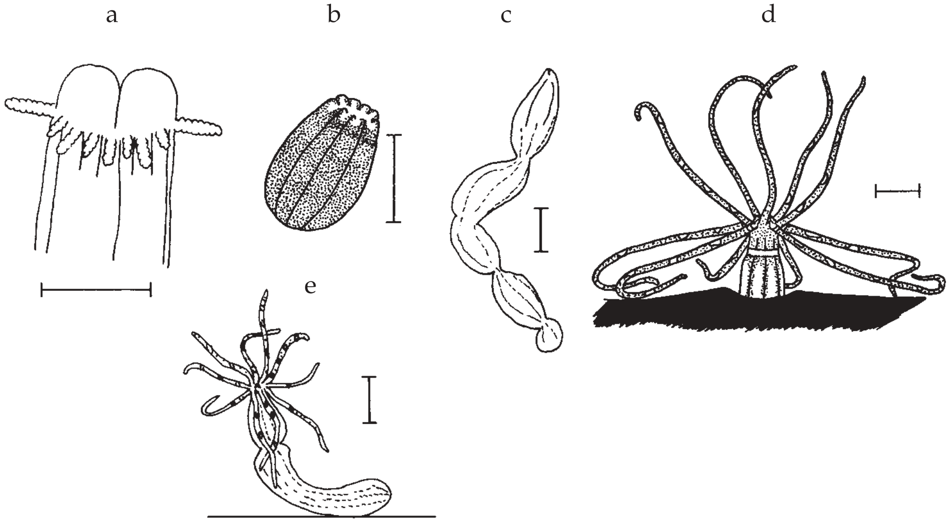

Fig. 2. *N. vectensis* during various phases of behaviour. a) maximal contraction of tentacles and distension of oral disk in ammoniated water (note microcnemes and macrocnemes between the tentacles, and corrugations of tentacles). b) fully contracted small individual. c) fully contracted large individual (the constrictions shown are contortions, not peristaltic waves). d) anemone in typical posture, buried in mud. e) anemone fixed upright by rugae to glass. Scales = 2 mm.

Table 1. Passage times of peristaltic waves and their speeds along the column of *N. vectensis* of different sizes (n = 7; 21-23°C; 33 p.p.t.) in strong artificial light (SAL) or weak natural light (WNL). SD = standard deviation of the mean.

Anemone length (mm)	Passage time of wave (s)	Mean passage time of wave ± SD (s)[1]	Mean wave speed ± SD ($\mu m\ s^{-1}$)[2]	Maximum no. of simultaneous waves
2 (SAL) (a)	75 78 86 98 99	87.2 ± 11.08	23.2 ± 2.95	1
3 (WNL) (b)	79 83 97 97 109	93.0 ± 12.08	32.7 ± 4.26	2
3.5 (SAL) (c)	95 96 107 108 114	104.0 ± 8.22	33.8 ± 2.69	2
5 (WNL) (d)	101 131 141 171 286	166.0 ± 71.59	34.0 ± 11.78	2
6 (SAL) (e)	150 180 198 200 221	189.8 ± 26.57	32.2 ± 4.90	3
10 (SAL) (f)	165 191 203 228 243	206.0 ± 30.69	49.5 ± 7.64	3
10 (WNL) (g)	130 160 244 257 282	214.6 ± 65.85	51.0 ± 17.97	3

[1] Differences (ANOVA; $P < 0.05$) between mean wave passage times: a *vs.* e, f & g; b *vs.* e, f & g; c *vs.* e, f & g

[2] Differences (ANOVA; $P < 0.05$) between mean wave speeds: a *vs.* f & g; e *vs.* g

mucus secreted by the adherent rugae, forming a fragile tube. This does not persist after the anemone is removed from the mud, but a hole remains if the anemone is stimulated to retract into the substrate. On removal from the mud, anemones usually have some lightly adhering material remaining on the physa and lower part of the scapus (see Williams, 1975b, fig. 1). If placed in a dish without mud, the continual peristaltic waves eventually dislodge this material.

Although normally found in mud, *N. vectensis* was once seen (30 May, 1971) adhering to the submerged leaves of the sea grass *Zostera marina* in Abraham's Creek at Wells-next-the-Sea (Norfolk, England, UK). These anemones had no mud adhering to the column. At the same time, other individuals were found in the mud, which had a well marked oxidizing layer.

The fundamental elements of locomotion in *N. vectensis*

Two anatomical features were demonstrated to be essentially responsible for all the modes of locomotion of *N. vectensis* when lying free on any surface, comprising penetrative burrowing, creeping and climbing. They are the endodermal circular muscle sheet of the column and the ectodermal column rugae, which function to provide direct peristaltic muscular waves and *points d'appui* by adhesion, respectively. These two complementary features were analysed qualitatively and quantitatively as appropriate, and are described separately below.

Column peristalsis

Each experiment was carried out on a single mud-free anemone in a glass dish of clean well-oxygenated water, and observations were made using a dissecting microscope. Seven individuals, from 2 mm to 10 mm long, were examined on two consecutive days in September to measure the time taken for a peristaltic wave to pass along the length of a measured anemone. Waves were initiated at the mid-level of the actinopharynx, passing down the column towards the physa, and the anemone progressed physa first. The waves were, therefore, direct (Trueman, 1975; Elder, 1980).

Five consecutive waves were timed for each individual. Four individuals were examined at 23°C in strong artificial light (SAL - illuminated with a standard microscope lamp from below the microscope stage, in a room lit by ceiling fluorescent lights); three others were examined at 21°C in weak natural light (WNL - no artificial light and microscope placed before a window just before dusk using natural light, focused from below with a concave mirror). The results are summarized in table 1.

Analysis of variance revealed statistically significant differences ($P < 0.05$) between mean passage times of waves in all the anemones from 2.0 to 3.5 mm long and all those from 6 to 10 mm long. However, there were no significant differences within either of those two size ranges, nor between the 5 mm long anemone and any of the others (table 1). There were statistically significant differences ($P < 0.05$) between mean wave speeds in the anemone of 2 mm and the two of 10 mm in length, and also between that of the 6 mm individual and the 10 mm individual in WNL (table 1).

The different light intensities and slightly different temperatures appeared to have no effect on the wave speeds, as revealed by fig. 3 which shows all the peristaltic wave speeds measured in all the anemones tested under the different conditions. There was, however, a statistically significant direct linear correlation between wave speed and anemone size in SAL:

$y = 2.9192x + 18.984$

$r = 0.8475$; $P < 0.01$

and in WNL:

$y = 2.7623x + 22.633$

$r = 0.5790$; $P < 0.05$

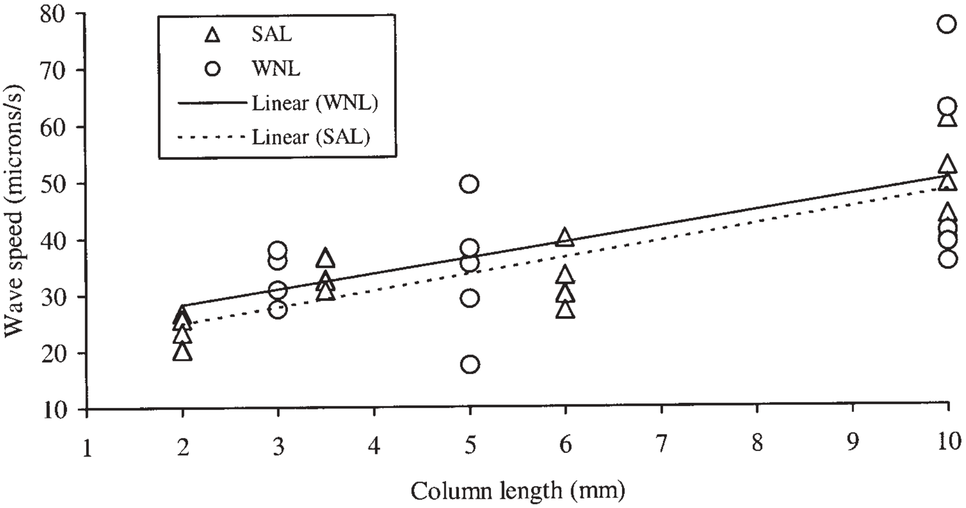

Fig. 3. Relationship between peristaltic wave speed and size of anemone in *N. vectensis* in strong artificial light (SAL) and weak natural light (WNL) at 21-23°C. Statistically significant in SAL ($P < 0.01$) and WNL ($P < 0.05$).

The mean wave speed for a 10 mm long anemone was about twice that for one of 2 mm long (table 1 and fig. 3).

The overall mean wave speed for all anemones tested in both lighting conditions at 21-23°C and salinity of 33 p.p.t. was 36.6 µm s^{-1}, ranging from 17.5 µm s^{-1} to 76.9 µm s^{-1} (calculated from individual data in table 1). Close observation showed that the speed of a wave was not quite constant during its passage, there being a distinct momentary pause just before the wave reached the tip of the physa. This pause was apparently caused by the strong adhesion of the particularly dense physal rugae, which at first held the physa down, then pulled free allowing the wave to pass.

Table 2 summarizes four experiments to measure the frequency with which peristaltic waves were initiated in WNL at 21°C and a salinity of 33 p.p.t. These experiments, carried out on the second day of the previous experiment (table 1), utilized different anemones, but from the same maintenance batch. For each of the four anemones tested, the time lapses between the initiations (near the middle of the actinopharynx) of six consecutive waves were measured. The overall frequency was one every 54.2 s, ranging from 27 s to 90 s (table 2). There were no statistically significant differences between the mean wave initiation intervals for anemones of different sizes (table 2, ANOVA, $P = 0.249$). This was confirmed by regression analysis (fig. 4; $r = 0.102$; $P > 0.1$). The theoretical maximum number of peristaltic waves that might pass simultaneously ranged from two, for an individual 3 mm long, up to four, for an individual 10 mm long (table 2). This compares with the actual maximum number of three observed for a 10 mm long anemone (table 1).

In summary, the mean wave initiation interval was constant at 54.2 s for anemones between 3 and 10 mm long; mean wave passage time and mean wave speed increased with greater body size for anemones in the range 2-10 mm long; and

Table 2. Frequencies of initiation of column peristaltic waves in *N. vectensis* of different sizes (n = 4; 21°C; 33 p.p.t.) in weak natural light. SD = standard deviation of the mean.

Anemone length (mm)	Wave intervals (s)	Mean wave initiation interval ± SD (s)[1]	Theoretical maximum number of simultaneous waves[2]
3	49 51 60 67 90	63.4 ± 16.53	1/2
5	27 27 32 58 66	42.0 ± 18.59	3/4
5	32 44 54 77 78	57.0 ± 20.27	2/3
10	47 50 56 59 60	54.4 ± 5.68	3/4

[1] Overall mean = 54.2 s. There are no statistically significant differences between any of the mean wave initiation frequencies (ANOVA; P = 0.249)

[2] Calculated from mean wave initiation interval (table 2) and mean wave passage time (table 1) for anemones of the same size. See fig. 14 for explanation of calculation and expression of results

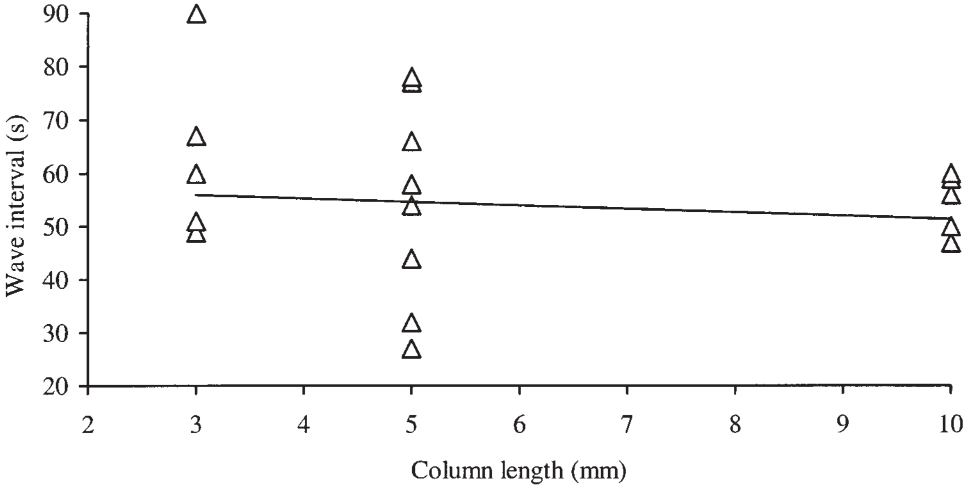

Fig. 4. Relationship between peristaltic wave initiation interval and size of anemone in *N. vectensis* in weak natural light at 21°C. Not statistically significant (P > 0.1).

light intensity had no discernible effect on peristalsis. The number of simultaneous waves increased with anemone size, with actual and theoretical maxima of three and four, respectively, in a 10 mm long individual.

Ruga adhesion

The adherent rugae that cover the surfaces of the physa and the lower part of the scapus were shown, by direct observation and by specific staining of whole anemones with mucicarmine, to secrete mucus. No batteries of cnidae were associated with the rugae, but basitrichous nematocysts were uniformly distributed throughout the column ectoderm. Live anemones were able to use any part of the column below the lower scapus to attach themselves to the bottom of a clean dish, maintaining the upper part of the scapus upright with outspread tentacles (fig. 2e), but most individuals tended to remain horizontal most of the time.

To examine ruga adhesion more closely, six anemones were placed on microscope-slide cover slips at the bottom of a glass dish. On microscopical examination of the cover slips 24 h later, trails of mucus left by the anemones as they had crept over the glass could be seen. Bacteria and ciliated protozoans (particularly *Euplotes alatus* (Williams, 1973b), probably feeding on the bacteria) were concentrated on the mucus trails. Very rarely, an isolated undischarged basitrichous nematocyst was found in the mucus.

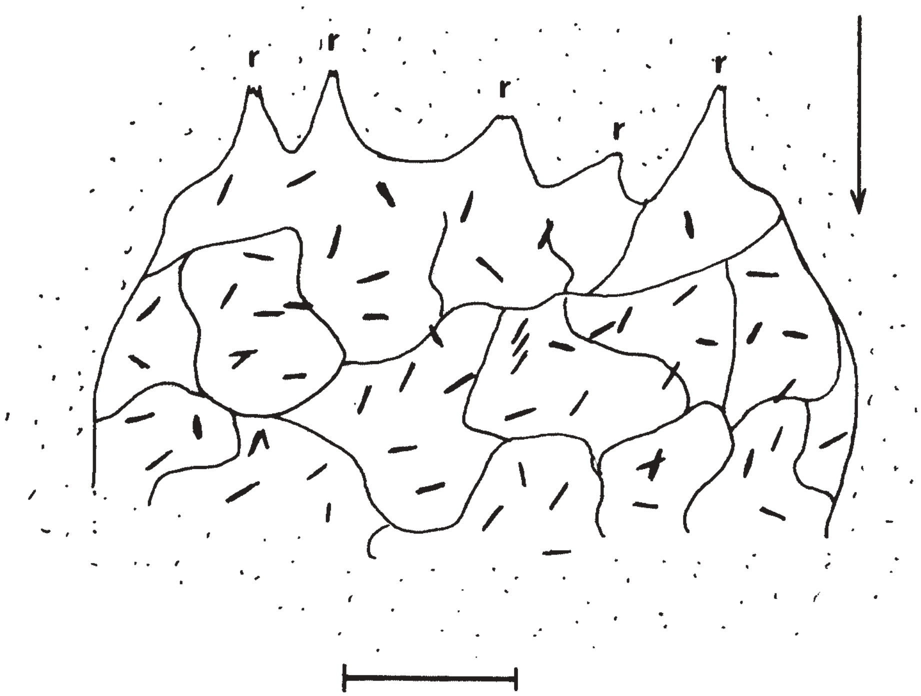

Fig. 5. Wrinkled column wall of live *N. vectensis* fixed to glass, showing adhesion of rugae (r) as a peristaltic wave passes in direction of arrow (note undischarged basitrichous nematocysts). Stippled area is out of focus. Scale = 50 µm.

Microscopical examination of anemones attached to the undersides of cover slips placed on cavity slides confirmed that adhesion was effected by the mucus-secreting rugae. Although basitrichous nematocysts were visible in the ectoderm of the column, none was discharged and they clearly played no part in adhesion. When an anemone was stationary, the wrinkled column wall (normally smooth when unattached to anything), was seen, through the cover slip, to take on the appearance of "crazy paving" (fig. 5). As the anemones moved, and especially when a peristaltic wave passed, the ectoderm pulled free, stretching out to points at the rugae (fig. 5) before contact with the glass was suddenly broken. When a wave had passed, the stretched ectodermal points sprang back to their original positions and the rugae immediately re-adhered to the glass.

Penetrative burrowing in *N. vectensis*

This section describes how the two complementary anatomical features described above are deployed during penetrative burrowing, and investigates how the effects of light and substrate characteristics may modify this behaviour.

Method of penetrative burrowing

When a specimen of *N. vectensis*, taken from its natural position within mud, was placed on the mud surface under water, it contracted maximally (figs 2b, 2c). Up to a minute was usually required for recovery and for the column to be re-extended. Peristaltic waves then began to be initiated below the capitulum at the level of the middle actinopharynx, passing aborally down the column towards the physa.

With the anemone lying horizontally on the mud, its tentacles extended, the column was anchored by the rugae on the lower side of the physa, forming a *point d'appui*. With the passing of each direct peristaltic wave, water in the coelenteron was forced into the physa, distending it further beyond the *point d'appui*. Since the anemone was anchored also by frictional forces between the substrate and the tentacles at the oral end, the column arched upwards and the tip of the physa was pushed downwards into the thixotropic mud, aided by the adhesion of the dense physal rugae (fig. 6a). Subsequent waves forced the physa deeper into the mud (fig. 6b). Occasionally, adhesion by the physa was not achieved (as when mud already adhering to the column shielded the rugae), in which case the physa did not turn downwards, but the tip instead extended horizontally, and with subsequent waves the anemone simply crept along the mud surface in the direction of the physa. After some time, however, the peristaltic waves dislodged the adherent material and normal burrowing continued.

Fig. 6. Stages (a-d; left to right) in penetrative burrowing behaviour of *N. vectensis*.

Table 3. The effects of light or darkness on the burrowing success of *N. vectensis* ($n = 2 \times 10$; sizes 4-8 mm; 21-26°C; 29 p.p.t.).

Time elapsed (h)	Anemones in light[1]	Anemones in darkness[1]
0.5	3 buried, 7 burrowing	4 buried, 6 burrowing
1.0	4 buried, 6 burrowing	5 buried, 5 burrowing
1.5	5 buried, 5 burrowing	5 buried, 5 burrowing
2.0	5 buried, 5 burrowing	5 buried, 5 burrowing
2.5	5 buried, 5 burrowing	6 buried, 4 burrowing
3.0	7 buried, 3 burrowing	6 buried, 4 burrowing
23.0	9 buried, 1 partly buried	8 buried, 2 partly buried

[1] There are no statistically significant differences (χ^2 test, $P > 0.05$) between the numbers of anemones buried in light or in darkness after any of the times elapsed

When about one-quarter to one-third of the lower column was buried, the anemone pulled itself up into a vertical or nearly vertical position, presumably utilizing the parietal muscles (fig. 6c). Occasionally, a premature attempt was made and it fell over again. Once the animal was approximately upright and the rugae all around the physa were able to be utilized, burrowing was faster and continued by direct peristalsis (fig. 6d). No obvious massive contractions of the retractors were seen to be involved in burrowing. Peristaltic waves ceased when the anemone was correctly positioned in the mud, with the whole surface of the scapus and physa in contact with the substrate, and the tentacles remaining exposed at the mud-water interface. In well oxygenated mud at 21-26°C, many anemones took from about 0.25 h to 1 h to achieve their natural posture, and most achieved it within 3 h, although some took considerably longer (table 3).

Lack of effect of light on penetrative burrowing

Ten anemones were placed on the mud surface in each of two dishes of natural mud and water. The experiment was carried out in April, and commenced during late afternoon, when the dishes were placed in direct sunlight. One of them was further illuminated by a microscope lamp directly focused onto it, whilst the other was covered by a light-proof box. The water temperature in both dishes was about 21°C at the beginning of the experiment, but reached almost 26°C at the end, after 23 h. Half-

Table 4. Particle size analysis of sieved mud from Half-Moon Pond, a natural habitat of *N. vectensis*.

Particle size (µm)	Particle description[1]	Sieved fraction weights (g): Fraction	Cumulative[2]	As %[2]
>1180	Plant debris	0.0844	-	-
>600	Plant debris	0.1189	-	-
>300	Medium sand	0.3742	0.3742	3.1
>150	Fine sand	1.3516	1.7258	14.5
>75	Very fine sand	1.7770	3.5028	29.4
>38	Silt and clay[3]	1.6692	5.1720	43.4
≤38	Silt and clay[3]	6.7322	11.9042	100.0

[1] After Morgans (1956)

[2] Excluding plant material

[3] Flocculent

Table 5. Relationships between particle size and interstitial water content of mud and sand used in *N. vectensis* burrowing experiments. SD = standard deviation of the mean.

Substrate sample source	Particle size (µm)	Mean % water (± SD)	Replicates
Mud (Half-Moon Pond)	Median 30	84.3 ± 1.00	6
Sand (Wells Beach)	<1180>600	25.0 ± 2.60	3
Sand (Wells Beach)	≤600>300	19.9 ± 0.85	3
Sand (Wells Beach)	≤300>150	19.9 ± 1.56	3
Sand (Wells Beach)	≤150>75	21.2 ± 1.37	3

hourly examinations of the burrowing progress of all anemones were made up to 3 h, with a final check after 23 h. The results are shown in table 3.

After 3 h, 70% of the anemones in the light had successfully burrowed, as had 60% of those in the dark. All the remaining unburied anemones were still actively burrowing after 3 h; by 23 h, 90% of the anemones in the light and 80% of those in the dark were buried. There were no statistically significant differences between the numbers of anemones that had burrowed successfully in the light or dark at any of the times when they were examined (table 3).

Light had no obvious effect either in stimulating or inhibiting burrowing. Further observations revealed that when a bright artificial light or direct sunlight was suddenly directed onto shaded anemones *in situ* in mud, there was no apparent response. Neither withdrawal into the mud nor any tentacle movement occurred.

Effects of substrate characteristics on penetrative burrowing

Three substrate characteristics were shown to influence the penetrative burrowing behaviour of *N. vectensis*: the particle size, the interstitial water content and the degree of oxygenation.

A sample of the mud from Half-Moon Pond, which was used as the natural control substrate for burrowing experiments, was subjected to particle size analysis (table 4). About 57% of the sample comprised particles of <38 µm diameter after plant fragments had been removed. Graphical analysis (Morgans, 1956) indicated a median particle size of about 30 µm. Ungraded beach-sand from Wells-next-the-Sea, used as an experimental substrate, had a median particle size of 234 µm; fractions of graded sand were also used for penetrative burrowing experiments. Table 5 shows the relationships between particle size and interstitial water content of the various substrates used in burrowing experiments. The natural mud had a water content of about 84% and was thixotropic, whilst the experimental non-natural substrates, graded beach-sand samples, contained about 20% to 25% water and were dilatant.

Table 6 summarizes eleven experiments, using 4-10 anemones in each, in which the success of penetrative burrowing was recorded in various substrates, some natural and some non-natural, in a glass dish. Substrates c) and e) were muds from the natural habitat of *N. vectensis*, and substrate b) was a mud from a nearby lagoon uninhabited by anemones. Substrates a) and d) comprised ungraded beach-sand of median particle size 234 µm. The experiments were completed on separate occasions during December and January, using anemones from two different batches, at temperatures

Table 6. Numbers of *N. vectensis* that had successfully burrowed 12 h after being placed on various substrates ($n = 64$; sizes 3-8 mm; 21-23°C; 29 p.p.t.).

Oxygenation	Substrate type	
	Sand	Mud
Well oxygenated	a) 2/5 (40%)	b) 7/7; 3/5 (83.3%) c) 5/5; 4/4; 4/4; 4/4; 9/10; 8/10 (92%)
Poorly oxygenated or anaerobic	d) 0/5 (0%)	e) 0/5 (0%)

a) Clean sand from Wells Beach (median particle size 234 µm)
b) Fine mud from a lagoon at Cley-next-the-Sea (particle size not determined)
c) Mud from Half-Moon Pond (median particle size 30 µm)
d) Blackened sand smelling of hydrogen sulphide (median particle size 234 µm)
e) Black mud smelling of hydrogen sulphide (median particle size 30 µm)

between 21°C and 23°C. Anemones were placed on the test substrate during early morning under fluorescent ceiling lighting and burrowing success was assessed 12 h later. The anemones could burrow easily in the well oxygenated muds, b) and c), but their burrowing capability in well oxygenated sand, a), was poor (statistical significance of difference, χ^2 test, $P < 0.05$). However, anemones could not burrow at all in mud, e), or sand, d), that was anoxic or anaerobic.

Results of a further twelve simultaneous experiments during 24 h in experimental non-natural substrates of well-oxygenated, graded beach-sand at 21°C are shown in table 7. These results, obtained during December, indicate that *N. vectensis* is sometimes able to penetrate fairly coarse sands, but not by burrowing in the normal way. The two coarsest samples tested (>300<1180 µm particle size) were covered copiously with mucus, indicating that the anemones had crept over most of the surface before some of them had managed to penetrate the substrate. A few individuals had climbed the glass dish wall. Those that had succeeded in penetrating the sand (50% overall in the two coarsest samples) took >17 h to do so; they appeared unhealthy and had retracted their tentacles (table 7). Only individuals <4 mm long managed to penetrate; others up to 8 mm long failed. Although 87% of anemones managed to burrow into the two finest sand samples (≤ 300>75 µm particle size), they took many hours to do so, and clearly penetration was more difficult than into mud from their natural habitat. The difference between the percentages of anemones that were able to penetrate the coarsest samples (>300 µm) and the finest samples (≤ 300 µm) was highly statistically significant (χ^2 test, $P = 0.002$).

Table 7. Numbers of *N. vectensis* that had successfully burrowed 24 h after being placed on sand of various grades (triplicate experiments) ($n = 60$; sizes 3-9 mm; 19-21°C; 31-33 p.p.t.).

Particle size (µm)	Anemones buried[1]	Notes
<1180>600	2/5; 2/5; 4/5 (53%)	Not true burrowing; physa inserted into large interstitial spaces; anemones sick
≤ 600>300	1/5; 1/5; 5/5 (47%)	Not completely buried; anemones sick
≤ 300>150	4/5; 5/5; 4/5 (87%)	Burrowing very slow and laborious
≤ 150>75	5/5; 3/5; 5/5 (87%)	Burrowing very slow and laborious

[1] Difference between proportions of anemones buried in sand of particle sizes of >300 µm and ≤ 30 µm is statistically significant (χ^2 test, $P = 0.002$)

When the water overlying a mud substrate was unaerated, the oxidizing layer gradually became shallower and the black hydrogen sulphide-bearing boundary approached and finally reached the mud-water interface. When buried anemones were kept in such conditions, they left their burrows and came to lie on the mud surface. It was not observed exactly how they achieved this, although it is probable that reversed peristalsis was employed. Once lying on the surface of the mud, they adopted a greatly attenuated shape with the tentacles retracted, and all peristalsis ceased. I have never seen this occur in the field. After a few days, the anemones died, presumably from asphyxia. If the water was re-aerated in time, however, the oxidizing layer became re-established at the mud surface, peristalsis in the anemones was resumed and penetrative burrowing commenced to re-establish them in their typical posture in the mud. In a glass-"Plasticine" chamber (fig. 1a) containing blackened reduced mud and water, in which a high concentration of hydrogen sulphide had been allowed to accumulate, peristaltic waves were soon inhibited in an anemone placed directly onto the mud surface.

Hence, *N. vectensis* was able to penetrate thixotropic mud from its natural habitat only if it was well oxygenated. If already positioned in the mud, anemones were forced to leave it if oxygen tension in the substrate became lowered; if unable to escape further from the mud surface by climbing up some convenient object, they eventually died. *N. vectensis* could penetrate well oxygenated coarse sand by creeping into interstitial spaces, and could burrow in fine sand, but in both cases, the process was slow, inefficient and at the obvious expense of much energy and mucus production. Anemones could not penetrate any substrate that was not well oxygenated, and were paralysed by hydrogen sulphide.

Creeping and climbing in *N. vectensis*

This section explains how creeping and climbing are involuntary modifications of penetrative burrowing behaviour, and investigates whether the effects of gravity, touch or light influence these behaviours.

Method of creeping and climbing

The adherent rugae on the column of *N. vectensis* have already been shown to play an essential role in anchoring anemones during penetrative burrowing. They are equally important in creeping and climbing. In fact, creeping and climbing are effected in essentially the same way as burrowing. However, on a firm surface such as glass or a leaf of a plant, penetration by the physa is obviously impossible and the nett progress of the animal is therefore necessarily parallel to the solid substrate. Fig. 7 shows a sequence of

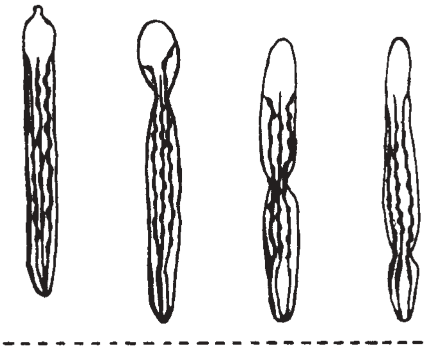

Fig. 7. Stages (right to left) in climbing behaviour of *N. vectensis*, with tentacles retracted, progressing physa-first vertically upwards on glass. Scale = 1 cm.

positions of an anemone climbing on a vertical glass plate, viewed through the glass. The animal moved physa first, the physa expanding beyond the *point d'appui* due to the increased hydrostatic pressure in front of the direct peristaltic waves. The tentacles were either retracted or remained expanded.

Lack of effects of gravity and tactile stimuli on creeping and climbing

Experiments were carried out to investigate what stimuli might be involved in creeping or climbing behaviour. Anemones were studied in apparatus without mud, so that creeping or climbing were their only locomotory options. Observations of anemones in the pipette apparatus containing only water (fig. 1b) showed that gravity had no effect in daylight on the initiation or passage of peristaltic waves. At whatever angle anemones were held through 360° in the vertical plane, waves were initiated and continued to pass. Furthermore, when the apparatus was fixed vertically, anemones progressed tail-first upwards or downwards, according to their orientation in the pipette. This showed that climbing is neither a negative nor a positive geotaxis, and that its direction is random.

Next, some aspects of specific elements of creeping were examined. It has already been noted that in experiments on column peristalsis there is a pause just before a wave reaches the end of the physa, and that during the early stages of penetrative burrowing the tip of the physa turns downwards into the mud (fig. 6a). Experiments were therefore carried out to ascertain whether gravity or tactile stimuli might direct the flexion of the physa towards the substrate as each peristaltic wave reaches the end of the physa.

Accordingly, in an experiment carried out during December at 21°C, eight anemones were placed in a glass dish of water in daylight and were allowed to adhere to the bottom. Using a dissecting microscope, peristaltic waves were observed as they progressed from the actinopharynx to the tip of the physa. Fifty random waves were recorded in various anemones; 48 times, the physa turned towards the glass (downwards) as the wave reached the end, and only twice did the physa not flex. Observed from the side with a magnifying glass, it was clear that what caused the physa to be turned downwards was the adhesion of the dense rugae at the tip of the physa. This adhesion, combined with the increased internal hydrostatic pressure in front of the advancing peristaltic wave, caused the physa to expand and turn towards the surface to which it adhered. However, further up the column, the rugae pulled free as the peristaltic wave passed, as previously observed (fig. 5). The adherence of the rugae near the tip of the physa also appeared to be the cause of the momentary pause in the progression of the wave just before it reached the physal extremity, since if, as occasionally happened, the physal rugae did not adhere well, the wave passed unimpeded right to the end of the physa, which therefore did not flex.

When anemones were lying on top of a horizontal substrate of mud, glass, etc., their orientation was such that the flexion of the physa, as a peristaltic wave reached it, was always downwards, i.e., towards the surface to which the physa adhered. That the downward flexion of the physa is not a geotactic response was confirmed by allowing anemones to adhere to a microscope slide in daylight, then inverting it on supports in a dish of water, so that the anemone was suspended out of contact with the bottom of the suspension chamber (fig. 1c). Normal peristaltic waves passed, but

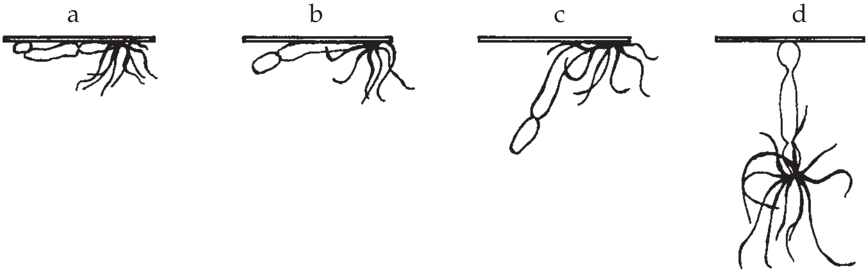

Fig. 8. *N. vectensis* showing various degrees of adhesion in a glass suspension chamber. a) rugae adhering along whole length of column. b) rugae of physa detached from glass. c) rugae of physa and lower scapus detached from glass. d) all rugae detached except those at tip of physa.

in this case, the physa turned upwards towards the glass to which it was adhering (fig. 8a).

Experiments in the same apparatus (fig. 1c) in daylight also demonstrated that physa flexion does not result from any tactile stimulus that might cause parietal muscle contraction. In fact, it is caused solely by the powerful adhesion of the rugae, without which physal flexion does not occur. If the physa of an anemone in a suspension chamber fell free under the influence of gravity, waves passed along it with no upward or downward flexion of the physa (fig. 8b). Furthermore, if the lower scapus also became detached, it and the physa hung down under the influence of gravity, and waves similarly passed without any physal flexion (fig. 8c). Sometimes, all the rugae but those at the very tip of the physa became detached, when the anemone dangled vertically from the physa tip with the tentacles hanging downwards, and peristaltic waves passed unimpeded directly upwards with no physal flexion (fig. 8d).

Overall, these results showed that climbing is not stimulated by gravity; that initiation and passage of peristaltic waves are not responses to gravity or tactile stimuli; and that flexion of the physa towards the substrate is neither a geotactic nor a tactile response. Physal flexion depends solely on ruga adhesion.

Lack of effect of light on creeping

Experiments were carried out to examine the effects of light and dark on creeping behaviour. In an experiment carried out in February, glass dishes containing single anemones in water were placed upon squared graph paper. Depending on the treatment, each dish either was left exposed to daylight or was covered with a light-proof box. The subsequent positions of each anemone were then recorded at intervals. Tables 8 and 9 show the cumulative distances travelled simultaneously by two lots of eight anemones of 4-5 mm column length kept at 16°C in either daylight or under a light-proof box, respectively. There were no statistically significant differences (by a *t*-test) between the mean speeds of creeping in daylight (table 8) or darkness (table 9) after 2 h ($P = 0.79$), 3 h ($P = 0.83$), 4 h ($P = 0.32$) or 5 h ($P = 0.21$). During a period of 5 h (in darkness) or 6 h (in daylight), there was no change in the mean speed of creeping (fig. 9). Regression analysis revealed no correlation of mean speed with the time spent in creeping ($r = 0.0332$ in darkness, $P > 0.1$; $r = 0.1634$ in daylight, $P > 0.1$). The directions in which anemones crept were quite random and changed frequently during

Table 8. Cumulative distances crept in daylight and mean speeds of eight individual *N. vectensis* at hourly intervals ($n = 8$; sizes 4-5 mm; 16°C; 29 p.p.t.).

Time elapsed	Cumulative distance (mm) travelled by each of 8 anemones at times shown								Mean speed (mm h^{-1})
	1	2	3	4	5	6	7	8	
2 h	3.5	9.0	2.0	1.5	5.0	2.5	3.5	1.5	1.78
3 h	7.5	13.0	3.0	3.0	11.5	6.0	5.5	3.0	2.19
4 h	9.0	17.0	5.5	7.5	13.5	10.0	10.5	6.5	2.48
5 h	13.5	19.0	6.5	9.0	15.5	15.0	12.5	7.0	2.43
6 h	15.0	22.5	7.0	10.0	17.0	16.0	13.0	7.5	2.25

Table 9. Cumulative distances crept in darkness and mean speeds of eight individual *N. vectensis* at hourly intervals ($n = 8$; sizes 4-5 mm; 16°C; 29 p.p.t.).

Time elapsed	Cumulative distance (mm) travelled by each of 8 anemones at times shown								Mean speed (mm h^{-1})
	1	2	3	4	5	6	7	8	
2 h	2.5	2.5	4.0	11.0	2.5	1.5	4.0	3.5	1.97
3 h	5.0	5.0	5.0	16.0	3.5	2.5	6.0	6.0	2.04
4 h	6.5	7.5	6.0	17.5	3.5	5.5	9.0	7.5	1.97
5 h	7.0	8.5	7.5	18.5	4.0	8.5	9.5	11.5	1.88

Table 10. Numbers of *N. vectensis* in each of four 90° quadrants (see fig. 10 for example) after creeping on glass under different conditions of lighting for 24 h ($n = 3 \times 8$; sizes 4-5 mm; 16-21°C; 29 p.p.t.).

Illumination type	Numbers of anemones in each quadrant[1]			
	I	II	III	IV
Daylight	4	0	4	0
Dark-box	2	3	1	2
Slit-box	2	2	1	3

[1] Combining treatments, there are no statistically significant differences (one-sample χ^2 test, $P > 0.10$) between the numbers of anemones in each quadrant

several hours (fig. 10). After 24 h, the numbers of anemones in each 90° quadrant were counted (table 10) and no statistically significant difference (by Fisher's exact test) was found between the distributions of anemones in daylight and darkness ($P = 0.614$).

To investigate phototaxis, a third group of eight anemones from the same batch was kept at 21°C, on a separate occasion, under a slit-box with a microscope lamp directed into the slit. The numbers of individuals in each quadrant were counted after 24 h and were compared with those in the two groups in daylight or darkness after the same period (table 10). No statistically significant difference (by Fisher's exact test) was found between the distributions of anemone groups in daylight or darkness combined, or the group in the slit-box ($P = 0.846$).

Overall, these results showed that locomotion was random in direction, whether in uniform conditions of daylight or darkness; that daylight or darkness had no influence on speed of locomotion, which was maintained unchanged for at least 6 h; and that no phototactic response to unidirectional light was demonstrable.

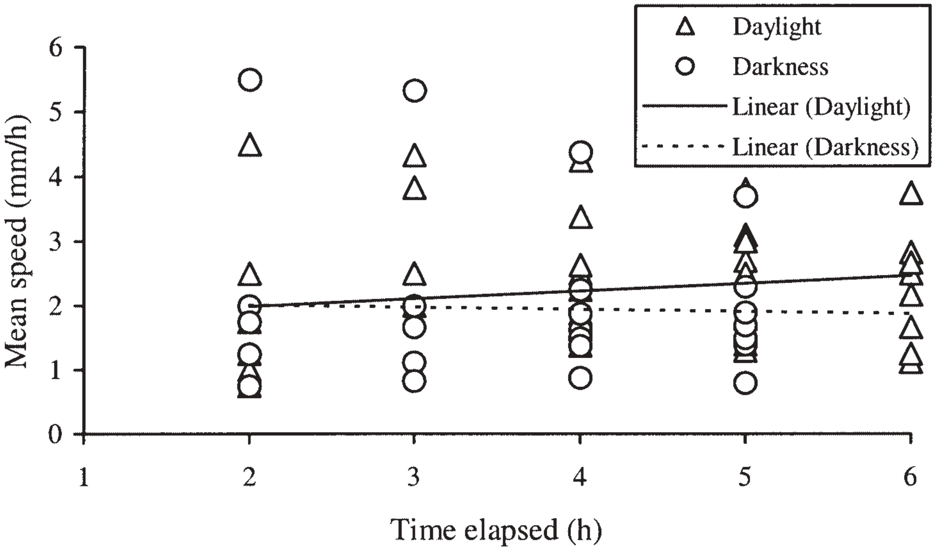

Fig. 9. Relationship between mean speed of progression and the period spent creeping by *N. vectensis* in daylight or darkness at 16°C. Not statistically significant in daylight or darkness (P > 0.1).

Intrasubstrate burrowing in *N. vectensis*

Casual observations showed that when anemones were collected with large amounts of mud (10 cm deep) and water in glass jars, they reappeared normally orientated at the newly established interface 12-24 h later (Williams, 1976). Clearly, the mode of locomotion employed to regain the mud surface must be quite different from penetrative burrowing. An experiment was therefore carried out to investigate how anemones respond when suddenly buried in deep mud. Twenty anemones were placed in clean water around the perimeter of a glass dish at 22°C, where they stretched out horizontally on the bottom. They were then covered with 2.2 cm of well oxygenated mud from their original habitat. Times taken for anemones to surface were recorded, and the method of locomotion was able to be observed, in some cases, through the side of the dish. All the anemones reached the mud surface in <3 h. The mode of locomotion employed is termed intrasubstrate burrowing and, unlike penetrative burrowing, it does not include the elements of creeping behaviour.

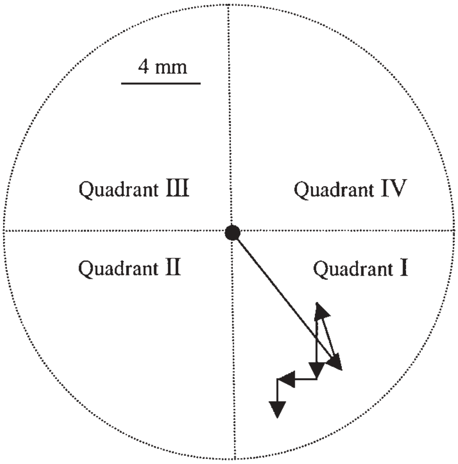

Fig. 10. Example of random path taken during 6 h by an individual of *N. vectensis* creeping on glass in daylight at 16°C (anemone no. 2, Table 8).

Anemones progressed head-first by

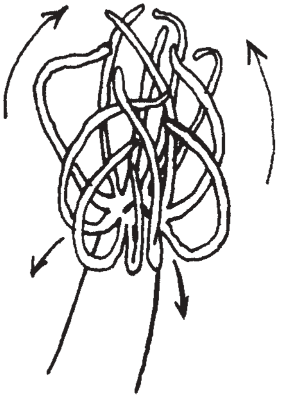

Fig. 11. Attitude of tentacles of *N. vectensis* when burrowing head-first, or extending the scapus, through thixotropic mud. Arrows show directions of water currents generated by cilia of the oral disk and tentacles in the mud-free zone around the oral end of the column.

alternate introversion and eversion of the capitulum and scapus, with the tentacles held as in fig. 11 during the eversion phase. Hence, ciliary action on the oral disk and tentacles assisted progress by increasing the fluidity of the thixotropic mud. A *point d'appui* was established by the rugae about the point where the physa joins the scapus. This allowed the tentacles to be thrust forward, and the physa was drawn behind it, by longitudinal contraction. Sometimes, however, the physa also exhibited reversed peristalsis, the waves originating at the widest part of the physa and ceasing part way up the scapus. These peristaltic waves were very slow and spasmodic, quite unlike those seen during penetrative burrowing.

All anemones surfaced tentacles first. They took between 1.5 h and 2.7 h to surface from a depth of 2.2 cm. Progression was not necessarily continuous; sometimes an individual remained stationary, with the scapus introverted, and exhibiting no peristalsis for up to 2 h. Thus mean speeds of progression towards the mud surface by intrasubstrate burrowing were between 0.8 cm h^{-1} and 1.5 cm h^{-1}. These are certainly underestimated, because progression was neither directly vertical nor continuous.

Further observations on behaviour of *N. vectensis*

Inhibition of peristalsis by ammonia

It has already been described how anemones can be paralysed by exposure to hydrogen sulphide. When drops of ammonia solution were added to the water, it was observed that peristalsis was immediately inhibited. All endodermal longitudinal muscles were maximally contracted, and the ectodermal radial muscles of the oral disk and the endodermal circular muscles of the column were relaxed (fig. 2a). Relaxation of the circular muscles may have been a reciprocal response to the contraction of the longitudinal muscles, causing indirect inhibition of peristalsis.

Reversed peristalsis

On two occasions, anemones completely covered by mud in the pipette apparatus (fig. 1b), and held horizontally, moved a short distance head-first. Peristaltic waves passed in the opposite direction from normal, i.e., the anemones exhibited reversed peristalsis. Since whole body progress was in the direction of the peristaltic waves, they were still direct, rather than retrograde, waves. These reversed waves were very slow and apparently poorly co-ordinated. Nevertheless, they are apparently deployed when anemones become accidentally buried and attempt to regain the mud-water interface, as described for intrasubstrate burrowing. It also seems likely that such reversed waves occur when anemones are forced to vacate their burrows by increased hydrogen sulphide concentrations in the mud.

Escape from accidental burial

In nature, *N. vectensis* may be accidentally buried on occasions, such as when wind-generated waves disturb the mud substrate in shallow water and redeposit it, or when foraging wading birds are probing the mud, or when arenicolid worms eject mud-casts. Somehow, a buried anemone must re-establish itself at the mud-water interface in order to be able to feed. The magnitude of this task may vary between that faced by an anemone that becomes covered by just a few millimetres of mud whilst still maintaining its typical posture and an anemone that is displaced from its burrow and buried by several centimetres of mud.

Experiments were therefore carried out to discover how anemones are able to escape burial at different depths. First, two types of apparatus were used to observe the behaviour of normally orientated anemones that were then lightly covered with mud. The glass-"Plasticine" chamber (fig. 1a) had the advantage that anemones were little restricted laterally and hence were able to bend sideways, whilst the pipette apparatus (fig. 1b) was more restrictive but gave a much clearer view of an anemone.

Twenty-four hours after placing four anemones on the mud surface in the glass-"Plasticine" chamber (fig. 1a), they had burrowed successfully, the temperature being 21°C. Then, after marking on the glass wall the level of the mud from which the anemones' tentacles protruded, 2.5 mm of mud was added. The anemones' first response was to retract into their burrows, then after 1-5 minutes all four animals emerged from the raised mud surface. After another 30 minutes, a further 2 mm of mud was added, evoking the same response as previously; three of the anemones resurfaced in 5-15 minutes, but the fourth did not reappear on that occasion. Another 30 minutes later, 2.5 mm more mud was added, and all four anemones resurfaced in 7-15 minutes.

Resurfacing was effected simply by elongating the scapus. By removing the mud with a Pasteur pipette, it was seen that the physa had remained at the bottom of the chamber. As the scapus was elongated, the oral end of the column was not introverted and the tentacles remained partly extended, though incurved as shown in fig. 11. Thus, the water currents generated by the cilia of the tentacles and the oral disk assisted in re-emergence from the thixotropic mud by increasing its fluidity. A zone clear of mud was maintained around the tentacular crown as the column elongated. Similar use of the tentacles was also observed during experiments using the pipette apparatus filled with mud and water (fig. 1b).

Incidentally, on several occasions, anemones established in about 1 cm of mud in the glass-"Plasticine" chamber (fig. 1a) were found to have retracted and then pushed their way to the mud surface at a point between 0.5 cm and 1 cm away from the entrance to the original burrow. This occurred overnight, without the stimulus of burial. When mud was removed with a Pasteur pipette, the physa was always found to be at the bottom of the chamber directly below the entrance to the original burrow.

However, when anemones are so deeply buried by mud disturbance that column extension is inadequate for regaining the surface, they employ head-first intrasubstrate burrowing, as previously described. Escape responses therefore vary according to the depth of burial. In summary, if anemones established in their burrows are covered with a thin layer of mud, their first response is simply to extend the column until the tentacles break the new surface. If, however, the disturbance is much greater, such that anemones are displaced from their burrows and suddenly engulfed in deep mud, they actively burrow head-first towards the mud surface, employing alternate introversion and eversion of the scapus, sometimes aided by reversed peristaltic waves. In both sets of circumstances, ciliary action on the oral disk and tentacles assists head-first progress by increasing the fluidity of the thixotropic mud.

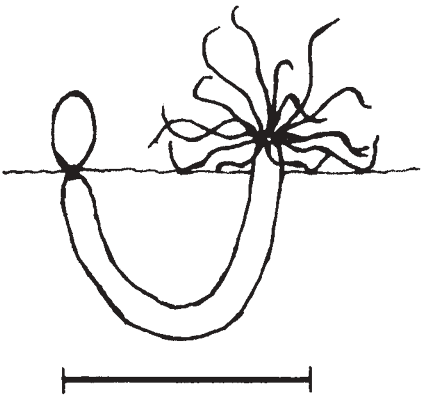

Fig. 12. Position of *N. vectensis* in mud during transverse fission. Scale = 1 cm.

Simultaneous feeding and penetrative burrowing

Both casual and experimental observations showed that anemones were capable of catching prey and ingesting it while burrowing into mud, without direct peristalsis being inhibited. After feeding anemones with pieces of chironomid midge larva in clean glass dishes, a reversed wave moving upwards from the physa was observed on rare occasions, but normal locomotory peristalsis was soon resumed.

Asexual reproduction by transverse fission

N. vectensis habitually reproduces asexually by transverse fission. This was recorded on seven occasions in anemones kept in mud, during January 1975 and October 1976, always overnight. The process was apparently the same in every case. An anemone remained buried in its typical posture with the tentacle crown exposed at the mud surface. Then in some way that was never observed, the physa was brought up to the mud surface so that the column assumed a U-shape, with the physa protruding from the mud by 2 or 3 mm. The physa then constricted at about the level of the mud surface, and became detached (fig. 12).

Detached physae had no obvious precursors of an actinopharynx or tentacles immediately after separation, but nevertheless irregular peristaltic waves were observed passing from the separated end to the tip. This did not result in burrowing because the shortness of the physa did not allow the full deployment of the elements of burrowing behaviour. Although not actually observed, it seems likely that the peristaltic waves in an asexually produced physa continue to be initiated while the new musculature and oral end are developing. Two detached physae removed to a dish of water developed an actinopharynx and stubby tentacles within four days of separation. Presumably, penetrative burrowing can commence as soon as the physa fragment is sufficiently developed.

The anatomy of *N. vectensis*

The relevant anatomy of *N. vectensis* was examined for correlation with the observed behaviour. Its description is based upon studies of more than 100 living specimens, two series of stained longitudinal sections, four series of transverse sections and three stained whole mounts. See Stephenson (1928) for terms used here to describe the anatomy.

The gross structure of *N. vectensis* is that of a typical athenarian anemone, as described by Stephenson (1928). Specimens collected from the field were mostly between 2 and 20 mm long, but exceptionally were up to 41 mm. The column is divided, though not very distinctly, into a very short capitulum, a scapus and an imperforate physa. The physa and lower part of the scapus are densely covered with adherent rugae from 3 to 7 µm in diameter, demonstrable in whole mounts stained with mucicarmine. Further up the column, the rugae become progressively less densely crowded. The ectodermal ciliation of the column is sparse. The physa and scapus have no cilia. A narrow band of cilia, continuous with the disk cilia, encircles the upper part of the column to just below the actinopharynx when the anemone is relaxed. The cilia do not remain exposed, however, when the upper part of the column (capitulum and upper part of the scapus) is introverted. The cilia on the oral disk and along the whole length of the tentacles beat centrifugally, and those on the upper column beat aborally.

The musculature of *N. vectensis* and its functions are summarized in table 11. Neither a marginal nor an oral sphincter exists. The ectodermal radial muscles of the oral disk extend over the oral cone and, for a short distance, into the upper part of the actinopharynx, but other than that, the

Table 11. The musculature and its functions in *N. vectensis*.

	Ectodermal muscle		Endodermal muscle	
Anatomical entity	Type	Function	Type	Function
Column wall	—	—	Circular	Elongates column or generates peristaltic waves
Macrocnemes	—	—	Retractors	Shorten column and introvert disk, capitulum and scapus
			Parietals	Shorten and bend column
			Transverse	Opens actinopharynx
Microcnemes	—	—	Parietals	Assist eversion of disk
Tentacles	Longitudinal	Shorten or bend tentacles	Circular	Elongates tentacles
Oral disk	Radial (extend over oral cone)	Open labia	Circular[1]	Constricts disk and closes labia
Actinopharynx	Longitudinal (upper region only, continuous with radial muscles of oral disk)	Open labia	Circular[1]	Closes labia and actinopharynx or generates peristaltic waves

[1] No sphincter muscles

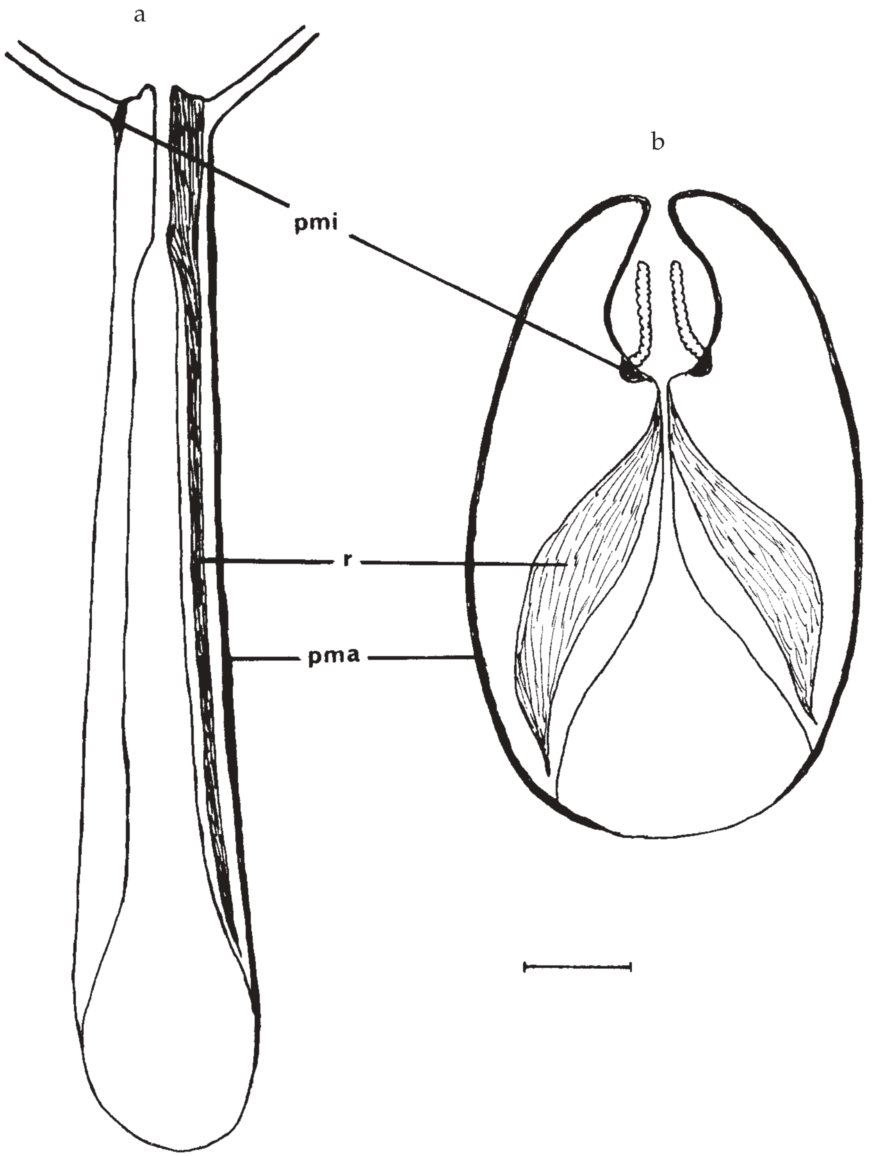

Fig. 13. Major longitudinal endodermal muscle groups of *N. vectensis*. a) relaxed, small individual. b) contracted individual of same size. r = retractors of macrocnemes; pma = parietals of macrocnemes; pmi = parietals of microcnemes. Scale = 1 mm.

actinopharynx has no ectodermal muscle. In longitudinal sections of relaxed anemones, the endodermal circular muscle of the column appears as a continuous sheet. Observations on living animals showed that the whole sheet can contract simultaneously to lengthen the column while the longitudinal retractors relax. Peristaltic waves are generated by the circular muscle sheet, which initially contracts in a circumferential band about 0.5 mm broad at the level of the middle actinopharynx, then simultaneously relaxes at its oral edge and contracts at its aboral edge, so that the constricted band progresses without any change in its breadth down the column towards the physa. A photograph of such a moving band is shown in Williams (1975b, fig. 2a). Even in relaxed anemones, there always appears to be some general uniform tonus in the circular muscle sheet. There is no ectodermal muscle associated with the column.

Fig. 13a shows the disposition of the longitudinal endodermal muscle series and mesenteries when the anemone is situated in its tube, and fig. 13b shows the positional changes that occur when it is fully retracted. Transverse sections of the column reveal endodermal longitudinal retractor muscles with up to 15 folds around the mesogloeal plates on one face of each macrocneme. These retractors are rather weak and extend from the oral disk to just below the junction of the physa and scapus. They are circumscript-diffuse in the main, tending more towards diffuse with smaller, widely spread muscle folds at the level of the actinopharynx. Where they meet the underside of the oral disk, they are diffuse. There is a pronounced thickening of the mesogloea where the macrocnemes join the actinopharynx. The weak endodermal parietal muscles possess from two to four small folds around the mesogloeal plates on each side of the macrocnemes where they join the column wall, except at the extreme upper end where they occur only on the side bearing the longitudinal retractor. They extend below the extremities of the retractors in the physa, but not right to the end of the physa, and stop just short of the oral disk at the upper end of the column, at the lower limits of the microcnemes (fig. 13). The longitudinal retractors and parietals of the macrocnemes become progressively weaker towards the physa.

The microcnemes may be seen to advantage in living anemones if a few drops of ammonia solution are added to their water. The oral disk and capitulum become greatly distended and transparent, revealing the microcnemes through the capitulum (fig. 2a). They occur in the region of the tentacle insertions, one between any two tentacles that are not separated by a macrocneme. Their longitudinal limits extend from the upper side of a tentacle aborally for a distance of up to twice the diameter of the base of the tentacle, i.e., they are restricted approximately to the externally poorly demarcated capitulum. They bear weak non-folded endodermal parietal muscles on both faces.

The tentacles possess ectodermal longitudinal and endodermal circular muscles throughout their lengths and are completely retractile. This was demonstrable by placing an anemone in water perfused with ammonia, when maximal tentacle-shortening without column introversion occurred. The tentacles contracted to a length slightly less than the radius of the oral disk (fig. 2a). Ammoniation of the water also caused apparently maximal contraction of longitudinal retractors and parietals, with relaxation of the radial muscles of the oral disk and the circular muscles of the column. When the tentacles retract naturally, they shorten without any bending; this is so even when the scapus is introverted (fig. 13b). When maximally contracted, the tentacles have a circumferentially corrugated appearance (figs 2a, 13b). The tentacles can shorten to about 13% of their maximum length and the column length can be reduced to 60% or less. When not buried, an individual of about 1 cm in length when expanded may contract to a pyriform or subglobular shape (figs 2b, 13b). Larger individuals can contract only to a lesser extent, forming an irregularly contorted cylinder (fig. 2c).

The constrictions shown in fig. 2c are not peristaltic waves, but are the result of the buckling of the maximally contracted column.

Discussion

Synthesis of locomotory behaviour of *N. vectensis*

The two essential anatomical elements that facilitate normal locomotion in *N. vectensis*, whether penetrative burrowing, creeping or climbing, are the circular muscles of the column, which generate direct peristaltic waves, and the column rugae, which provide adhesion at the *points d'appui*. These facts have been established from the present behavioural and anatomical study. The locomotory behaviour of *N. vectensis* was interpreted in a physiological context, based upon work on other cnidarians. This interpretation facilitated a broad synthesis of the behaviour, with its various stimuli and taxes, and an assessment of their survival values.

The spontaneous initiation of peristaltic waves in *N. vectensis* is presumably controlled by an endogenous pacemaker system in the nerve net (Josephson, 1965, 1974), although such a system in *N. vectensis* remains to be confirmed by electrophysiological studies. Josephson (1974) has suggested that, in cnidarians, multiple potential pacemakers are connected to a common conducting system, so that when any single pacemaker fires, it activates the whole system, hence resetting all the other potential pacemakers. This hypothesis is supported by the observation that in sea anemones, spontaneous contractions occur in rings of tissue excised from the column (Batham & Pantin, 1954; Needler & Ross, 1958; Hoyle, 1960).

Such a hypothesis would explain how peristaltic waves continue to be initiated in an asexually produced physa of *N. vectensis*. There is a constant, rhythmical initiation of peristaltic waves at the level of the actinopharynx in intact anemones of different sizes. Yet the waves in a recently separated physa still progress "aborally", although no actinopharynx is present. This suggests that the pacemaker system in *N. vectensis* is diffuse rather than localized, but it nevertheless appears to be polarized. The usual polarity seems to be reversed, however, during head-first intrasubstrate burrowing after accidental burial.

Previous evidence for endogenous pacemakers in cnidarians is the finding that spontaneous electrical potentials are still produced by hydroids long after magnesium anaesthetization has inhibited muscular contraction (Josephson, 1962; Ball & Case, 1973). Furthermore, the thenarian anemone *Bunodosoma cavernata* exhibits nerve net electrical activity in either aerobic or anaerobic conditions (Mangum, 1980). The present work provides further supportive evidence. Thus, *N. vectensis* is paralysed by hydrogen sulphide, yet when returned to reoxygenated water, peristalsis is resumed at the previous rate. However, rhythmical muscular activity is also inhibited naturally when *N. vectensis* is in its typical posture buried in the mud, but with its tentacle crown exposed. Presumably, this is a negative tactile response to contact of the column with the substrate on all sides, resulting in inhibition of peristalsis.

Reciprocal inhibition between parietal and circular muscles, as observed in the thenarian anemone *Metridium senile* by Batham & Pantin (1954), presumably also occurs in *N. vectensis*. Evidence comes from various sources. Thus, peristalsis never occurs in fully contracted individuals (note the legend to fig. 2c). Also, when *N. vecten-*

sis is paralysed by hydrogen sulphide, the longitudinal retractor and parietal muscles relax, whilst the circular muscle sheet appears to retain tonus. Furthermore, ammonia causes apparently maximal contraction of longitudinal retractors and parietals, with relaxation of the radial muscles of the oral disk and the circular muscles of the column, resulting in inhibition of peristalsis (fig. 2a).

R.L. Manuel (in Shelton, 1982) suggested that increasing the fluidity of the substrate may be an important aid to efficient burrowing, and that this might be achieved by the expulsion of water from an anemone at the digging site. This might involve release of water from the mouth during head-first burrowing, or from pores in the column during tail-first burrowing. However, it is difficult to see how expulsion of water could occur without compromising burrowing efficiency, since it is necessary to maintain a certain internal hydrostatic pressure for either head-first or tail-first burrowing. In fact, *N. vectensis* does increase the fluidity of the mud during head-first intrasubstrate burrowing, but it uses the cilia of the oral disk and tentacles. It cannot use a similar mechanism during tail-first penetrative burrowing, because there are no cilia on the column below the level of the capitulum. Water expulsion from pores would in this case be impossible since the column is imperforate.

Of the several types of behaviour listed for actiniarians in the Introduction, some cannot occur simultaneously in the same individual. For instance, cnida discharge and feeding behaviour in some species may be inhibited during locomotion, e.g., during the swimming response of *Stomphia coccinea* (Ross & Sutton, 1964). This has a survival value, since swimming in *S. coccinea* is an emergency escape response. However, *N. vectensis* can capture prey normally while burrowing, so cnida discharge and muscular activity in the tentacles are clearly uninhibited during locomotion. Since penetrative burrowing is not an escape response in *N. vectensis*, the ability to continue feeding during burrowing maintains its own survival value. Similarly, anemones climbing amongst underwater vegetation must be able to continue feeding (see later). With regard to cnida discharge, however, the column cnidae play no part in locomotion and remain undischarged during the generation of peristaltic waves (fig. 5).

The effects of various stimuli in controlling locomotion are now considered. Table 12 summarizes the various presumed taxes of *N. vectensis* demonstrated during the present work, with their respective stimuli and resulting behaviours. No specific photoreceptor cells have been identified in anthozoans, but extraocular photosensitivity may control phototaxis, expansion, contraction and spawning (Martin, 2002). In general, however, light cannot be considered to have any consistent effect on all sea anemones (Shelton, 1982). Hargitt (1907) reviewed his own work and previous studies on reactions of various anemones to light. He found that some species retracted on exposure to light and others were indifferent to it, but there was no correlation with the burrowing or free-living habits of anemones. Later work by Parker (1916) showed that the thenarian *Metridium marginatum* shortens its column on exposure to light, whilst Marks (1976) found that the same stimulus causes the burrowing anemone *Calamactis praelongus* to extend its tentacles. However, Ellehauge (1978) observed no diel activity, nor any reaction to sudden light or shadow in the athenarians *Edwardsia danica* and *E. longicornis*.

Light appeared to have no observable effect on the behaviour of *N. vectensis* (table 12). Thus, the speed of peristaltic waves is not affected by different lighting conditions

Table 12. Presumed taxes of *N. vectensis* in response to various combinations of stimuli and environmental conditions.

Position of anemone	Physical conditions	Stimuli operating			Responses to stimuli		Resulting behaviour
		Touch	Light	Gravity	Peristalsis	Use of scapus	
Column buried up to the capitulum, tentacles exposed above mud surface	Well oxygenated mud and water	Whole column in contact with mud, tentacles free in water	Neutral	Neutral	Inhibited	Inhibited	Maintains typical posture in burrow
Lying horizontally on mud surface	Well oxygenated mud and water	Side of column contacting mud	Neutral	Neutral	Direct waves	Inhibited	Penetrative tail-first burrowing into mud
Whole animal below mud surface	Well oxygenated mud and water	Whole column and tentacles contacting mud	Neutral	Negative geotaxis	Reversed direct waves	Alternate intro- and eversion	Intrasubstrate head-first burrowing toward mud surface
Column buried up to the capitulum, tentacles exposed above mud surface	Low oxygen tension in mud, well oxygenated overlying water	Whole column in contact with mud, tentacles free in water	Neutral	Negative geotaxis (?)[1]	Reversed direct waves (?)[2]	Inhibited	Vacates burrow and lies on mud surface
Lying horizontally on mud surface	Anoxic mud and water conditions	Side of column contacting mud	Neutral	Neutral	Inhibited	Inhibited	Lies paralysed on mud surface

[1] Not demonstrated experimentally. May be a negative chemotaxis.
[2] Not actually observed.

(table 1 and fig. 3), and speed of burrowing does not vary in daylight and darkness (table 3). Furthermore, the speeds of creeping are not significantly different in daylight or darkness (tables 8 and 9), the random direction of creeping is similar in uniform or unidirectional light and in darkness (table 10 and fig. 10), and prolonged or sudden exposure to light of the tentacle crown of a buried anemone does not elicit retraction into the mud. However, these results should be interpreted with caution, because they might have been influenced by the particular maintenance conditions of the anemones. Furthermore, no effort was made to control the artificial light intensities used, and daylight conditions were obviously dependent on the season. Nevertheless, despite these provisos, the consistency of the findings is remarkable.

Responses to gravity in *N. vectensis* apparently vary according to the prevailing conditions. There is no geotaxis during creeping, and the downward physal flexion during penetrative burrowing is passive, being caused solely by ruga adhesion. However, it appears that when an anemone is completely buried, a negative geotaxis enables it to reach the mud surface by intrasubstrate burrowing, since light presumably cannot penetrate mud far enough to stimulate phototaxis (table 12). In any case, phototaxis was not demonstrable in *N. vectensis* during the present study. The essential condition necessary for negatively geotactic intrasubstrate burrowing seems to be a tactile stimulus on the tentacles while they are extended in thixotropic mud (table 12). As the head-first alternate introversion and eversion of the scapus proceeds, it is as if the occasionally extended tentacles are testing the conditions ahead of the animal. When the tentacles finally emerge from the mud into clear water, scapus activity is inhibited again and geotaxis is apparently neutralized. If light or darkness were a contributory factor in intrasubstrate burrowing, resurfacing and re-establishment in a new burrow would not be achievable during both day and night; however, observations have shown that, on the contrary, this does occur.

The possibility that intrasubstrate burrowing in order to regain the mud-water interface is a negative chemotaxis should not be discounted (table 12). It is probable that in nature there is a concentration gradient of hydrogen sulphide in the mud, the concentration increasing with depth. This might force anemones up to the oxidizing layer at the mud surface. One observation made during the present study militates against this hypothesis, however, viz., anemones still moved to the mud surface when experimentally buried with well oxygenated mud. Nevertheless, it is possible that a hydrogen sulphide gradient began to develop in the 1.5-3.0 h during which the anemones were resurfacing. *N. vectensis* may be sensitive to concentrations of hydrogen sulphide that are too low to blacken mud or to be detected by the human sense of smell. It is perhaps much more likely that a negative chemotaxis operates to expel established anemones from their burrows when the substrate becomes anoxic. The question of chemotaxis with regard to intrasubstrate burrowing must, therefore, remain open until definitive experiments have been carried out.

Physical conditions essential for survival of *N. vectensis*

Although Frank & Bleakney (1978) were unable to keep *N. vectensis* alive in dishes without mud, Hand & Uhlinger (1992) have kept well-fed specimens in such conditions for well over a year, during which spawning and asexual reproduction were frequently observed. Burrowing *per se*, therefore, is not an absolute requirement for the survival of *N. vectensis*, but this habit presumably ensures that anemones remain in an environment that provides both a refuge from predators and stable conditions that facilitate feeding and reproduction.

It is concluded from the present results that *N. vectensis* requires the following physical conditions to maintain its burrowing habit: 1) Thixotropic mud of median particle size about 30 µm, 2) A well defined oxidizing layer at the mud surface, 3) Well oxygenated water overlying the mud substrate. Such a combination of conditions is only to be found in the usually calm waters of salt marsh lagoons and creeks in estuaries and sheltered bays, hence the restriction of this species to such localities (Williams, 1973a, 1976; Hand & Uhlinger, 1994).

It has been clearly demonstrated that *N. vectensis* is capable of burrowing only in very fine, thixotropic mud. However, in coarse sand or gravel, it can insinuate, though with obvious difficulty, the physa into interstitial spaces, much like the larvae of *Halcampa duodecimcirrata* (see Nyholm, 1949). This behaviour may be regarded as a form of creeping. However, the energy required for this, and for the production of increased amounts of mucus from the rugae, seems to be so considerable that the behaviour has a deleterious effect on the health of the anemone (table 7).

The importance of a well oxygenated substrate and overlying water has also been demonstrated (table 6). Inhibition of peristalsis occurred in unburied anemones when they were starved of oxygen experimentally by the presence of ammonia or hydrogen sulphide in the water. Progressive oxygen depletion of the mud stimulated *N. vectensis* to leave the substrate and eventually they laid apparently paralysed on the mud surface; I have never seen this happen in the field. Interestingly, Lindsay (1975) recorded individuals of *N. vectensis* moving over the bottom of pools. Since he further noted burrowing in “anaerobic muds”, perhaps he saw individuals that had recently emerged from a substrate that was becoming depleted of oxygen. Other observers (Frank & Bleakney, 1978; Bleakney & Meyer, 1979) have also seen anemones lying free on the mud surface, but there was no suggestion that they were dying. Perhaps, in nature, hydrogen sulphide rarely reaches concentrations as high as those that were produced in mud and water during the laboratory experiments described here.

Sheader et al. (1997) suggested that climbing into free-floating vegetation is an adaptation for escaping anoxic conditions. However, at least in isolated lagoons, if anemones were forced, by increasing hydrogen sulphide concentrations, to vacate their burrows and failed to make contact with vegetation soon enough, they might become paralysed and be stranded on the mud surface, as demonstrated experimentally herein. But in salt marsh pools that are flooded at least on spring tides, anemones exposed on the mud surface might be revived by oxygenated water or even washed into other pools; the same might happen to anemones in vegetation, if present.

It is assumed that, of the various locomotory behaviours described, the one having the primary survival value is burrowing, since that allows the anemones to employ the substrate as a stable refuge. Climbing, therefore, would seem to be an incidental behaviour, resulting from the accidental contact of an anemone with some impenetrable surface during an attempt to burrow. This is perhaps why climbing does not occur very commonly in nature. Although it may help anemones to escape anoxic conditions (Sheader et al., 1997), it is not known whether such behaviour is a true adaptation or is accidental, because it has not been demonstrated experimentally that climbing in nature is a negative chemotaxis. In the laboratory, it occurs consistently if anemones are kept in well oxygenated water, but without any mud in which to burrow, since it is but a variation of creeping. It also occurred when anemones wandered randomly about during failed attempts to burrow in sand (table 7). It is notable that there were no obvious anoxic conditions in the substrate when I found *N. vectensis* on *Zostera marina* in Abraham's Creek during the month of May.

Asexually produced physae can develop an actinopharynx and short tentacles within two days (Lindsay, 1975; Frank & Bleakney, 1978; Hand & Uhlinger, 1995) or up to four days (present results), but sometimes it takes up to 50 days (Hand & Uhlinger, 1995). It is possible that climbing might accidentally occur when such physae happen to come into contact with underwater vegetation immediately they make their first creeping movements. They would presumably wander continuously among the vegetation until they encountered soft mud again, but would still be able to feed in the meantime. Hand & Uhlinger (1995) found that some physal fragments could develop sufficiently well to capture and ingest food in as few as three days from the time of fission. In addition to the present record, *N. vectensis* has previously been observed attached to vegetation by G.F. Selwood (in Stephenson, 1935), Bailey & Bleakney (1966), Lindsay (1975), Frank & Bleakney (1978), Bleakney & Meyer (1979) and Sheader et al. (1997). Pending the demonstration of a negative chemotaxis to hydrogen sulphide, it seems to be a moot point whether climbing is a true adaptation for survival.

To assess the survival value of the burrowing habit of *N. vectensis* during drought, Williams (1976) examined the reactions of anemones living in dishes of 1 cm deep mud, after removal of the overlying water. Under these experimental conditions, the mud became rapidly desiccated, but not anaerobic. Because of this, the anemones retracted increasingly further into the mud to keep below the lowering water table, rather than attempting to vacate their burrows to escape asphyxia. Three days after the water was removed, they were all lying horizontally at the bottom of the dishes; when the original water was added to the drying mud, the tentacles of all the anemones were seen to be expanded at the mud surface within 15 minutes. As expected, they achieved this by column extension, but during penetration of this fairly hard mud, the scapus was introverted. In thixotropic mud, the tentacles would have been expanded during this process, as observed in the present study. After five days, the mud had become so hard that no anemones were able to regain the surface, and only 35% of those originally in the dish survived when they were dug out. No anemones survived desiccation for six days or more. Field observations, however, suggested that the main limiting factor in nature is more likely to be anoxia than desiccation, because the great depth of mud retains water for much longer so that bacteria continue to metabolize, and survival in a dried out lagoon is unlikely to exceed about one week (Williams, 1976).

The burrowing habit in relation to survival has some bearing on the present description of asexual reproduction in *N. vectensis*. Most previous records of transverse fission involved anemones that were not buried (Lindsay, 1975; Frank & Bleakney, 1978; Hand & Uhlinger, 1992, 1995). However, the present description (and the record of Williams, 1976) involved anemones buried in mud. Fission always occurred at night, as previously observed by Frank & Bleakney (1978) and Hand & Uhlinger (1995). A remarkable feature was that the physa became curved upwards to emerge from the mud before fission occurred (fig. 12). It is not known whether this would have happened in mud deeper than the 2 cm in which it was observed during the present work. However, such behaviour, if consistent, would ensure that the detached physa is not released within the substrate, from where it would be incapable of regaining the surface to feed. This modification of asexual reproductive behaviour seems, therefore, to be an adaptation in response to the burrowing habit, to help ensure survival of the species.

The significance to *N. vectensis* of calm water is probably quite complex. Since anemones kept *in vitro* can feed and reproduce without difficulty for long periods (Hand & Uhlinger, 1992; Fritzenwanker & Technau, 2002), their dependence on calm water may be only indirect, by virtue of the requirement for a fine substrate in which to burrow for protection. Certainly, persistently vigorous wave action precludes the formation of stable thixotropic mud. Sheader et al. (1997) measured flow rates of water just above the mud in lagoons inhabited by *N. vectensis*, and found that anemone abundance was greatest at flow rates below 0.1 cm s^{-1}, whilst no anemones were present where the flow rate exceeded 0.18 cm s^{-1}. Unfortunately, however, such calm, enclosed waters are more susceptible to pollution than open coastal waters. Chemical or biological effluents can easily upset the ecological balance, destroying the oxidizing layer at the mud surface (Williams, 1973a, 1976). If such a situation were to continue for long, the anemones would presumably die.

Previous observations on locomotion of *N. vectensis*

Crowell (1946) was the first to note peristaltic waves in *N. vectensis*, but the only previously published description of locomotion is that by Lindsay (1975), based on American anemones. However, Lindsay's description is greatly at variance with the present results. Only forms of creeping were described. He did not consider penetrative burrowing, but noted that anemones could reach the mud surface, emerging tentacles first, after being buried (i.e., intrasubstrate burrowing), although he did not observe how it is achieved. The rate of progression was about 0.3-0.4 cm h^{-1}, considerably slower than the rate recorded herein for head-first intrasubstrate burrowing (0.8-1.5 cm h^{-1}).

Lindsay (1975) recorded the head-first progression of an individual across the mud surface, using a stepwise process that he called the "wind-up and cast" method. First, the whole body was contracted by bringing forward the physa, retracting the scapus and withdrawing the tentacles. Then, with the physa fixed in its forward position, the scapus was slowly curved backwards towards the physa before being thrust forwards as the tentacles re-emerged, with a nett forward progression of about 3 mm. This two-phase "step" took about 5 minutes, and an anemone was seen to move about 90 mm in 9 hours by this method, i.e., at a rate of 1 cm h^{-1}. Clearly, however, the anemone did not make continuous progress in this way, because the potential distance travelled in 9 h would have been about 32 cm, based upon the time and distance recorded for one step. Nevertheless, the rate of progress recorded was about five times that observed in the present experiments for tail-first creeping (tables 8 and 9).

Lindsay (1975) also observed a second mode of creeping on the mud surface. Essentially, this was similar to that already described, except that after the first phase, the scapus was simply thrust straight forward, with a gain of about 1 mm. It was not stated whether the tentacles were retracted or not during this process.

Although Lindsay (1975) mentioned seeing peristaltic waves, he did not describe how they were involved in either of the creeping processes that he observed, nor are any waves shown in his fig. 1. Little experimental detail was provided, particularly with regard to the number of times the behaviours were analysed. Having observed hundreds of *N. vectensis* from several English localities, I have never seen anything remotely similar to the "wind-up and cast" behaviour. Lindsay's (1975) other description of head-first creeping on the mud surface has some similarity to intrasubstrate burrowing but, from results reported herein, tail-first progression would have been expected for creeping.

Published photographs of laboratory-reared *N. vectensis* provide an opportunity to assess the predictive value of the wave parameters established here (tables 1 and 2) from wild-caught anemones. The theoretical maximum number of simultaneous waves possible in anemones about 10 mm long was three or four in the present experiments (table 2). Individuals >16 cm long may be grown in laboratory cultures (Hand & Uhlinger, 1992). Available photographs show anemones up to 29 mm long (Hand & Uhlinger, 1992, fig. 1), up to 24 mm long (Hand & Uhlinger, 1994, fig. 1), up to 47 mm long (Hand & Uhlinger, 1995, fig. 4) and up to 13 cm long (Fritzenwanker & Technau, 2002, fig. 3a). It is admittedly difficult to tell whether the various narrowings on the columns of most of the photographed anemones are moving peristaltic waves or stationary constrictions. However, those shown in fig. 1 of Hand & Uhlinger (1994)

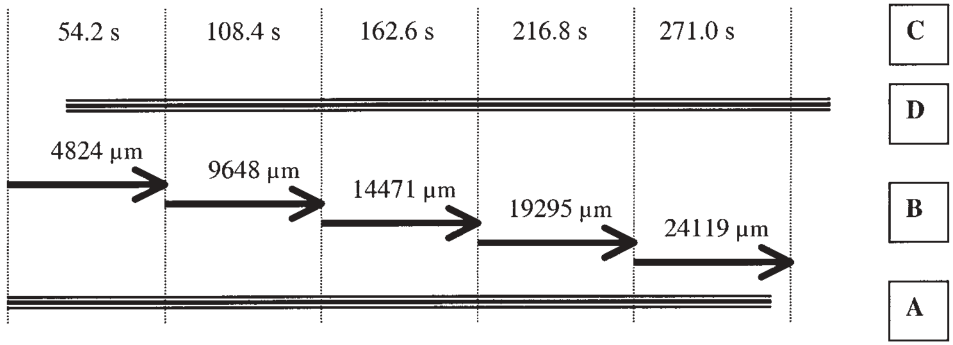

Fig. 14. Calculation of the maximum number of peristaltic waves able to pass simultaneously down the column of an individual of *N. vectensis*. "A" represents the 24 mm (= 24,000 μm) column length of an anemone. "B" represents the distances travelled along the column by successive waves during constant wave initiation intervals. "C" shows the cumulative time taken, in the example of "A", for five waves to be initiated. Because the column length is just shorter than the distance that can be travelled by a wave in the time taken for another four following waves to be initiated, the number actually passing immediately before a new wave is initiated is four (see "A"). However, at almost any other time between wave initiations, there are five waves passing simultaneously in the same anemone (example "D"). For this reason, the theoretical maximum number of waves able to pass simultaneously is expressed as 4/5 in this particular anemone.

appear to be true peristaltic waves; a 24 mm long individual apparently has four passing simultaneously. Parameters derived from the present results were therefore used to calculate the theoretical maximum for an individual of that size. Using a mean peristaltic wave initiation interval of 54.2 s, which is fairly constant regardless of anemone size (table 2), and a wave speed of 89 µm s^{-1} cautiously extrapolated from fig. 3, a theoretical maximum of four or five waves was calculated. Fig. 14 demonstrates how this was calculated, and explains why the maximum number may differ according to the phase of the wave initiation. In this case, the actual and predicted maximum numbers of simultaneous waves are in accord.

The question remains whether there may be some ergonomic limitation to burrowing capability in athenarian anemones over a certain size. For instance, Panikkar (1936) noted that only small individuals of *Phytocoeteopsis ramunnii* could burrow, and that adults up to 20 cm long were practically helpless when extracted from the mud and could not repenetrate it. It is not known whether the unnaturally large specimens of *N. vectensis* bred in the laboratory by Hand & Uhlinger (1992) and Fritzenwanker & Technau (2002) are able to burrow.

Functional morphology of *N. vectensis*

The locomotory behaviour of *N. vectensis* comprises burrowing of two types (penetrative and intrasubstrate), creeping and climbing. The animal depends for locomotion on its musculature working against the hydrostatic skeleton, used in conjunction with the adherent rugae on the column. Being a small animal with a relatively weak musculature, burrowing is limited to very fine, thixotropic muds. The major muscle system employed is the endodermal circular sheet of the column, which produces direct peristaltic waves from actinopharynx to physa during penetrative burrowing, creeping and climbing. The longitudinal retractor muscles do not seem to be involved in penetrative burrowing. The parietal muscles probably help to bring the anemone upright once burrowing is well under way, but are otherwise not involved.

The mucus-secreting rugae are essential for the establishment of *points d'appui* during burrowing and creeping. Flexion of the physa into the mud during penetrative burrowing results from adhesion of the rugae during the passage of a peristaltic wave, rather than from geotaxis or any tactile stimuli. Cilia play no part in tail-first penetrative burrowing, but those on the oral disk and tentacles are important for increasing the fluidity of thixotropic mud during head-first intrasubstrate burrowing.

A polarized nerve pacemaker system probably initiates peristaltic waves continually in the circular muscle sheet of the column, as long as the anemone remains unburied. The location of such a pacemaker is not known. However, it appears to be diffuse because, as indicated by the generation of peristaltic waves, it is active not only in whole anemones, but also in newly detached physae resulting from asexual reproduction (transverse fission). When penetrative burrowing is completed, and an anemone is ensconced in its new burrow, with its column in contact with the substrate over its whole surface (the tentacles remaining exposed), peristalsis ceases, so the pacemaker would be inhibited. However, if an anemone becomes accidentally buried, head-first intrasubstrate burrowing to regain the mud surface commences, which is possibly a negative geotaxis, triggered by tactile stimulation of the tentacles that also apparently reverses the polarity of the nerve pacemaker system. It is not known how polarity is controlled in "double anemones" and other anomalous individuals with multiple tentacle crowns (Williams, 1975b; Hand & Uhlinger, 1995); certainly they cannot burrow.

Comparative anatomy and behaviour of athenarian anemones

Pantin (1955) suggested that comparative analysis of the functional morphologies of animals to construct an anatomical classification might shed light on "the question of classification and the sequence of forms presented by evolution." Accordingly, an attempt is made here to correlate morphology with behaviour, to investigate whether Pantin's (1955) concept might be instructive when applied to athenarian sea anemones. Table 13 gives some examples of the great variety of types of musculature already described from athenarian anemones. To avoid confusion, the scientific names used in the original publications have been retained, although some may not currently be regarded as valid. The family Ilyanthidae (see Carlgren, 1949) is now known as Haloclavidae (see Manuel, 1988). The few published descriptions of locomotion in four athenarian families are now summarized and compared.

Family Haloclavidae

Most of the detailed reports of the burrowing behaviour of athenarian anemones are concerned with the haloclavid *Peachia hastata* (Holdsworth, 1859; Gosse, 1860; Faurot, 1895; Ansell & Trueman, 1968). This anemone is a robust species (table 13), up to 25 cm long, that can generate an internal hydrostatic pressure of 50 mm of water during tail-first burrowing and penetrates sand quite easily in about 1.25 h (Ansell & Trueman, 1968). Holdsworth (1859) shrewdly remarked that "It is probably by this hydrostatic pressure that all burrowing soft-bodied animals having a water-circulation are enabled to work their way into the ground."

During burrowing, direct peristaltic muscular waves are initiated in the upper scapus, passing down the column one at a time. The powerful retractor muscles also play a major part in pulling the animal down into the sand, once a firm physal anchor is established (Ansell & Trueman, 1968). It was also suggested that ciliary action was involved in moving a collar of sand up the column during burrowing (Ansell & Trueman, 1968), but no experimental evidence was provided.

Elmhirst (1915) used powdered carmine to study ciliary currents in *P. hastata* and observed that "There is no ciliary current noticeable on the column, and only a very faint incurrent between the tentacles." Other relevant observations made by Haddon & Dixon (1885) on *P. hastata* were that "Sometimes when it projects perpendicularly out of the hole it encircles itself with a collar about one inch [2.54 cm] in depth of slime set thickly with particles of sand." And when kept without sand in which to burrow, "They generally lie at the bottom of the tank, surrounded more or less with collars of slime interwoven with the confervoid growth that gathers in an aquarium; sometimes they hold themselves erect."

Faurot (1895) observed that a peristaltic wave of *P. hastata* was initiated every five or six seconds, which is much more frequently than the one every 1.5 minutes recorded by Ansell & Trueman (1968). Unfortunately, the ambient temperature was not stated in either case, and the original habitat and laboratory maintenance conditions were

Table 13. Comparison of retractor and parietal muscles of *N. vectensis* with those of some other athenarian anemones. Only the numbers of major muscle folds are given; some or all of them may be ramified.

Family	Species	Retractor folds	Parietal folds	Authority
Edwardsiidae	*Nematostella vectensis*	9-14	4	Stephenson (1935, Fig. 48; p. 50)
		8	4	Crowell (1946, Fig. 3)
		6-12+	Not recorded	Hand (1957, p. 411)
		Up to 15	2-4	Present paper
Edwardsiidae	*N. polaris*	12-15	6?	Carlgren (1921, Figs 80, 81; pp. 65, 67)
Edwardsiidae	*N. nathorstii*	10	4 (plus on column mesogloea)	Carlgren (1921, Figs 84, 85; pp. 67-69)
Edwardsiidae	*Edwardsia callimorpha*	8-19	25	Stephenson (1928, Figs 15, 21)
	(= *E. claparedii*)	16-17	21-26	Stephenson (1935, Figs 49, 50)
Edwardsiidae	*E. danica*	12-14	8 (plus on column mesogloea)	Carlgren (1921, Figs 30-32)
Edwardsiidae	*E. delapiae*	20-30	15-20	Stephenson (1935, Figs 49, 51; p. 65)
Edwardsiidae	*E. ivelli*	17	4-5	Manuel (1975, Figs 3, 4; p. 708)
Edwardsiidae	*E. longicornis*	13-20	11-22 (plus on column mesogloea)	Carlgren (1921, Figs 20-24; pp. 33-35)
Edwardsiidae	*E. meridionalis*	3-8	6-10 (plus on column mesogloea)	Williams (1981, Figs 4-7; p. 332)
Edwardsiidae	*E. tecta*	10-12	12	Stephenson (1935, p. 67)
Edwardsiidae	*E. callianthus* (= *E. timida*)	25-30	5-7	Rawlinson (1935, Figs 6, 7, 8; p. 141)
		25-30	"few processes"	Stephenson (1935, p. 403)
Edwardsiidae	*Milne-Edwardsia carnea*	12-20	"very strong"	Stephenson (1935, p. 38)
Edwardsiidae	*M. dixonii*	12-18	Not recorded	Stephenson (1935, p. 43)
Halcampactiidae	*Phytocoeteopsis ramunnii*	"strong, highly ramified"	14	Panikkar (1936, Fig. 6; p. 241)
Halcampidae	*Halcampa chrysanthellum*	10-15	Not recorded	Stephenson (1935, p. 74)
Halcampoididae	*Halcampoides abyssorum*	"very strong"	"strong"	Riemann-Zürneck (1993, p. 34)
Haloclavidae	*Ilyanthus mitchellii*	76	53	Stephenson (1935, Fig. 58)
Haloclavidae	*Eloactis mazeli*	30	Not recorded	Stephenson (1935, p. 94)
Haloclavidae	*Peachia hastata*	ca 80	ca 20	Stephenson (1928, Plate XIV)

considerably different. Despite this difference in the rate of peristaltic wave initiation, Faurot (1895) recorded that *P. hastata* took about 1 h to burrow successfully, a similar period to that of 1.25 h observed by Ansell & Trueman (1968).

Family Halcampoididae

The burrowing behaviour of an unidentified *Halcampoides* species (up to 7 cm long) from Signy Island (Antarctica) has been studied by Ansell & Peck (2000). Unfortunately, no anatomical description is available, but the related *H. abyssorum* (table 13) has a very strong musculature (Riemann-Zürneck, 1993), which may be similar to that of the unidentified species. *Halcampoides* sp. burrows very slowly (up to 17 h or more at 0°C) tail-first in medium to fine sand of grain size 200-300 µm (Ansell & Peck, 2000).

It exhibits two types of direct peristalsis, the first involving only the physa during the initial phase of burrowing, and the second being generated in the upper scapus and being co-ordinated with retractor muscle activity. At first, peristaltic waves pass one at a time, but later, more than one wave may be seen simultaneously on the column. Similarly to *P. hastata*, penetration is aided by periodic contraction of the longitudinal retractor muscles. Ansell & Peck (2000) also considered that ciliary action and mucus secretion were involved in moving sand up the column during burrowing, but presented no experimental evidence for such a mechanism. They concluded that *Halcampoides* sp. and *P. hastata* burrow in closely similar ways, except that the rates of initiation of peristaltic waves and retractor muscle contractions are very different. This they reconciled by calculating the temperature coefficient, Q_{10}, for the Antarctic *Halcampoides* sp. at 0°C and the temperate *P. hastata* at 11-14°C, which was reasonably close to the expected value of 2.

Family Halcampidae

Halcampa chrysanthellum has relatively weak retractors (table 13), but can burrow tail-first into, and also creep over, mud or muddy sand (Faurot, 1895). Its locomotion is perhaps most similar to that of *N. vectensis* (family Edwardsiidae), facilitated by complementary peristaltic waves and adhesion of the column. However, unlike *N. vectensis*, it has two types of adhesive structure, rugae and tenaculi. Tenaculi are rather more complex than rugae (Stephenson, 1928), and are somewhat larger, those of *H. chrysanthellum* being, according to Gosse (1860), about 0.002 inch [51 µm] in diameter (*cf*. the rugae of 3-7 µm in *N. vectensis*).

Faurot (1895) observed that *H. chrysanthellum* can creep faster over a substrate than *P. hastata* can, despite the great difference in size (*H. chrysanthellum* is up to only 8 cm long). He was unable to discern the precise method of substrate penetration by *H. chrysanthellum*, but noted that it took up to 3 h to burrow (*cf*. usually up to *ca* 3 h herein for *N. vectensis*). Interestingly, whilst *H. chrysanthellum* burrows in finer muddy sand, its congener *H. duodecimcirrata* burrows in coarse sand or shell-gravel (Nyholm, 1949; Ellehauge, 1978). Apart from the fact that penetrative burrowing is tail-first also in *H. duodecimcirrata*, no detail was recorded.

Family Edwardsiidae

N. vectensis is a small (up to 2 cm long) anemone with a much less robust anatomy and a weaker musculature than either *P. hastata* or *Halcampoides* sp., but is very similar in these respects to *H. chrysanthellum* (table 13). It can burrow effectively only in thixotropic mud. The co-ordinated peristaltic waves lead to creeping or climbing if a solid substrate such as underwater vegetation is encountered. This could not be achieved by heavier-bodied types like *P. hastata*, which, however, creeps relatively ineffectively over the sand surface before it begins to burrow (Holdsworth, 1859; Faurot, 1895).

Although, during penetrative burrowing, *N. vectensis* normally uses the physa, like the representatives of the families Haloclavidae, Halcampoididae and Halcampidae already discussed, other edwardsiids of sizes fairly similar to that of *N. vectensis* may burrow in quite different ways, whether in mud, sand or gravel. For instance, *Edwardsia ivelli* (up to about 2 cm long) has a musculature similar to that of *N. vectensis* (table 13) and also lives in the fine mud of poikilohaline lagoons, but penetrative burrowing is achieved head-first by alternate introversion and eversion of the scapulus, only occasionally involving the tentacles (Manuel, 1975). Burrowing is rapid, and an individual may bury itself in one minute or less. During intrasubstrate burrowing, long horizontal galleries are constructed, from which vertical excursions are made to the mud surface where the tentacles are then expanded (Manuel, 1975).

The much larger (up to 18 cm) edwardsiid *Scolanthus callimorphus* also progresses head-first during both penetrative and intrasubstrate burrowing, at a rate from about 1 inch [2.54 cm] per hour (Gosse, 1853, 1860) to at least 15 cm per hour (Manuel, 1981). Unfortunately, neither Schmidt (1979, as *Alfredus lucifugus*) nor Manuel (1981) described the musculature of *S. callimorphus* in their taxonomic studies of this species. *S. callimorphus* is exceptional in using the head-first method of burrowing in sand or fine gravel, probably because its physa is covered with a periderm that makes it useless as a digging organ since it cannot adhere to particulate matter (Manuel, 1981).

Edwardsia claparedii (up to 13 cm long) can burrow in a much wider range of substrates than most species of edwardsiid, and exhibits two types of penetrative burrowing, depending on the substrate. When in glutinous mud, it burrows head-first, with the tentacles withdrawn, which may be a way of maintaining the high internal pressure necessary for digging into that kind of substrate (D.N. Huxtable in Shelton, 1982). In comparison, *N. vectensis* uses the tentacles during head-first intrasubstrate burrowing, but its normal habitat is thixotropic, not glutinous, mud. Manuel (1981) confirmed that *E. claparedii* burrows head-first in mud but pointed out that when in sand or gravel it burrows tail-first with the physa. It has a fairly strong musculature (table 13).

A number of other *Edwardsia* species, viz., *E. danica*, *E. longicornis*, *E. meridionalis* and *E. timida*, all of similar sizes, are known to burrow tail-first in sand and gravel, but no unusual aspects of this behaviour have been recorded (Ellehauge, 1978; Williams, 1981; Manuel, 1981).

In contrast with other family members, *Milne-Edwardsia carnea* is a small (up to 4 cm long) aberrant edwardsiid that does not burrow, but lives in tiny holes and cracks in rocks. Despite its small size, it has a relatively strong musculature (table 13). Its young can creep over hard surfaces using rugae and peristaltic waves (Gosse, 1860, as *Halcampa microps*; Stephenson, 1935), but it is not clear whether the waves are direct or retrograde.

Comparative analysis of functional morphology

Faurot (1895) was apparently the first to make comparisons between burrowing behaviours of anemones, which he also described anatomically. He observed that *P.*

hastata and *H. chrysanthellum* both employed direct peristaltic waves in locomotion. (The statement by Clark (1964), based on Faurot's paper, that those species progress oral end first, so that the waves would be retrograde in both species, appears to result from an error in translation.)

Manuel (1981) discussed the efficiencies of various methods of locomotion employed by athenarian anemones in relation to the substrates in which they burrow. Citing the use of the physa by *E. claparedii*, *E. timida*, *H. chrysanthellum* and *P. hastata* for penetrative burrowing in sand or gravel, and noting that *E. ivelli* burrows head-first into mud, he concluded that tail-first burrowing is less effective in mud than in coarser substrates. Furthermore, *E. claparedii* is unusual in having two alternative burrowing methods; the fact that it burrows head-first in mud but tail-first in gravel might be regarded as supporting evidence. However, such a general hypothesis is rendered untenable by the fact that *N. vectensis* uses the physa to burrow into thixotropic mud, and cannot penetrate coarse sand or gravel at all. It should also be recalled that *S. callianthus* employs head-first burrowing in gravel (Gosse, 1853), which also militates against the hypothesis. It is now known that *N. vectensis*, like *E. claparedii*, also exhibits two methods of burrowing, but only in mud, progressing tail-first during penetrative burrowing and head-first during intrasubstrate burrowing.

Following Pantin's (1955) advocacy of comparative functional morphology, the available data have been tabulated here (table 14). What conclusions may be drawn from them? First considering the eight edwardsiids, six species employ the tail-first mode of penetrative burrowing, but the range of substrates includes the finest mud to gravel (table 14). Furthermore, the strength of the musculature of these species (table 13) appears to bear no consistent relationship to the substrate in which they dwell, although they are all about the same size (table 14). Moreover, two of them, *N. vectensis* and *E. claparedii*, employ a head-first burrowing mode as well as tail-first, depending on the substrate or other circumstances (table 14). The musculature of *S. callimorphus* has not been described, but it is a much larger edwardsiid than the others, and burrows head-first in gravel (table 14); because of its periderm-covered physa, it probably cannot do otherwise (Manuel, 1981). However, *E. ivelli*, which is similar in size, musculature and habitat to *N. vectensis*, and so different in these respects from *S. callimorphus* (tables 13 and 14), burrows in the same way as *S. callimorphus*, rather than like *N. vectensis* (table 14).

The representatives considered of the families Halcampidae, Halcampoididae and Haloclavidae all employ tail-first penetrative burrowing in sand or gravel (table 14). Yet, despite their similar locomotory behaviours and habitats, they are quite different in size (table 14) and musculature (table 13). Comparing them with the Edwardsiidae, there are again some matches and some mismatches in all aspects (tables 13 and 14). The only broad conclusion possible is that the major behavioural difference between anemones that generally burrow head-first and those that burrow tail-first is that the former appear to be able to burrow much faster. However, this conclusion is based only upon the limited knowledge of burrowing speeds of only two habitual head-first burrowers, *S. callimorphus* and *E. ivelli*, no relevant data being available about the burrowing speed of *E. claparedii* (see Shelton, 1982). In this regard, the head-first intrasubstrate burrowing of *N. vectensis* is not relevant because that is not a habitual burrowing behaviour; nevertheless, it seems significant that it is the behaviour used as an escape response to burial. Hence, head-first burrowing may be more efficient than

Table 14. Comparison of sizes, typical substrates and burrowing behaviours of athenarian anemones of various families (mps = median particle size in µm).

Family	Species	Column length	Typical substrate	Mode of burrowing	Data sources
Edwardsiidae	*Nematostella vectensis*	2 cm	Mud (mps = 30)	Tail-first (penetrative) Head-first (intrasubstrate)	Present paper
Edwardsiidae	*Scolanthus callimorphus*	18 cm	Gravel	Head-first (penetrative and intrasubstrate)	Gosse, 1853; Manuel, 1981
Edwardsiidae	*Edwardsia claparedii*	13 cm	Glutinous mud	Head-first (penetrative and intrasubstrate)	Manuel, 1981; Manuel in Shelton, 1982
			Sand/gravel	Tail-first (penetrative)	
Edwardsiidae	*E. danica*	4 cm	Sand (mps = 70-180)	Tail-first (penetrative)	Ellehauge, 1978
Edwardsiidae	*E. ivelli*	2 cm	Fine mud (15% organic content)	Head-first (penetrative and intrasubstrate)	Manuel, 1975
Edwardsiidae	*E. longicornis*	4 cm	Sand (mps = 70-180)	Tail-first (penetrative)	Ellehauge, 1978
Edwardsiidae	*E. meridionalis*	4 cm	Sand/gravel (mps = 170-570)	Tail-first (penetrative)	Williams, 1981 and unpublished
Edwardsiidae	*E. timida*	6 cm	Sand/gravel	Tail-first (penetrative)	Manuel, 1981
Halcampidae	*Halcampa chrysanthellum*	8 cm	Mud/muddy sand	Tail-first (penetrative)	Faurot, 1895; Manuel, 1981
Halcampidae	*H. duodecimcirrata*	3 cm	Sand (mps = 70-180) or shell gravel	Tail-first (penetrative)	Nyholm, 1949; Ellehauge, 1978
Halcampoididae	*Halcampoides* sp.	7 cm	Sand (mps = 250)	Tail-first (penetrative)	Ansell & Peck, 2000
Haloclavidae	*Peachia hastata*	25 cm	Sand	Tail-first (penetrative)	Faurot, 1895; Ansell & Trueman, 1968

tail-first burrowing in any substrate, although there is no obvious correlation with the anatomy of the species concerned.

Concluding remarks

Shelton's (1982) speculation, that burrowing in different anthozoans may be achieved by different mechanisms, has proved to be true. Indeed, there are sometimes different burrowing behaviours in the same species, depending on the environmental conditions. It is perhaps not surprising that burrowing behaviour in anemones is achieved in so many different ways, since it has already been shown that during the shell-climbing behaviour of some thenarian anemones, various species may assume different characteristic postures (Ross, 1974), and it has also been demonstrated that different neuronal conduction systems are involved in the co-ordination of such behaviour in *Stomphia coccinea* (Lawn & McFarlane, 1976) and *Calliactis parasitica* (McFarlane, 1976). Similarly, behavioural differences between swimming anemones are so remarkably constant that Ross (1979) has suggested that behaviour patterns might be valuable in systematic descriptions. Manuel (1981) found this approach valuable in his taxonomic study of *S. callimorphus*.

Nevertheless, it must be concluded not only that burrowing behaviour in athenarian anemones is extremely diverse, but that there is no consistent association between modes of burrowing, types of substrate, musculature and familial relationships (tables 13 and 14). Pantin's (1955) suggested application of the study of functional morphology has therefore turned out to be unenlightening in the present case, but that is not to say that descriptions of the locomotion of yet more anemones would not lead to a more fruitful conclusion in the future.

Most published descriptions of locomotion pertain to the Edwardsiidae and the variability in behaviour, even within this family, is considerable (table 14). A recent attempt to explain the evolution of actiniarian body plans has shown that the anatomy of edwardsiids is a mosaic of retained primitive and derived features (Daly et al., 2002), which although consistent with, does not explain, their behavioural inconsistencies. The Edwardsiidae are but one of at least nine families of athenarian anemones (Carlgren, 1949). Furthermore, there are only three examples of detailed behavioural studies within the remaining eight athenarian families, and each example is from a different family, viz., the Halcampidae, Halcampoididae and Haloclavidae (Faurot, 1895; Ansell & Peck, 2000; Ansell & Trueman, 1968). Hence, what the currently available information on burrowing might tell us about adaptive evolution in athenarian sea anemones is still far from clear.

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