OFFSHORE FOULING COMMUNITIES AND SETTLEMENT AND EARLY GROWTH IN *Tubularia larynx* (ELLIS AND SOLANDER) AND *Pomatoceros triqueter* (L.).

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DECLARATION

This work has not been accepted, and is not concurrently being submitted for any other degree, and is a record of the work carried out by the candidate himself.

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ABSTRACT

Offshore Fouling Communities and Settlement and Early Growth in *Tubularia larynx* (Ellis and Solander) and *Pomatoceros triqueter (L)*.

The horizontal and vertical distribution of the macro-fouling organisms, both flora and fauna, associated with offshore structures was studied. Offshore buoys located around the coasts of Britain (38) and Norway (10), were used as sites for the collection of material. Sites were visited annually, when possible, over the period 1978-1983.

Numerical clustering techniques and indicator species analysis were used to reveal broad geographical categories in the fouling community data. Eight major Site Groups were identified amongst U.K. sites, and three from Norway; whilst within each site group characteristic species were also identified.

The frequency and abundance of *Tubularia larynx* and *Pomatoceros triqueter* at both the British and Norwegian sites indicated that each species was a 'successful' member of the fouling communities examined, and able to rapidly colonize newly immersed hard substrata. Light microscopy, histochemical techniques and electron microscopy were used to investigate the mechanisms of substrate attachment and settlement in both species. It is postulated that the secretion of an acidic 'adhesive' mucosubstance prior to the secretion of the calcareous tube in *Pomatoceros* may contribute to the successful colonization of hard surfaces; whilst a rapid chitin tanning process was identified during 'growth' of the aboral pole of the settling actinula larva in *Tubularia*, as well as in the subsequent vegetative growth of 'primary' stolon tips. In addition, a gregarious settlement-response was identified in *Pomatoceros* larvae.

Several growth experiments were performed on each animal and a multifactorial approach was used to investigate the conditions for early growth in each organism. Both species were capable of rapid growth immediately following settlement. 'Tubegrowth' was recorded in *Pomatoceros* under a wide range of temperature and salinity conditions; being greatest amongst animals maintained in combinations of high temperature (15-20°C) and high salinity (26-34°/oo), whilst the secretion of new tube was much reduced at 6°C and 10°/oo. In *Tubularia*, although growth was rapid between 6°C and 18°C., the hydroid was more strictly marine and both stolonic growth and hydranth production were restricted in salinities below 28°/oo.

CHAPTER 1

Introduction

The present study is firstly a quantitative analysis of the offshore algal and sessile marine invertebrate communities of Britain and Norway; together with a detailed investigation of the mechanism of attachment and settlement, and the conditions for early growth, in two of the most common species, the hydroid *Tubularia larynx* (Ellis and Solander) and the serpulid *Pomatoceros triqueter L.* The organisms constituting these communities inhabit hard substrata, often forming dense assemblages of mixed species composition.

As fouling organisms they also colonize submerged parts of ships, harbour installations and other man-made structures (Woods Hole Oceanographic Institute, 1952). Since the 1920's studies in various parts of the world have been directed at these communities, or at major component species, in order to control or prevent fouling of materials (Visscher, 1927). Information on the general extent of fouling was obtained by inspecting the epifauna of the hulls of ships, and from floating structures such as pontoons, and more recently, raft-borne panel arrays and buoys (Woods Hole Oceanographic Institute, 1952). Such studies yield data on settlement, seasonal abundance and growth of common fouling species, which form the base line for testing the performance of antifoulants (Kuhl, 1957; Stubbings and Houghton, 1964).

Fouling community development has been widely documented (Scheer, 1945; Aleem, 1957; Ito, 1959; Kawahara, 1961; Haderlie, 1969 and 1974) and the relationships between the physical environment and species distribution and fluctuations in abundance over time have commonly been investigated (e.g. Woods Hole Oceanographic Institute, 1952). More recently, the importance of biological inter-relationships, such as

predation and competition for attachment space has been reported and new considerations regarding the dynamics of epifaunal communities were put forward (Sutherland, 1974, 1977, 1978; Standing, 1976; Connell and Slayter, 1977; Jackson, 1977a, 1979; Osman, 1977; Sutherland and Karlson, 1977; Anger, 1978; Dean and Hurd, 1980; Schmidt, 1983a; Hughes, 1984).

The fouling communities of offshore structures have been less intensively studied. Of particular interest at the present time are the effects of marine growths on the jackets of offshore oil and gas production platforms (Houghton, 1978). Fouling of these structures increases the fluid loading, impedes inspection and maintenance and may accelerate corrosion (Freeman, 1977; Ralph and Goodman, 1979; Leitch, 1980; Oldfield, 1980; Ralph and Troake, 1980; Hardy, 1981; Ralph, Goodman and Picken, 1981).

Permanent offshore structures for oil and natural gas production are an expanding feature of the coastal marine environments of the world. Algal spores and invertebrate larvae rapidly colonize submerged portions of platform structures, establishing a fouling assemblage (Houghton, 1978). The seasonal vertical distribution patterns of such assemblages or communities associated with offshore platforms have been described for the northern Gulf of Mexico (Gunter and Geyer, 1955; Pequenat and Pequenat, 1968; Gallaway, Howard, Martin and Johnson, 1978; Heideman and George, 1981) and for Californian waters (Wolfson, Van Blaricom, Davis and Lewbal, 1979). The fouling communities present on offshore platforms in the North Sea have been described in a number of studies, including Freeman (1977), Ralph and Goodman, (1979); Ralph, Goodman and Boyle (1979); Leitch (1980); Oldfield (1980), Ralph and Troake, (1980), Hardy (1981) and Goodman and Ralph (1981). The type of community

occurring was found to be dependant upon a range of variable factors, (Freeman, 1977; Oldfield, 1980; Kingsbury, 1981). Oldfield (1980) suggested that the single most significant factor affecting the fouling community on offshore structures, was that of depth. He reported that with increasing depth, the resultant reductions in temperature, light penetration and food availability, all served to depress the metabolism and rate of growth of both the algal and faunal components of the fouling community. In addition, water currents, seasonal changes, direct temperature effects, and distance from the shore were other important factors which determined both the development and type of fouling community found (Freeman, 1977; Oldfield, 1980; Kingsbury, 1981).

According to Oldfield (1980) and Kingsbury (1981) when the speed of water travelling across a surface exceeded 0.5 m/sec., many larvae were unable to attach themselves. Earlier, Dooch and Smith (1951) and Wood (1955) had reported differences in preferred water velocities amongst fouling organisms. Algal spores, for example, were found to be capable of settling even when the water flow was "considerably greater than 2 m/sec.", (Houghton, Pierman and Tierney, 1972). In the case of fixed structures, Oldfield (1980) and Kingsbury (1981), stressed the importance of periods of slack water, during which larvae have the opportunity to settle and attach.

Seasonal variations in the species composition of fouling communities has been the subject of a number of investigations (Graham and Gay, 1945; Mook, 1976, 1980; Iwaki, 1977; Menon, Katti and Shetty, 1977; Osman, 1978; Bastida De Mandri, De Bastida and Stupak, 1980; Ehler and Lyke, 1980). A direct result of temperature and length of day, seasonal changes were most obvious amongst the flora and fauna of surface waters. In British waters, Houghton (1978) reported that most

fouling species tended to grow rapidly during the period from April to the end of October. During this time also, maximum rates of spawning and re-colonization were observed.

Temperature was found to directly affect both the growth rate of the community as a whole, and that of its component members (Oldfield, 1980; Kingsbury, 1981). A rise in temperature usually increased the rate of growth. Oldfield (1980) estimated that the overall growth rate of a fouling community doubled through every $10^{o}C$ rise in temperature, but stated that nearly all fouling organisms have a lethal limit of between $30^{o}C$ and $35^{o}C$.

Most of the fouling species constituting the communities found on offshore platforms in the North Sea, originated from shore and sub-littoral populations (Oldfield, 1980; Kingsbury, 1981). Liberated spores and larvae depended upon currents for distribution. With increasing distance from the shore, the greater was the duration of the pelagic phase, with consequent reduction in availability of spores and larvae due to mortalities and dispersal. Oldfield (1980) suggested that as a result, surfaces beyond the 40 m depth contour were not rapidly colonized by coastal organisms. However, species whose spores or larvae were capable of immediate attachment might continue to recolonize the same structure. In addition, settlement from bottom living organisms, if present, may be another source contributing to the community (Houghton, 1978).

Aleem, (1957) reported differences in both quantity and diversity between fouling communities with depth, as well as with distance from the shore. His findings, later confirmed by the observations of Oldfield (1980) and Kingsbury (1981), indicated the importance of light variations in limiting the growth of many fouling organisms. The

influence of light on seasonal and vertical distribution patterns in fouling communities was further shown by Zevina (1972). In a study of fouling growths at buoy sites in the Caspian Sea, he found that those sites located in turbid waters supported a much smaller biomass of algae than did those buoys situated in more exposed areas. Zevina (1972) also described a characteristic zonality of the fouling communities on buoys, which he attributed principally to light conditions. He distinguished three zones whose width varied depending upon the conditions of light penetration.

1. The illuminated zone, where growth consisted mainly of algae.

2. The zone where growth consisted mainly of animal species; characteristically, bivalve molluscs, hydroids, bryozoa, barnacles and annelids.

3. The sea bottom zone, where growth was restricted mainly to a few specialised animal groups.

According to Zevina (1972) animals were the predominant component of the fouling growth in poorly illuminated regions, whilst algal species were mainly represented in well illuminated places.

The influence of the translucence of water on the depth of penetration of algae had been previously recorded by Milne (1940). He found that the algal zone growing on buoys at the mouth of the Tamar estuary (England), became narrower compared to the algal zones observed on buoys in more open sites. He attributed this to the conditions of higher turbidity found at the mouth of the estuary, restricting light penetration in that area.

The dependance of algae on light for photosynthesis determines that they occupy mainly surface zones. Light intensity also effects the activities of many difference animal groups (Klugh and Newcombe, 1935;

McDougall, 1943; Barnes, 1952; and see Kinne, 1971c). There is now considerable literature regarding the responses to light of marine invertebrate larvae. In a comprehensive review, Thorson (1964) found that most species, including many intertidal species became photo-negative during metamorphosis and settlement, and preferred shaded areas and dark surfaces for attachment. More recently, a number of experimental studies of the effect of light on the larvae of common fouling groups have been carried out. These include observations by Wisely (1959), Crisp and Ghobashy (1969), Forbes, Seward and Crisp (1969); Crisp and Ritz (1978).

Naturally occurring fouling in British waters has been documented from ships (Bengough and Shepheard, 1943; Bishop, Pyefinch and Spooner, 1949; Pyefinch, 1950); from fixed structures such as pilings (Corlett, 1948) and oil and gas production platforms (Freeman, 1977; Eikers, 1978; Goodman and Ralph, 1979, 1981; Ralph and Goodman, 1979; Ralph, Goodman and Boyl 1979; Leitch, 1980; Oldfield, 1980; Pipe, 1980; Ralph and Troake, 1980; Hardy, 1981; Kingsbury, 1981; Moss, 1981; Moss, Tovey and Court, 1981) and from floating structures such as marinas (Fletcher, 1980a, 1980b), and buoys (Grieve and Robertson, 1864; Knight and Parke, 1931; Fraser, 1938; Milne, 1940; Tittley and Price, 1977). Many of these studies included references to the importance of algal growths in the fouling community (Milne, 1940; Hardy, 1981). Of the animal organisms reported, hydroids, annelids, barnacles, molluscs and bryozoa were identified as important fouling groups; and the widespread occurrence and often high levels of abundance of common sedentary species such as *Tubularia larynx, Pomatoceros triqueter, Balanus crenatus* and *Mytilus edulis* noted.

In a study of fouling communities around the north coast of Britain, Meadows (1969) found that at a given site the relative abundance of species was predictable from year to year, although the absolute abundance was variable. He also reported that often only two to three species contributed a large proportion of the total abundance at any one site; a feature of fouling communities recorded from studies throughout the world (Scheer, 1945; Woods Hole Oceanographic Institute, 1952; Huve, 1953; Hedgepeth, 1957; Kawahara, 1969; Braiko, 1974; Tsuchiya, 1979). In addition, Meadows (1969) described a number of species (including *Pomatoceros triqueter* and *Tubularia larynx*), which were part of a "pool of potentially important fouling organisms, common to a widespread geographical area covering the northern coasts of Britain. He postulated that local conditions would then select from this 'pool' a few species that were best suited to the environment. These considerations might then produce distinctive communities of fouling organisms that might characterize or distinguish different sites and water bodies. Meadow's (1969) conclusions may be equally applicable to offshore fouling communities, where the levels of growth and type of fouling were variable, and a number of commonly occurring species analogous to Meadow's (1969) 'pool' species were recorded (Freeman, 1977; Oldfield, 1980; Ralph and Troake, 1980; Goodman and Ralph, 1981; Hardy, 1981; Kingsbury, 1981).

A number of investigators have studied the species composition and structure of fouling communities (Woods Hole Oceanographic Institute, 1952; Osman, 1977; Ehrler and Lyke, 1980; Mook, 1980; Schoener and Green, 1980; Dean, 1981; Moran, 1981; Kay and Butler, 1983). Most studies of this kind include statistical analyses of species composition and abundance. Methods of analysis are widely based upon

accepted techniques employed in vegetation ecology (Bray and Curtis, 1957; Boudouresque, 1971; Bunce and Shaw, 1971; Sneath and Sokal, 1973; Frenkel and Harrison, 1974; Mueller-Dombois and Ellenberg, 1974; Campbell, 1978). Numerical methods of classifying and sorting species groups in marine ecology, including epibentic communities, have been commonly used to aid the interpretation of data in the analysis of distribution patterns (Field and McFarlane, 1968; Field, 1970; Lie and Kelly, 1970; Day, Field and Montgomery, 1971; Hughes and Thomas, 1971; Velimirov, Field, Griffiths and Zoutenkyk, 1979; Hooper, South and Whittick, 1980). Field, Clarke and Warwick (1982) reviewed many of the numerical techniques that have been used, and put forward a strategy of statistical and numerical classification techniques "particularly well suited to the analysis of marine biological survey data."

Many studies of offshore fouling communities carried out in various seas around the world, have utilised buoy stations as sampling sites (Woods Hole Oceanographic Institute, 1952; Zevina, 1972; Gorin, 1975; Merrill, 1975; Il'in, 1976, 1978; Brykov, Levin, Orysannikova and Selin, 1981; Huang, Cai, Jiang, Cai and Wu, 1982), for British waters records of offshore fouling are made up principally of reports of the marine growths found on oil and gas production platforms in the North Sea (Freeman, 1977; Ralph and Goodman, 1979; Oldfield, 1980; Ralph and Troake, 1980; Goodman and Ralph, 1981; Hardy, 1981; Kingsbury, 1981). Of the communities associated with these platforms, Freeman (1977) described a "hard" and "soft" category of fouling species. The "hard category" fouling were the dense shelled animal organisms such as barnacles, tubeworms and mussels; whilst the "soft category" foulers included all plant species as well as certain animal organisms represented mainly in the Porifera, Coelenterata and Tunicata.

Hydroids were typical members of the "soft category" fouling growths, and dense growths of *Tubularia larynx* (Ellis and Solander) were frequently reported from the communities found on oil and gas platforms in the North Sea (Freeman,1977; Oldfield, 1980; Ralph and Troake, 1980; Goodman and Ralph, 1981; Hardy, 1981). The importance of hydroids, including *Tubularia* in the fouling communities of offshore platforms in Italian waters has also been recorded (Montanari and Morri, 1977). Standing (1976) described hydroids as "common members of marine epibenthic communities" and reviewed their role in fouling community structure. Extending earlier work by Coe (1932) and McDougall (1943), Standing (1976) concluded that hydroids were important in altering the surface characteristics of substrata, which might favour the settlement of certain other organisms; and also in forming a physical barrier, which might prevent settling larvae from other groups reaching the substrate. Keith (1971) and later Caine (1979) suggested that structural aspects of hydroids were important habitat requirements for caprellid amphipods. Osman (1977) noted that growths of *Tubularia larynx*, tended to trap sediment around the basal stolons. He observed that such a build-up of silt and sediment may smother intolerant species and deny settlement space to other groups. However, Field (1982) later reported that the accumulated sediment amongst the basal stolons of *Tubularia crocea* canopies formed a substratum readily colonized by tubiculous amphipods. In a fouling community at Delaware (Dean and Hurd, 1980), hydroids enhanced the settlement of tunicates, and the resultant tunicate-hydroid assemblage then enhanced settlement of mussels. These findings were later confirmed by Schmidt (1983b)who found that canopies of *Tubularia larynx* growing on experimental panels at Portsmouth, England, greatly enhanced the settlement of the solitary ascidians,*Ciona* and *Ascidiella*.

It is clear that hydroid colonies may play an important role in the species composition and distribution patterns of fouling communities. However, for *Tubularia*, probably the most widespread and abundant hydroid in British waters, especially the North Sea (Freeman, 1977;Ralph and Goodman,1979; Oldfield, 1980; Ralph and Troake, 1980; Goodman and Ralph, 1981; Hardy, 1981) and "of major importance as a fouling species" (Stubbings and Houghton, 1964), fundamental aspects of its general biology such as the mechanism of settlement and early growth, remain to be clearly defined.

Tubeworms, including *Pomatoceros triqueter* (L), have also been identified as a conspicuous component of the fouling growths found on North Sea oil and gas installations (Freeman, 1977; Leitch, 1980; Oldfield, 1980; Ralph and Troake, 1980; Goodman and Ralph, 1981; Ralph, Goodman and Picken, 1981), and are included in the "hard category" foulers of Freeman's (1977) classification. *Pomatoceros triqueter* is commonly found on rocky coasts in temperate waters and extends from the surface to depths of over two hundred metres (Dons, 1927, 1943; Thomas, 1940; Segrove, 1941; Woods Hole Oceanographic Institute, 1952; Føyn and Gjøen, 1954; Lewis, 1964; Dybern, 1967; Relini and Sara, 1971; Klockner, 1976, 1978; Relini, 1976; Castric-Fey, 1982). The calcareous tubes secreted by *Pomatoceros* (as well as other serpulids) provide "secondary free space" (Dayton, 1971) within fouling communities, which is "particularly susceptible to epifaunal colonization ... and ... some colonial animals may even preferentially settle upon the tubes of serpulids". (Jackson, 1977a). According to Jackson (1977a) this susceptibility to overgrowth increases the substrate surface area and thus "the potential biomass and reproductive output of colonial animals is enhanced". In addition, some tube worms may exhibit a gregarious

settlement-response. (Straughan, 1972; Crisp, 1977; Scheltema, Williams, Shaw and Loudon, 1981) and sometimes form very large aggregates (see ten Hove, 1979; Bosence, 1979) which may deny space to other fouling groups.

Clearly, *Pomatoceros triqueter* may also play an important role in fouling community structure and development. However, to date, as is the case for the hydroid *Tubularia larynx*, the mechanisms of attachment and settlement and the early growth pattern have not been fully reported. These early life history processes directly affect the ability of marine invertebrate larvae to successfully and rapidly colonize the surfaces of hard substrata (Osman, 1977), and part of this study aims to identify the mechanisms of attachment and settlement, and investigate the conditions for early growth in each species.

1.1 *The Settlement and Growth of Tubularia larynx (Ellis and Solander).*

The life cycle of *Tubularia crocea* (Agassiz) has been outlined by Berrill (1952) who added to the original work of Agassiz (1862). Essentially, following discharge from the gonophore, the actinula larva settles and gives rise to a colony by a combination of ramifying stolonic growth and hydranth production. In a study of fouling organisms at Beaufort, Carolina, McDougall (1943) estimated a period of 26 days from settlement to the shedding of second generation actinulae in T. *crocea*. Newly settled polyps of *T. larynx* were reported to have developed gonophores within 26 days in Plymouth Sound, England (Orton 1914); and in "just over three weeks" at Millport, Scotland (Pyefinch, 1950). Pyefinch and Downing (1949) found that laboratory cultures of *Tubularia larynx* matured in 24 days.

The duration of the larval period following liberation, was usually short-lived (Ciamician, 1879, in Pyefinch and Downing, 1949; Pyefinch, 1950; Hawes, 1958). Ciamician (1879, in Pyefinch and Downing, 1949) found that the actinulae of *T. mesenbryanthemum* became attached within 4 to 6 hours after release from the gonophore. This 'free' larval period may vary, however, and Pyefinch and Downing (1949) in a study of *T. larynx*, reported that "a considerable time interval may elapse before an appreciable proportion of actinulae become attached permanently to the substratum". They observed that some individuals did not settle until 46 hours after liberation.

Early reports suggested the actinulae of *Tubularia larynx* could select a site for settlement, and could swim (Hincks, 1868; Allman, 1871). Pyefinch and Downing (1949) reported some limited free movement over the substratum, which was effected "by the aboral tentacles". They estimated a 'crawling rate' in *T. larynx* of 0.3 cm per hour. However, Hawes (1958) recognised no site selection behaviours, and concluded that any observed tentacle movements in the actinula larva were unco-ordinated and of no significance. He described the actinula in *T. larynx* as "not a true larva but a juvenile polyp", and reasoned that "no specialized pattern of behaviour involving the change from a free-living to a sedentary way of life" was necessary. Other hydrozoan larvae do, however, exhibit site selection behaviours. In their review of attachment and settlement in Coelenterates, Chia and Bickell (1977) recognised two major behavioural phases which commonly occurred during the larval period in laboratory populations of hydrozoan planula larvae. The first was a period of active swimming near to the water surface. Later, the larvae moved to the bottom of the culture vessel and swimming ceased. Thus most young planulae were found to be photopositive, a response which was reversed in most species prior to settlement.

The settling larvae of many coelenterates also responded to substrate texture and contour (Williams, 1965; Nishihira, 1967; Lewis, 1974; Cargo, 1979). Pyefinch and Downing (1949) found *Tubularia larynx* was capable of settling both on smooth and roughened glass surfaces. Later, Mackie (1966) confirmed these observations in a study of *T. crocea*, where actinula larvae readily settled on "even the smoothest glass surface". Barnes and Powell (1950) noted that *Tubularia* actinulae were able to settle on fibreglass mats that precluded the settlement of *Pomatoceros* trochophores and barnacle cyprids. The ability of *Tubularia* larvae to settle on a wide variety of substrata, including slime films was confirmed by Pyefinch (1950). Recently, Fry (1975) suggested that *Tubularia* actinulae may settle preferentially on slime films.

Two types of substrate attachment are now recognised in the settlement activity of coelenterate larvae (Chia and Bickell, 1977). The first type of attachment or "temporary attachment", was described as "weak and transient", and was effected by mechano-sensitive nematocysts. Such "temporary attachments" were described in the settlement of *Tubularia larynx* (Pyefinch and Downing, 1949; Hawes, 1958). Pyefinch and Downing (1949) showed that the temporary attachment was effected by the discharge of large numbers of nematocysts contained in the swollen tips of the aboral tentacles. Ewer (1947) had previously demonstrated that the tentacles of buds in *Hydra vulgaris attenuata*, became "attached to the substratum by means of atrichous isorhizas". Similarly, mechano-sensitive nematocysts have been implicated in the initial attachment phase of the planula larva in *Hydractinia echinata*. (Teitelbaum, 1966; Muller, Wieker and Eiben, 1974) and in the reef coral, *Pocillopora damicornis* (Vandermeulen, 1974). In a study of the symbiotic hydroid *Proboscidactyla flavicirrata*, Donaldson (1974) identified a "contact-chemical" response in the nematocysts of the

planulae to sabellid worm tubes.

Chia and Bickell (1977) described the second type of substrate attachment as "much stronger and sometimes permanent". Called the "settlement attachment" it was mediated by gland cells of the aboral pole of planula larvae (Bonner, 1955; Nyholm, 1959; Vandermeulen, 1974, 1975). 'Settlement attachment' in *Tubularia* actinula larvae has been attributed to secretion of the chitinous perisarc (Ciamician, 1879, in Pyefinch and Downing, 1949; Berrill, 1952; Hawes, 1958). The experiments of Goldin and Barth (1941) with *Tubularia crocea*, and of Hauschka (1944), with *Campanularia flexuosa*, demonstrated the ability of hydroid perisarc to become attached to the surface of experimental vessels. The adhesive properties of newly secreted chitin were also recorded in a study of the growth process in *Obelia commissuralis* by Berrill (1949), who found that "stolon tips readily attached to solid surfaces". He described the surface of the stolon tips as "sticky" and observed that stolons already attached had hardened and were "difficult to detach without breaking". Berrill (1952) later reported the 'settlement attachment' of the actinula in *Tubularia crocea*. In this study, Berrill (1952) reasoned that the aboral pole of an attached actinula was "rapidly growing tissue, i.e. a stolon tip," and new chitinous perisarc was being continuously produced, which itself served as a "cement" as it was secreted. These findings were confirmed by Hawes (1958). He described the aboral pole of the actinula larva in *T. larynx* as little more than a stolon bud, the growth of which secured the larva to the substrate by means of the "perisarcal chitin."

The ability of hydroid stolons to attach, and grow, upon contact with the substratum was later confirmed by Tusov and Davis (1971). Knight (1968) reported that the hardening of the perisarc was a phenolic tanning process, or sclerotization. Earlier, Berrill (1949) had

described distinct "chitin-secreting" cells in the epidermis of growing stolon tips in *Obelia*. Subsequently, Knight (1970, 1971) demonstrated the presence of the orthodihydroxyphenol, dopamine, and the enzyme orthodihydroxyphenol oxidase in the "tanning cells" of the perisarc in the hydroid *Laomedea (Campanularia) flexuosa*.

Pyefinch and Downing (1949), however, disagreed with the view that the perisarcal chitin was sufficient to attach the *Tubularia* actinula to the substratum (Ciamician, 1879, in Pyefinch and Downing, 1949; Berrill, 1952; Hawes, 1958). Rather, Pyefinch and Downing (1949) suggested the possibility that an "extra-chitinous cement, similar to that found in arthropod groups" (Harris, 1946; see Crisp,D.J.,(1977)effected the 'settlement attachment'. In support of their considerations, Pyefinch and Downing (1949) cited a personal communication which reported the presence of dihydroxyphenols in the "attachment cement" of *Tubularia* actinulae, and concluded that the "muco-protein based attachment cement" was tanned by the action of a quinone upon settlement.

Whether the 'settlement attachment' is effected by the perisarcal chitin alone or an extra-chitinous "attachment cement" is necessary, it is clear that the tissues of the aboral pole are directly involved in successfully attaching the *Tubularia* actinula to the substrate. Lowe (1926) recorded that the ectodermal cells of the aboral pole of the actinula larva in *T. larynx* were distinctly columnar, contained large numbers of dark granules, and were probably secretory in function. Pyefinch and Downing (1949) confirmed these observations and suggested the ectodermal cells were responsible for the secretion of an "attachment cement". Later, Berrill (1952) described the "basal protrusion" (or aboral pole) of the actinula larva of *T. crocea*, as the "organ of attachment", and considered the "thickened epidermis" to be the active tissue in effecting the attachment. Contact of the aboral pole of the

Tubularia actinula larva with the substratum, was pre-requisite to the 'settlement-attachment' phase (Pyefinch and Downing, 1949; Hawes, 1958). Ectodermal gland cells were involved in the 'settlement attachment of a number of other coelenterate larvae, including *Phialidium gregarium* (Bonner, 1955); *Prontanthea simplex* (Nyholm, 1959); *Pocillopora damicornis* (Vandermeulen, 1974, 1975); and *Ptilosarcus gurneyi* (Chia and Bickell, 1977).

Once permanent attachment of the actinula of *Tubularia* has occurred, the animal is capable of very rapid growth. A number of reports have noted the fast rate of growth of *T. larynx* in British waters (Orton, 1914; Milne, 1940; Pyefinch and Downing, 1949; Barnes and Powell, 1950; Pyefinch, 1950; Fry, 1975; Ralph, Goodman and Boyle, 1979; Leitch, 1980; Goldie, 1981; Goodman and Ralph, 1981; Hughes, 1983). Similarly, a rapid rate of growth has been recorded in *T. crocea* in various seas (McDougall, 1943; Berrill, 1952; Mackie, 1966; Bastida, De Mandri, De Bastida and Stupak, 1980).

Laboratory observations of *Tubularia larynx* (Pyefinch and Downing, 1949) showed that newly settled actinulae underwent an initial, rapid elongation process. This was followed by a period of predominantly basal stolonic growth, which was maintained for "approximately eleven days after settlement". Subsequently, the rate of basal stolonic growth was reduced and chiefly stems (hydrocauli) and hydranths were produced. Mackie (1966) reported that in the newly settled actinula of *T. crocea* stolonic growth (which began in the form of a distinct bud from the aboral pole) did not occur until the hydrocaulus was about 4 mm long. Laboratory cultures of *T. crocea* exhibited a stolonic growth rate of 1 mm per day over the first seventeen days, immediately following settlement (Mackie, 1966).

The rate of growth in laboratory populations of colonial hydroids has been measured as the increase in hydranth number (Crowell, 1953, 1957; Loomis, 1954; Crowell and Wyttenbach, 1957; Fulton, 1962; Kinne and Paffenhofer, 1966; Tusov and Davis, 1971; Hundgen and Hartman, 1979; Stebbing, 1981a); and as the increase in the length of stolons (Berrill, 1949; Crowell, 1957; Crowell and Wyttenbach, 1957; Kinne, 1958; Fulton, 1961, 1962, 1963; Wyttenbach, 1968; Tusov and Davis, 1971). The use of both these criteria as an index of growth showed that the growth of most colonial hydroids was exponential. In a review of hydroid growth, Davis (1971) noted that the logarithmic growth rate constants (k) of most species fell within the range of 0.20 k to 0.35 k.

Both the rate of hydranth production and stolonic growth were influenced by environmental factors, principally temperature and salinity (Berrill, 1949; Loomis, 1954; Crowell, 1957; Crowell and Wyttenbach, 1957; Kinne, 1957, 1964, 1971; Fulton, 1962; Kinne and Paffenhofer, 1966; Paffenhofer, 1968; Tusov and Davis, 1971; Hundgen and Hartman, 1979; Stebbing, 1981a).

A general account of the effect of exposing invertebrates to different temperatures and salinities has been given by Kinne (1971ab), in which he reviews his own extensive work on the effects of salinity on the hydroid, *Cordylophora caspia*. The effects of reduced salinity on the growth of another hydroid, *Bougainvillia* were studied by Tusov and Davis (1971), whilst recently, Stebbing (1981a) has investigated salinity stress in *Campanularia* and fully demonstrated the growth response to reduced salinities. The effects of temperature on hydroid growth are well shown by the work of Paffenhofer (1968) on *Clava multicornis*. Earlier, Berrill (1949) demonstrated that the growth of free stolons in *Obelia geniculata* varied from 1.0 mm per day at 10°C to 12°C; to 10 mm per day at 16°C to 17°C; and up to 20 mm per day at 20°C. The combined effects of temperature and salinity on both hydranth structure

and stolonic growth in *Clava multicornis* were reported by Kinne and Paffenhofer (1965, 1966). Temperature and other environmental effects on *Tubularia* have been mainly restricted to studies on hydranth regeneration and reorganisation (Morse, 1909; Elmhirst, 1922; Moore, 1939; Berrill, 1948, 1952; Tardent, 1963). However, Mackie (1966) recorded a stolonic growth rate of 1 mm per day in newly established colonies of *Tubularia crocea*. This rate of growth was recorded from laboratory cultures of T. crocea held at 14°C, and in "the purest, natural sea-water available".

Field observations have shown that in unfavourable conditions some hydroids resorb or discard parts of their body in order to survive as a dormant stage from which regrowth occurs in the next growing season (Tardent, 1963). A winter dormancy has been reported for *Tubularia indivisa* (Allman, 1871; Corlett, 1948) and for *T. larynx* (Pyefinch and Downing, 1949). Orton (1914) recorded a maximum rate of growth in *Tubularia larynx* during August and September at Plymouth, England, while *T. crocea* may regress during the summer at Naples (Mackie, 1966). In a study of the distribution of hydroids in estuaries, Calder (1976) found salinity was a "master factor", and reported that *Tubularia crocea* was restricted to waters above 23°/oo to 25°/oo salinity.

1.2 *The Settlement and Growth of Pomatoceros triqueter (L).*

A number of studies have shown that the spawning and settling period of *Pomatoceros triqueter* may be variable but occurs mainly in the spring and summer months in temperate zones (Føyn and Gjøen, 1954; Dybern, 1967; Relini and Sara, 1976), although Klockner (1976) reported the main larval settlement in late summer to autumn. In British waters, the main settlement of *Pomatoceros triqueter* was observed in March to April (Segrove, 1941) and June to August (Pyefinch, 1950). In a study of both

the mainly sub-littoral *Pomatoceros triqueter*, and the predominantly intertidal P.*lamarkii*, Castric-Fey (1982) noted two settlement peaks occurred in both species in the waters around Brittany, France. These peaks in settlement were recorded during April to June and August to October, although both species were capable of settling all the year round.

Most serpulids have a planktonic larval stage of between six days and two months (see ten Hove, 1974). This period may vary depending upon the season or temperature (Segrove, 1941; Wisely, 1958; Hong, 1980; Scheltema, Williams, Shaw and Loudon, 1981), salinity (Hill, 1967) or food availability (ten Hove, 1979). The larvae of several serpulid species can delay settlement under unsuitable conditions (Hill, 1967), which may, however, result in a decreased discrimination during settling (see Knight-Jones, 1953).

According to Kinne (1971a,b)the effects of salinity and temperature are of major importance to the survival and rate of development of marine invertebrate larvae. A number of studies involving bivalve larvae have often shown a significant temperature or salinity effect, as well as interaction effects in the development and mortality of larvae (Nelson and Perkins, 1930; Davis and Calabrese, 1964; Haskin, 1964; Bohle, 1972; Lough, 1975; Hidu and Haskin, 1978). Lyster (1965) investigated salinity tolerance in polychaete larvae including *Pomatoceros triqueter*. Using late trochophores and "crawling stage" larvae he found *Pomatoceros* to be "fairly tolerant" to a range of salinities from 10^{o}/oo to 60^{o}/oo. He suggested larval salinity tolerance was an important limiting factor in the distribution of *Pomatoceros* in estuaries. In addition, Lyster (1965) described a significant salinity/temperature interaction effect in the larval

survival of *Pomatoceros*, and found that at 14°C ("possibly the physiological optimum for the species"), the maximum salinity tolerance was displayed. Similar salinity and temperature interactions have been recorded in the survival and metamorphosis of other serpulid larvae (Gaucher, Leone and Denoit, 1967; Gray, 1976).

The laboratory culture and development of *Pomatoceros triqueter* has been reported (Segrove, 1941; Føyn and Gjøen, 1954). Segrove (1941) found temperature to be important in the rate of development of *Pomatoceros* larvae. At "summer" temperatures, he reported the period from fertilisation to metamorphosis was three weeks, although fully-formed free-swimming stages could persist for up to six to eight weeks. According to Segrove (1941) late-stage trochophore larvae (or metatrochophores) performed "creeping" behaviours and descended to the bottom of the culture vessel. Immediately prior to settlement the larvae became "positively phototropic" and "the great majority" settled "in a window". Føyn and Gjøen (1954) disagreed with some of Segrove's (1941) findings, and in particular reported that metatrochophore larvae of *Pomatoceros triqueter* were photo-negative immediately prior to settlement and did so "mainly on the bottom of the culture bowl".

A number of field studies have shown that most serpulids were found on the lower surface of experimental plates (McDougall, 1943; Gaucher, Leone and Denoit, 1967; Sentz-Bracconot, 1968; Relini and Sara, 1971; Bosence, 1979). The brackish water serpulid, *Ficopomatus uschakovi* (as *Mercierella enigmatica*) was shown to prefer surfaces that were shaded, dark-coloured, rough and facing downwards (Straughan, 1968, 1972). Wisely (1958) reported similar settling responses in *Hydroides elegans* (as *Hydroides norvegica*). The response to light has been noted as a principal factor in influencing substrate settlement in serpulids,

and may lead to aggregation in the tidal zone in *Pomatoceros triqueter* (Thorson, 1950, 1957; Lewis, 1964). In addition to the laboratory observations of Segrove (1941) and Føyn and Gjøen, (1954), naturally occurring larvae of *Pomatoceros triqueter* were found to be "negatively phototactic" immediately prior to settlement (Klockner, 1976). Those studies indicated that serpulid larvae, including those of *Pomatoceros triqueter*, were photonegative upon settlement.

However, Sentz-Bracconot (1968) considered "it was probable" that the reactions of serpulid larvae to light were more complex, and were particularly dependent upon the nature and intensity of the light. Further, ten Hove (1979), reported that the habit of settling mainly on the underside of experimental panels and blocks etc., exhibited by serpulid larvae may be caused by a number of factors including light. His view that the final settling place of serpulid larvae "will certainly be a compromise between light-factors and several other biotic and abiotic factors" substantiates the earlier findings of Wilson (1952) and Dybern (1967), and coincides with the conclusions of Schroeder and Hermans (1975). Bosence (1979) has recently shown that the settling larvae of the tubeworm, *Serpula vermicularis* and algae are in competition in the shallow water zone.

It is well known that the nature of the substratum can also influence the attachment and settlement of epifaunal larvae. Early studies were mainly concerned with barnacle settlement (Visscher, 1928; Coe and Allen, 1937; McDougall, 1943; Crisp and Barnes, 1954; Wisely, 1959), and factors such as contour, colour, texture and angle of the surface were emphasised. Wilson (1948) reported the importance of the properties of the substratum in the settlement of a number of marine worms. Later, Pyefinch (1950), in a study of marine fouling organisms

settling on experimental panels, found that the tubeworm *Hydroides* settled equally readily on smooth and rough surfaces, whilst the settlement of *Pomatoceros triqueter* larvae was more restricted. Although settlement of *Pomatoceros triqueter* was recorded from "most non-toxic substrata", larvae did not settle on rough surfaces (Pyefinch, 1950) and were precluded from fibre glass matting (Barnes and Powell, 1950). Pyefinch (1950) also noted that settlement of *Pomatoceros* larvae on "slimy, smooth glass" surfaces or "greasy" surfaces was "insecure". More recently, Klockner (1976) reported that surface structure and material of substrate had no effect upon the settlement of *Pomatoceros triqueter*. However, Straughan (1968, 1972) found the settling larvae of *Ficopomatus uschakovi* (as *Mercierella enigmatica*) preferred rough, dark-coloured, opaque and downwardly-pointing surfaces.

The influence of water speed has also been implicated in the settlement of serpulid larvae. Pyefinch (1950) suggested that larvae of *Pomatoceros triqueter* settled only during periods of slack water. A similar relationship between water speed and larval settlement was recorded in *Ficopomatos uschakovi* (as *Mercierella enigmatica*) by Turpaeva (1961) and Straughan (1972).

Investigations of larval development and substrate attachment in tubeworms include a number of studies of Spirorbinae (e.g. Hoglund, 1952; Wisely, 1960; Gee and Knight-Jones, 1962; Nott, 1973). Studies of other serpulids include *Pomatoceros* (Segrove, 1941; Føyn and Gjøen, 1954), *Hydroides* (Wisely, 1958; Sentz-Bracconot, 1964; Gaucher, Leone and Denoit, 1967; Hong, 1980; Scheltema, Williams, Shaw and Loudon, 1981). *Galeolaria* (Andrew and Anderson, 1962; Grant, 1981; Marsden and Anderson, 1981), *Serpula* (Sentz-Bracconot, 1964), *Sabellaria* (Wilson, 1970), *Ficopomatus (Mercierella* : Straughan, 1972), *Pomatoleios* (Crisp,M.1977); whilst Crisp, D.J. (1977) recently presented a review of the mechanisms of substrate adhesion in fouling organisms, including tubeworms.

Intra-specific settlement responses are now well known for a number of invertebrate taxa (Crisp, 1974; Scheltema, 1974), and enable solitary forms, including tubeworms, to rapidly colonize hard substrata. The gregarious settlement response of Spirorbinae has been well reported (e.g. Knight-Jones, 1951; Knight-Jones and Moyse, 1961; Knight-Jones Bailey and Isaac, 1971; Knight-Jones, Knight-Jones and Al-Ogily, 1975 ; Knight-Jones, Knight-Jones and Kawahara, 1975). Only three other species of serpulid were reported to settle gregariously, *Ficopomatus uschakovi (Mercierella enigmatica:* Straughan, 1972), *Pomatoleios kraussi* (Crisp,M,1977), *Hydroides dianthus* (Scheltema, Williams, Shaw and Loudon, 1981).

Immediately prior to settlement, invertebrate larvae commonly perform 'searching' or 'seeking' behaviours designed to select a suitable settling site (see Crisp, 1974). Many studies reported the existence of such 'seeking' behaviours in serpulid trochophore larvae which were typically called 'crawling stage' larvae (Segrove 1941; Knight-Jones, 1951; Føyn and Gjøen, 1954; Turpaeva, 1961; Straughan, 1968, 1972; Nott, 1973). Such activities prior to metamorphosis and attachment in tubeworms may involve the use of a group of specialised (possibly sensory) cilia, the 'apical tuft', located centrally on the head of the trochophore larva (Shroeder and Hermans, 1975). Apical cilia in *Spirorbis* were implicated as possible chemosensory structures in the site selection habits peculiar to that genus (Nott, 1973). Grant (1981) and Marsden and Anderson (1981) found the apical tuft degenerated or was totally lost during the demersal (or 'seeking') larval phase in *Galeolaria caespitosa*. Instead, Grant (1981) recorded the appearance of other apical cilia, in groups or singly, on the head and pygidial segment, which he suggested played a sensory role in substrate selection.

Temporary attachments to the substratum by the secretion of adhesive mucous have frequently been reported in the settling larvae of serpulids (e.g. Segrove, 1941; Straughan, 1968, 1972; Nott, 1973; Crisp,M,1977; Marsden and Anderson, 1981). Mucous-mediated temporary (and permanent) attachments have also been recorded in settling pediveliger larvae of *Ostrea edulis* (Cranfield, 1973). The tubeworm, *Hydroides*, was described as "sticking" to the substrate. (Scheltema, Williams, Shaw, and Loudon, 1981). However, Wisely (1958) found no evidence of temporary attachments in *Hydroides*, but rather observed a direct and permanent ("mucin-mediated") attachment. Free-swimming serpulid metatrochophores (late stage trochophore larvae) have often been seen to secrete mucous threads from the posterior parts of their bodies, and to trail mucous threads (Knight-Jones, 1951; Wisely, 1958; Nott, 1973; Crisp,M., 1977; Marsden and Anderson, 1981). Segrove suggested some metatrochophore larvae in *Pomatoceros triqueter* adhered to the substrate by a secretion of mucus from the ventral mucus glands. He also considered that fixation to the substrate during settlement possibly resulted from the secretion of the "hyaline contents" of the anal vesicle.

The anal vesicle was a conspicuous feature of all the free-swimming larval stages in *Pomatoceros triqueter* (Segrove, 1941), and was lost once the animal had settled. The disappearance of the anal vesicle upon settlement was recorded in other serpulids (Wisely, 1958; Crisp,M., 1977; Marsden and Anderson, 1981; Scheltema, Williams, Shaw and Loudon, 1981). The view that the anal vesicle was implicated in attachment to the substrate in serpulid larvae was substantiated by observations that settling larvae did so mainly by their posterior ends (Segrove, 1941; Wisely, 1958; Marsden and Anderson, 1981). Grant (1981) noted that metatrochophore larvae in *Galeolaria caespitosa* often swam with the

anal vesicle pointed downwards. Similarly, for the same species, Marsden and Anderson (1981) recorded that metatrochophores swam so that the anal vesicle was "pulled across the bottom of the dish".

During the course of settlement and metamorphosis in *Galeolaria*, Marsden and Anderson (1981) observed "sticky masses" of secretory material around the neck region and posteriorly around the anal vesicle, which was now granular in appearance. They considered the anal vesicle was involved in the secretion of the primary, mucoid tube. Earlier, Wisely, (1958) reported that initial tube formation in *Hydroides* followed the disappearance of the anal vesicle. He noted that the tube became calcareous "3 to 4 days later". The mechanism of settlement in the sub-family Spirorbidae is more clearly defined. Knight-Jones (1951) described an "attachment gland", which Hoglund (1952) called the "primary shell gland", in the trochophore larva in *Spirorbis*. In a comprehensive study of settlement in *Spirorbis spirorbis*, Nott, (1973) described a number of glandular regions in the trochophore larva, associated with both the 'attachment gland' of the gut, and the anal vesicle. These mucous glands opened to the body surface through numerous pores, and their secretions were responsible for securing the larva to the substrate, (Nott, 1973). He reported that following formation of a primary mucoid tube, a calcareous tube was laid down by secretions from the ventral collar gland.

The calcareous tube of serpulids consists of two components: mineral deposition (the inorganic component, of calcium carbonate) which was produced from under the fold of the collar (Soulier, 1891; Thomas, 1940; Swan, 1950; Hedley, 1956a, 1956b; Vovelle, 1956; Neff, 1968, 1969), and mucous secretions of the ventral shield epithelium (the organic component), which together formed the tube-building material

(see Hedley, 1956a, 1956b and Neff, 1968, 1969).

Following settlement, in *Pomatoceros triqueter*, Segrove (1941) reported the elongation and 'growth' of the calcareous tube was often very rapid. Similarly, an initial fast rate of 'tubegrowth' was observed in *Hydroides dianthus* (Gaucher, Leone and Denoit, 1967) and *Ficopomatus uschakovi* (as *Mercierella enigmatica*, Hill, 1967; Dixon, 1980). Increases in tube length occurred by additions to the anterior end of the tube and could be very variable. (Hargitt, 1909, 1912). A number of early investigators carried out quantitative studies of the rate of 'growth' (usually measured as increases in tube length) of serpulids (Soulier, 1891; Hargitt, 1906, 1909, 1912; Harms, 1912; Dons, 1927). Recently, Klockner (1978) described the formation of tubes in the serpulidae as "a metabolic function which probably correlated mainly with body growth and was modified by many exogenous and endogenous factors".

A major environmental factor governing the rate of tube formation in serpulids was temperature. Several studies noted that many species of tubeworm from northern waters failed to produce tube or mineral, or produced it at a greatly reduced rate, during the winter period (Harms, 1912; Dons, 1927; Grave, 1933; Robertson and Pantin, 1938; Thomas, 1940; Vovelle, 1956). Grave (1933) reported the rate of growth of a number of invertebrates, including *Hydroides hexagonis* at Woods Hole, Massachusetts. He found that most of the 'tubegrowth' in *Hydroides* occurred during the summer months. According to Potts (in Robertson and Pantin, 1938) *Pomatoceros triqueter* produced tube only between March and August. However, Føyn and Gjøen (1954) reported that 'tubegrowth in *Pomatoceros* continued throughout the year (although at a minimum during December to February), and found laboratory populations of the

serpulid could produce new tube at temperatures between 2°C and 4°C. Earlier, Thomas (1940) reported that *Pomatoceros triqueter* was unable to secrete calcium carbonate when the water temperature was lower than 7°C. According to Pyefinch (1950), food supply was the dominant factor in governing the rate of growth in *Pomatoceros*, and he found "no direct correlation" between the rate of tube secretion and water temperature. Klockner (1976) showed that monthly rates of 'tubegrowth' in *Pomatoceros triqueter* were significantly higher at shallow water sites (7 m) than at depths of 30 m to 60 m. He attributed this to "higher temperatures and more food" in the surface waters. At temperatures above 25°C to 26°C, he reported a rapid decline in the formation of new tube in *Pomatoceros*. More recently, Castric-Fey (1982) observed that both *Pomatoceros triqueter*, and *P. lamarkii*, grew fastest during the summer months around the coast of Brittany, France.

Many investigators removed worms from their tubes and observed the attempts to build a new tube (Harms, 1912; Dons, 1927; Faouzi, 1931; Robertson and Pantin, 1936; Thomas, 1940; Swan, 1950; Vuillemin, 1954; Neff, 1968, 1969; Klockner, 1978). These studies reported the appearance within a few hours, of the mineral (inorganic) component of the tube under the collar. The production of this "mineral regenerate" (see Neff, 1968) was employed as an index of 'tubegrowth' in *Pomatoceros triqueter* (Neff, 1968, 1969; Klockner, 1978). According to Klockner (1978) tube regeneration was primarily a function of the experimental temperature, and maximum regeneration rates were recorded between 20°C and 24°C., whilst there was a rapid decline at temperatures above 26°C to 28°C. Klockner (1978) also noted that tube regeneration in *Pomatoceros* was always very much less than the corresponding natural rate of 'tubegrowth'.

The effects of salinity on 'tubegrowth' in serpulids has received rather less attention. According to Alexander, Southgate and Bassingdale, 1935, in Lyster, 1965), adults of *Pomatoceros triqueter* were able to survive exposure to salinities as low as 3^{o}/oo, but were more normally found in salinities ranging from 18^{o}/oo to 23^{o}/oo. In a study of fouling organisms in a Norwegian fjord, Dybern, (1967) reported that *Pomatoceros triqueter* readily settled and grew on experimental surfaces in salinities ranging from 15^{o}/oo to 18^{o}/oo. The ability of *Pomatoceros triqueter* to survive and grow under wide ranging conditions of salinity was pointed out earlier by Percival (1929) and Milne (1940) who described the tubeworm as "fairly tolerant" to salinity fluctuations. Studies of other serpulids have also reported the effects of salinity on 'tubegrowth' Neff (1968, 1969) found *Hydroides* failed to produce 'mineral regenerate' below a salinity of 20^{o}/oo. Recently, Mak and Huang (1982) reported the salinity tolerance in *Hydroides elegans*. Salinity was 'a major factor' in the rate of growth and tubelength in *Ficopomatus uschakovi* (as *Mercierella enigmatica* : Straughan, 1972).

The rate of 'tubegrowth' in serpulids was also influenced by the environmental concentration of calcium. Robertson and Pantin (1938) noted that *Pomatoceros triqueter* was unable to produce 'mineral regenerate' in artificial sea water containing less than 50% of the normal amount of calcium. According to Dew (in Straughan, 1972), the rate of growth of *Ficopomatus* (as *Mercierella*) could be increased by adding calcium to the seawater. Earlier, Neff (1968, 1969) had shown that above the 'critical' salinity of 20^{o}/oo., the rate of mineral production increased "with increasing salinity and environmental calcium concentration up to full strength sea water (34^{o}/oo salinity). In hypercalcinated sea water (0.490 mg Ca/ml.) "no significant increase" in the rate of mineral production was observed, and Neff

(1968, 1969) considered a non-linear relationship between the calcium concentration of sea water and the rate of tube formation operated, similar to that demonstrated in bivalve shell formation by Kado (1960).

Another factor known to influence the rate of mineral regeneration and tube formation in serpulids, was the effect of size (age) of the worms (Hill, 1967; Neff, 1968, 1969; Straughan, 1972; Klockner, 1978). These studies recorded that the rate of 'tubegrowth' was highest during the period immediately following settlement, and that the rate decreased as the worms grew larger. Similar findings were reported earlier in the shell growth of molluscs (Wilbur, 1964).

Clearly, a number of factors may influence the rate of 'tubegrowth' in serpulids, however, to date, there is no information regarding the effects of such environmental variables on the early production of tube in *Pomatoceros*.

1.3 *Aims of Present Study*

This study aimed, in part, to extend our knowledge of the distribution of fouling organisms associated with offshore structures. Meadows (1969) showed that fouling community structure varied from place to place around the northern coasts of Britain, and defined assemblages of fouling organisms that were characteristic of particular sites. However, Meadows' (1969) sites were coastal and related to harbour and port fouling. To date most information of the fouling growths associated with offshore structures has come from studies of the fouling communities of oil and gas platforms in the North Sea. Such reports, however, are most often based upon contributions from untrained divers in the form of underwater photographs and descriptions of the fouling growths. Few preserved samples were collected. According to Haywood (1981) most information on permanent record is taken from under-

water photographs of "variable quality and standard".

The utilisation of offshore buoys in the present study has enabled:

(i) more scientifically valid random sampling techniques to be employed.

(ii) the rapid preservation of fresh material, including delicate algae and soft-bodied animals, so eliminating the danger of dessication.

(iii) Samples to be taken throughout the water column, from the 'splash-zone' to the sea bed.

(iv) the opportunity to visit the same buoy site annually, over a period of years.

Further, this investigation was not confined to the fouling growths of North Sea sites, but encompassed sea areas around the whole of the British coastline, samples of fouling communities being taken also from offshore buoy sites in the Celtic Sea, Irish Sea, English Channel, the Hebridean Sea, as well as from offshore sites around the coasts of Norway.

Reports from the North Sea showed that the hydroid, *Tubularia larynx* (Ellis and Solander), and the serpulid, *Pomatoceros triqueter (L)*, were common members of the fouling communities of offshore structures. *Tubularia* was a member of the "soft" fouling component, and *Pomatoceros* was a representative of the "hard" fouling category in Freeman's (1977) classification. Initial observations in the present study from a range of buoy sites around Britain, confirmed the high frequency and levels of abundance of both species in offshore fouling communities. Both animals are clearly successful fouling organisms. However, fundamental aspects of the basic biology of each species such as settlement and early growth (important processes in effecting the rapid colonization of hard substrata) remain to be clearly defined.

These two animals were, therefore, selected and detailed studies made of the mechanisms of attachment and settlement. In addition, the conditions for early growth, including the effects of environmental variables, were investigated.

CHAPTER 2

Materials and Methods

2.1 *Distribution of Fouling Organisms*

The species composition of offshore fouling communities at selected sites around Great Britain and Norway was investigated. Admiralty and Trinity House buoys, and buoys under the jurisdiction of various Norwegian Port Authorities were utilised as sites for the collection of marine fouling growths. The sites were selected to cover the whole of the coastline in both countries and to be situated in a number of different sea areas or water masses. Figures 2.1.1 (for U.K. sites) and 2.1.3 (for Norwegian sites) illustrate the distribution of the buoy sites; whilst their geographical positions are listed in Table 2.1.2 (for U.K. sites) and Table 2.1.4 (for the Norwegian sites).

Most of the buoys in British waters were visited during the May, June and July period, over the four years 1978 to 1981. A few sites were sampled in 1982 and 1983.

Only one period of sample collection, in May 1981, was possible from the sites in Norwegian waters. Due to adverse weather conditions it was not possible to obtain samples of the marine growth from buoy sites off the north-western and northern coasts of Norway.

In all cases, the buoys had been in the sea for between 10 and 14 months. Samples of the macro-fouling organisms (both flora and fauna) were collected from the following depth zones at each of the buoy sites:

(i) The 'splash-zone'. This was the area of the buoy normally above the waterline. Samples of the fouling growth occurring were taken from a band occupying the area from the waterline to 0.3m above the

waterline.

(ii) From the waterline, to depth 0.5m.

(iii) 0.5m to 1.2m.

(iv) 1.2m (the underside of the buoy in U.K. sites).

(v) 1.2m to 1.6m.

(vi) 1.6m to 3.5m (the 'bridle' chains of U.K. buoys)

(vii) 3.5m to 5m.

(viii) 5m to 7.5m.

(ix) 7.5m to 10m.

(x) Samples were taken from the remainder of the buoy cable at 2m intervals through the water column to the sea bed. Samples of the fouling organisms found on the anchor, or 'sinker', securing the buoy to the sea bed were collected whenever possible. Figure 2.1.5 illustrates the structure and associated depth zones of both U.K. buoys and Norwegian buoys.

At each buoy site, ten areas or random 'quadrats' in each of the depth zones were sampled at different points around the buoy and cables, to equal an area of approximately $100cm^2$. The samples of fouling organisms were placed into collection jars and preserved in a 4% formalin solution. Each set of samples was collected immediately the buoy and cables were raised and brought onto the ship's deck.

Once in the laboratory, the specimens were sorted, identified and blotted dry. The fresh weight of each species was then determined using an analytical top pan balance. The fouling organisms were keyed to species level, except for a few which were identified to generic level only. The results were tabulated and stored on a computer file for subsequent analysis. Nomenclature followed the

Plymouth Marine Fauna (1957) unless superceded by a more recent source. Keys and references used in identification are listed in Appendix 1.

The large amount of raw data collected made it necessary to carry out the analysis of information from U.K. sites, separately from the Norwegian data. For both sets of data similarity measures and numerical classification techniques were used in the analysis of species composition between sites. Mean biomass values for each species within each site (both U.K. and Norwegian buoy stations) were calculated prior to analysis. The initial classification of sites was based on the strategy put forward by Field, Clarke and Warwick (1982). Following log transformation (after Clifford and Stephenson, 1975) to reduce the scores of abundant species, the similarity measure of Bray-Curtis (1957) was used to construct a similarity matrix.

The Bray-Curtis measure has the form:

$$\delta jk = \frac{\sum_{i=1}^{s} \left| Y_{ij} - Y_{ik} \right|}{\sum_{i=1}^{s} (Y_{ij} - Y_{ik})}$$

Where Y_{ij} = score for the ith species in the jth sample; Y_{ik} = score for the ith species in the kth sample; δjk = dissimilarity between the jth and kth samples summed over all s species. δ jk ranges from 0 (identical scores for all species) to 1 (no species in common) and is the complement of the similarity S_{jk}:

$$S_{jk} = 1 - \delta_{jk}$$

From the resulting similarity matrix the hierarchical sorting strategy of group-average sorting or Wards (error of sum of squares) was employed to produce a dendrogram. To complement this classification, a Principle Components Analysis was also run with each set of data. A disadvantage of this method of classification was that the clustering techniques employed could be used with a maximum of only 80 variables. Hence the data set was reduced for both the U.K. and Norwegian sites. According to Field, Clarke and Warwick (1982) to simply select the 80 overall most abundant species would introduce bias to the data set. They advocate selection on the basis of species having above an arbitrary % dominance at any one site. As a consequence, once single occurrences had been discounted, all species with less than 4% dominance (at any one site) were disregarded. The data set for U.K. sites was thus reduced to 65 species and for the Norwegian sites, to 45 species.

In order to retain all the information available from a full data set, and to identify any possible indicator or characteristic species, a more powerful computer program, TWINSPAN (Hill, 1979) was also used to classify the U.K. and Norwegian data. TWINSPAN (Hill, 1979) is a FORTRAN program for two-way indicator species analysis. The program uses a polythetic divisive method of classification, and ultimately identifies one to several species which are particularly diagnostic of each division in the classification. In addition, data such as the frequency of particular species, e.g. *Tubularia larynx* or *Pomatoceros triqueter*, could be readily extracted from the output. The indicator species analysis is based on the ordination method of reciprocal averaging (Hill, 1973, 1974). According to Hill, Bunce and Shaw (1975), reciprocal averaging was preferred to principal components analysis

because it has several technical advantages when applied to large heterogeneous data sets and is suitable for ecological survey data.

2.2 *Settlement and Growth in Tubularia larynx (Ellis and Solander)*

Small colonies of *Tubularia larynx* were collected by divers from a navigation buoy in Plymouth Sound at the end of March, 1980. The water temperature at the time of collection was 8.5°C., and the site was exposed to full salinity (34°/oo) sea water.

In the laboratory, colonies were subjected to a limited series of tolerance tests to determine the range of experimental temperatures and salinities that would be employed. Colonies held in sea water diluted by more than 25% tended to lose 'condition', i.e. noticeably the hydranths became paler and lost pigmentation, and tentacular movements became slower and less responsive to prey. At salinities of 26°/oo and below many hydranths were shed within 2 to 3 days following exposure. Similarly, temperatures greater than 20°C usually resulted in widespread hydranth autotomy, whilst regeneration was often minimal. The range of experimental temperatures and salinities finally adopted was also partly determined by considerations of colony maintenance and culture, and were designed to reflect seasonal temperature variations in British waters, and a range of salinity conditions.

Colonies of *Tubularia larynx* were divided into groups and acclimatized over a period of 10 days to one of each of the following combinations of temperature and salinity:

(i) At 18°C., 34°/oo., 30°/oo., 28°/oo.

(ii) At 12°C., 34°/oo., 30°/oo., 28°/oo.

(iii) At 6°C., 34°/oo., 30°/oo., 28°/oo.

During acclimatization, temperature changes were of $\pm 1^{o}$C., per day to target temperature. Salinity dilutions were achieved using distilled water, and the colonies were maintained in controlled environment cabinets under a 12 hour daylength regime. The sea water in each tank was changed daily, and the colonies were kept well aerated and fed once daily with first stage *Artemia* nauplii. *Tubularia* colonies acclimatized to each of the above salinity and temperature combinations, formed the 'stock' material for subsequent experiments designed to investigate the growth of *Tubularia* (i) immediately following larval settlement, hereafter called 'actinula' growth (see 2.2.3.1) and (ii) from the growth of a single nutritive hydranth, hereafter called 'colony' growth (see 2.2.3.2 to 2.2.3.4).

2.2.1 *Laboratory Settlement*

2.2.1.1 *The Effect of Substrate*

Four experimental materials were used to test for substrate preference in the actinula larva of *Tubularia*. These materials were rough perspex, smooth perspex, wood (pine) and tufnol. One plate of each 'substrate' (10cm x 10cm x 0.4cm) was suspended vertically in each of 5 tanks of sea water at 34^{o}/oo salinity. The settlement tanks were maintained at 18^{o}C., in a controlled environment cabinet. Gentle aeration was supplied by airstone to maintain circulation, and the tanks were exposed to a 12 hour daylength period (at light intensity, 4×10^{-3} lux). One hundred newly liberated actinulae, from the 'stock' colony held at 18^{o}C., and 34^{o}/oo salinity, were introduced to each tank. The number of larvae permanently settled on each of the experimental substrates after 20 hours was counted, and the mean %

settlement on each substrate calculated.

2.2.1.2 *Duration of Larval Period*

A further 5 experimental tanks were set up to measure the duration of the larval period in *Tubularia* actinulae. The seawater was again maintained at $18^{o}C/34^{o}/oo$ and gently aerated, under a 12 hour day-length period (4×10^{-3} lux). Three settlement plates of smooth perspex (10cm x 10cm x 0.4cm) were suspended vertically in each tank. One hundred newly released actinula larvae (collected from the 'stock' colony at $18^{o}C/34^{o}/oo$) were added to each of the tanks. The number of permanently settled individuals at the end of each of the following time periods: 2, 4, 8, 12 and 20 hours, was recorded. Counts of settled polyps included individuals attached to the sides of the tanks and the aerators etc.

2.2.2 *Field Settlement and Growth*

Field observations were restricted to a study carried out in the river Yealm, near Plymouth. A 'turtle' raft belonging to International Marine Coatings Ltd., was used to investigate the influence of light and substrate on the settlement and growth of *Tubularia larynx*, and to record the seasonal pattern of settlement. The raft was moored in the lower reaches of the Yealm estuary (S. Devon) and was subject to tidal influences with salinities ranging from $30.5^{o}/oo$ to $33.6^{o}/oo$ (see Table 4.5.1 for salinity and temperature readings) during the period of study.

The 'turtle' raft is octagonal in section and designed to afford surfaces which receive varying degrees of incident light (Lovegrove, 1978 and Fig. 2.2.1). Settlement panels of smooth perspex, rough

perspex, tufnel, and wood (marine plywood) were fixed to the facets of the 'turtle' (one of each material, giving four settlement panels per facet on the raft). Each settlement panel was 25cm x 25cm. Those panels fixed to the waterline facets received more intense light and for longer periods than the panels fixed to the intermediate or 'turn' facets. The panels fixed to the bottom facets were shaded and received the lowest light levels (Lovegrove, 1978). Wire mesh screens (mesh size 2.5cm x 2.5cm) were bolted to a framework fixed to the raft, to cover the panels at a height of 70mm to reduce mullet grazing. With the turtle submerged, the centre of the waterline facet was about 0.5m below the surface, the turn facet about 1m deep and the bottom facet at about 1.5m.

Observations were made over the period, April to September 1980, at fortnightly intervals. With the 'turtle' raised to just below the surface the facets could be rotated and the settlement and growth of *Tubularia* colonies examined. The % cover of *Tubularia* was estimated using the point sampling method widely used in plant ecology (Wratten and Fry, 1980) and frequently employed in studies of epibenthic communities (Sutherland and Karlson, 1977; Jackson, 1977b; Russ, 1980; Field, 1982; Schmidt, 1983a). Regularly spaced points were used. A transparent perspex sheet (20cm x 20cm x 0.2cm), grid marked at 1cm intervals to give 400 locations or points (at the intersections of the grid) was used to estimate the % cover. The grid was slotted into a holding frame on the 'turtle' and lowered to a depth of 70mm above the experimental panels. The number of points overlying the *Tubularia* colonies were counted and the % cover, in relation to the total number of points, recorded. Care was taken to estimate the % cover marked by the boundaries of basal stolonic growth, and not canopy

overhang. Only actively growing colonies (i.e. those bearing hydranths) and newly settled *Tubularia* were included in the count. Dormant growths due to die-back were ignored. Since the canopies could not be cut there were problems of accuracy, consequently % cover readings were always rounded down to the nearest 5%. At the time of inspection all other fouling organisms, including any predatory nudibranch molluscs; were removed so that 'free space' was always available for *Tubularia* settlement and growth.

2.2.3 *Laboratory Growth*

2.2.3.1 *'Actinula Growth'*

Actinula larvae were collected on smooth perspex plates (10cm x 10cm) placed in vertical series in each of the twelve colony holding tanks, containing 'stock' material. Each plate was examined under a binocular microscope between 6 and 8 hours after exposure to liberated larvae. Of the newly settled actinulae, twenty individuals were left on each plate, whilst the others were brushed off. Care was taken to use only those larvae which had achieved settlement attachment, and were securely attached to the substrate with the aboral tentacles raised in the feeding posture. These new 'uprights' represented the starting point on which measurements of stolonic growth (basal and upright) were made. Four perspex plates supporting twenty young polyps were transferred to their respective experimental tanks, so that initially 80 'uprights' were available for observation at each temperature and salinity combination. Throughout the experiment the tanks were gently aerated, and exposed to a 12 hour daylength period. Seawater was changed once every two days, and feeding was at the rate

of one *Artemia* nauplius (first-stage) per hydranth per day.

Increases in stolon length (basal and upright) were measured every 2 days over a 14 day period of observations. For each set of experimental conditions, the perspex plates were laid horizontally in a dissecting tray and stolonic growth measured (in mm) using a vernier micro-scale (Balzer-Union Ltd.) and a binocular microscope at X40. Measurements of upright stolon growth were carried out in the experimental tanks. The mean of 10 observations per experimental temperature/salinity combination was calculated.

2.2.3.2 *The Effect of Temperature and Salinity on 'Colony Growth'*

For each of the nine experimental tanks, twelve 'uprights' with hydranths were randomly selected from 'stock colonies' of *Tubularia* in the corresponding temperature and salinity holding tanks. Care was taken to use 'healthy' hydranths (those showing active tentacular and feeding movements). A small piece of the basal stolon was also taken so that each 'upright' with hydranth could be secured to a smooth perspex plate (10cm x 5cm) with a piece of thread (after Wyttenbach, 1968). The water in the tanks was changed once daily, and the animals were exposed to a 12 hour daylength period. From day one, the original hydranth, and later subsequent hydranths resulting from colony growth, were fed one first stage *Artemia* nauplius per day. The food was presented to the hydranths by pipette to ensure capture. For each set of salinity and temperature combinations, the number of hydranths (and hydranth buds) produced over the first 36 days following explant, was recorded at 3 day intervals.

2.2.3.3 *The Effect of Light*

The effect of light on the rate of 'colony growth' (measured as hydranth production) in *Tubularia larynx* was also monitored at 3 day intervals over a 36 day experimental period. Three sets of light conditions were employed:

(i) continuous light (4×10^{-3} lux)

(ii) continuous darkness

(iii) 'shaded' conditions, where the light intensity was reduced to 4×10^{-2} lux.

The control tank was exposed to a 12 hour daylength period (at 4×10^{-3} lux). Twelve excised hydranths plus basal stolon, were again used for growth observations in each experimental tank. All four tanks were maintained at 12°C., with full salinity sea water (34°/oo) and the feeding regime remained at one first stage *Artemia* nauplius per hydranth per day. The water in each tank was gently aerated and changed daily.

2.2.3.4 *The Effect of Ration*

A parallel experiment was carried out to investigate the effects of increased feeding frequency (ration) on the temperature-salinity curves in Experiment One (see 2.2.3.2). The growth of *Tubularia* at the two higher temperatures of 18°C., and 12°C., only was studied at each salinity level. Each experimental tank contained 12 newly cut *Tubularia* hydranths (from 'stock' colonies) explanted onto perspex slides. For each set of experimental conditions a 12 hr., daylength period (light intensity 4×10^{-3} lux) was imposed and the water in each tank gently aerated, and changed daily. Developing *Tubularia* were

fed 3 times a day (one *Artemia* nauplius per hydranth, three times daily) by pipette. The rate of growth of the developing colonies, measured as the rate of hydranth production was monitored over a 36 day period of observations at 3 day intervals.

In all experiments of *Tubularia* growth, where numerical data were collected, means and standard deviations of replicates were calculated. Appropriate significance tests were carried out using analysis of variance techniques.

2.2.4 *Light Microscope Studies*

In this part of the study a range of standard histochemical tests were used to investigate the nature of adhesion to the substrate in *Tubularia* actinulae. Most methods were used with resin sections. Details of fixation and preparation of wax embedded material appear in Appendix 2.

Actinula larvae were fixed in phosphate buffered formalin (Carson, Martin and Lynn, 1973) for four hours at room temperature. Dehydration through to 3 changes of absolute ethanol preceded embedding in a 2-hydroxyethyl methacrylate (HEMA) resin. Serial sections (1-2 μm thick) were dry cut on a Reichart-Jung 'Autocut' bench microtome and floated on a 60^{o}C water bath before mounting on glass slides.

Sections stained strongly in 1% toluidine blue in borax, and were used to reveal morphological details in the actinula larvae. These sections were examined under an Olympus-Vannox Photomicroscope and photographs taken using Ilford Pan f film at 32 A.S.A. Negatives were developed using 'Perceptol' and 'Acufix' reagents to the manufacturer's recommendations.

In addition a range of histochemical methods were carried out (Table 2.2.2). According to Berrill (1949) chemical tests for chitin were crude and difficult to interpret, whilst Hackman (1964) could find no histochemical method for chitin suitable for sectioned tissues. However, Dennell and Malek (1955) and more recently, Chapman (1968) have used Mallory's triple stain to identify tanned and untanned chitin in the cuticle of the cockroach *Periplaneta*, and the podocyst cuticle in *Aurelia*, respectively. Chapman (1968) outlined the action of Mallory's stain on chitinous tissue and found that, as a result of the physical properties of the dye, heavily tanned tissue did not react, moderately tanned chitin was stained red, and untanned chitin was stained deep blue. However, earlier observations by Manton (1941) and Pyefinch and Downing (1949) in *Obelia* and *Tubularia* reported that the perisarcal sheath in these hydroids stained blue with Mallory's. Pearse (1968) found the alkaline tetrazolium method, although "grossly unspecific" was positive to all chitins he tested. Chitin free from phenolic substances (and also SH groups and reducing lipids) gave an intense blue reaction, whilst tanned chitin did not react. Tanned protein-chitin complexes were dispersed in 10% sodium hypochlorite solutions (Brown, 1950b), and Chapman (1968) reported that the podocyst cuticle in *Aurelia* was 'dissolved' within 33 hours.

Staining methods for the histochemical techniques of Table 2.2.2, followed Pearse (1968) and no modifications were necessary. Settled *Tubularia* (for studies of the 'stolon bud' and 'primary' stolon tissue) were obtained from actinulae settled on polycarbonate membrane filters (Bio-Rad Laboratories, Richmond, U.S.A.). These specimens were fixed and embedded as previously described. A number of permanently settled polyps (i.e. up to 24 hours after 'settlement

attachment') were exposed to 10% sodium hypochlorite solutions in sea water and the rate of 'dissolving' observed.

A further series of whole mount histochemical methods (Knight, 1968, 1970, 1971) were carried out to investigate the possible presence of a phenolic tanning process in the settlement of *Tubularia* actinula larvae (Table 2.2.3). Material was fixed in 4% glutaraldehyde in 0.1M sodium cacodylate buffer containing 0.25M sucrose solution (pH 7.2) for 2 hours at room temperature before transfer to the histochemical reagents.

2.2.5 *Electron Microscopy*

2.2.5.1 *Transmission Electron Microscopy*

Actinula larvae, newly settled larvae and young polyps were fixed for 2 hours at 4°C., in 4% glutaraldehyde in 0.1M sodium cacodylate or 0.2M Sorensen's phosphate buffer containing 0.25M sucrose solution (pH 7.2). Settled polyps showing attachment of the aboral pole to polycarbonate membrane filters were used. After rinsing in buffer and sucrose solution, post-fixation was carried out in 1% osmium tetroxide in 0.1M sodium cacodylate or 0.2M phosphate buffer (pH 7.2) at 4°C., for one hour. Following thorough rinsing in the buffer and sucrose solution, dehydration was carried out in an ethanol series followed by a brief wash in propylene oxide before embedding in Spurr's resin (Spurr, 1969).

Specimen blocks were sectioned on a Porter-Blum MT2-B Ultramicrotome on to water. The sections were expanded with Chloroform vapour and gold/silver sections transferred to uncoated 100 μm copper grids (Taab Laboratories, Reading). The sections were stained for 15

minutes each in uranyl acetate and Reynolds lead citrate prior to examination under a Philips EM300 Transmission Electron Microscope operated at 80 Kv. Photographs were taken using Kodak D19 developer and Kodafix fixative to the manufacturer's recommendations.

2.2.5.2 *Scanning Electron Microscopy*

Free *Tubularia* actinula larvae, newly settled ('temporary attachment') and young polyps ('settlement attachment') on polycarbonate membrane filters and thin perspex slides (1mm thick) were collected. Fixation methods were the same as already described in the section on transmission electron microscopy (2.2.5.1). Following gradual dehydration through a series of ethanols ranging from 5% to 3 changes of absolute ethanol the specimens were critically point dried and mounted on S.E.M. stubs with silver dag paint. The specimens were then gold coated (15 nm) in a Polaron sputter coating unit before examination in a Jeol JSM 35 Scanning Electron Microscope operated at 25 Kv.

2.3 *Settlement and Growth in Pomatoceros triqueter (L.)*

Experiments designed to investigate the larval development and settlement, and early 'tube growth' in *Pomatoceros triqueter* were carried out. Large numbers of larvae were readily obtained for laboratory observations from only a few adult tubeworms.

Adult *Pomatoceros triqueter* individuals growing on loose stones were collected from the sub-littoral zone at Wembury Bay, South Devon. Selected animals were divided into groups and acclimatized over 7 days to one of each of the following temperature and salinity combinations.

(i) $20^{o}C.$, $34^{o}/oo.$, $30^{o}/oo.$, $26^{o}/oo.$, $22^{o}/oo.$, $18^{o}/oo.$

(ii) $15^{o}C.$, $34^{o}/oo.$, $30^{o}/oo.$, $26^{o}/oo.$, $22^{o}/oo.$, $18^{o}/oo$

(iii) $10^{o}C.$, $34^{o}/oo.$, $30^{o}/oo.$, $26^{o}/oo.$, $22^{o}/oo.$, $18^{o}/oo.$

(iv) $6^{o}C.$, $34^{o}/oo.$, $30^{o}/oo.$, $26^{o}/oo.$, $22^{o}/oo.$, $18^{o}/oo.$

Acclimatization was carried out in controlled environment cabinets where the animals were exposed to a 12 hour daylength period in well aerated tanks.

2.3.1 *Larval Development*

Following the seven day period of acclimatization, the calcareous tubes were carefully prised from the stones and adult worms removed from the tubes wholly intact. Within each salinity and temperature group, male and female worms were separated. Groups of tubeless worms were placed into crystallizing basins containing natural sea water at their respective salinity/temperature combinations. The removal of worms from their tubes invariably led to the release of gametes from the abdominal segments. Eggs from 3 to 4 females were transferred to a 50ml beaker at each temperature/salinity combination and fertilized by mixing with a few drops of sperm solution obtained from 2 to 3 males. Each beaker was covered. After one hour the fertilised eggs were removed to 500ml beakers and allowed to develop. Within twenty-four hours after fertilization large numbers of motile larvae from each set of temperature and salinity conditions were transferred by pipette to 12 beakers. Each group of larvae were transferred by pipette to fresh sea water every three days.

By day 16 after fertilization, many larvae were exhibiting settling behaviours (i.e. 'creeping' along the bottom of the vessels and performing 'temporary attachments' on occasions and a few individuals had settled and secreted a clacareous tube in the group held at $20^{o}C/34^{o}/oo$. Day 16, therefore, was considered as the earliest end of the larval period under the experimental conditions employed. On day 16 the surviving larvae from each temperature and salinity category were transferred to their respective settlement tanks. During the 16 day larval development period the mean length of the *Pomatoceros* larvae (the mean of 10 observations) from each temperature/salinity group was measured every 4 days by split-image microscopy.

Throughout the larval rearing process, the animals were fed once daily with the green unicellular alga, *Isochrysis galbana*, and maintained under a 12 hour daylength period (at 4×10^{-3} lux). Feeding levels were constant at 2ml/vessel/day at a concentration of 2,000-3,000 cells per ml to day 10. After day 10, the larvae were fed at the rate of 5ml per vessel per day, at a concentration of 4,000-5,000 cells per ml.

2.3.2 *Laboratory Settlement*

In the laboratory, the settlement of *Pomatoceros* was minimal on a range of materials, including smooth and rough perspex, wood and tufnel. However, appreciable numbers readily settled on 'slides' of old roofing slate (10 cm x 3 xm). At each temperature and salinity combination, the slate was allowed to build up a microbial slime film (over 3 to 4 days) prior to transfer to the respective settlement tanks. In each experiment, settlement tanks were provided with two tiers of slate 'slides'. One layer covered the bottom of the tank, and another layer was placed in mid-water. The slates were arranged in such a way

as to ensure both tiers received incident light when applicable (figure 2.3.1).

2.3.2.1 *The Effect of Temperature and Salinity.*

Experiments on the effects of different temperature and salinity combinations on larval settlement in *Pomatoceros* were restricted to combinations of 4 temperatures (20°C., 15°C., 10°C., and 6°C.), and three salinities (34°/oo, 26°/oo, and 18°/oo). In the reduced salinity tanks the calcium concentration was maintained at 'normal', full salinity levels (430 mg/L) by adjustment with $CaCl_2$. Each tank contained 6 litres of water and a 12 hour daylength period (at 4 x 10^{-3} lux) was maintained. Feeding levels remained at 5 ml/tank/day of *Isochrysis galbana*, at a concentration of 4,000-5,000 cells per ml.

Up to 200 larvae were transferred to each settlement tank (the minimum number used was 116, at 10°C.,/26°/oo. The percentage settled worms, 48 hours after transfer to the settlement tanks was recorded in each set of temperature and salinity conditions, by observing the number of calcareous tubes. Larvae settling on the sides or bottoms of the tanks were included in the count. Under each set of conditions any larvae which settled over a further 3 day period from day 18 to day 21 were also recorded.

2.3.2.2 *The Effect of Hypercalcinated seawater.*

A parallel series of experiments was carried out with the larvae reared under the same 12 temperature and salinity combinations. Feeding levels and aeration remained constant. On day 16 in the experiment, the larvae were transferred to their respective settlement tanks, and

the calcium concentration then adjusted to 490 mg/L., using Calcium Chloride. The amount of $CaCl_2$ needed to make the adjustment was calculated after initial concentrations had been estimated using a flame photometer. Re-adjustment to target salinity was carried out with distilled water. The % settlement in each experimental tank was again recorded at day 18 and between day 18 and day 21 after fertilization.

2.3.2.3 *The Effect of Light*

Four sets of light conditions were used to investigate the effect of light on the settlement of *Pomatoceros triqueter* larvae. In each case, the larvae were reared, and settlement tanks maintained at $20^{o}C.$, in full salinity ($34^{o}/oo$) sea water. Aeration and feeding levels in the settlement tanks (and the feeding levels during larval rearing) remained as previously described.

Groups of one hundred larvae were transferred to settlement tanks for each set of observations. Group I larvae were reared under conditions of continuous light (4×10^{-3} lux), and Group II larvae under conditions of continuous darkness. Group I larvae were transferred to a settlement tank maintained in conditions of continuous light (4×10^{-3} lux) on day 16, whilst the settlement tank for Group II larvae was kept in total darkness. Group III larvae were reared in a continuous light regime (4×10^{-3} lux) until day 16, after which they were transferred to a settlement tank held in conditions of continuous darkness. Group IV larvae were reared under a 12 hour daylength period (4×10^{-3} lux) until day 16. They were then transferred to a settlement tank exposed to a 12 hour daylength regime (4×10^{-3} lux). However, half the settlement tank here was shaded with black polythene.

Under each set of light conditions, the number of *Pomatoceros* larvae which had settled by day 18, and between days 18 and 21 after fertilization was recorded.

2.3.2.4 *The Effect of Resident Pomatoceros triqueter*

Larvae were reared at 20^{o}C.,/34^{o}/oo salinity, under a 12 hour daylength period (at 4×10^{-3} lux), and 100 larvae transferred to each of 3 settlement tanks, maintained under the same conditions of temperature, salinity and light, on day 16 after fertilisation. Feeding levels during larval rearing and in the settlement tanks remained as previously described.

Three pairs of slate 'slides' were placed on the bottom and a further 3 pairs in mid-water in the settlement tanks, in the usual arrangement (see figure 2.3.1). Each pair of 'slides' consisted of an experimental surface (a slate with attached resident individuals of *Pomatoceros triqueter*) and a control surface (a 'clean' slate, with no other organisms attached, save the 'slime film'.) The number of *Pomatoceros* larvae settled by day 18 and between days 18 and 21 was again recorded.

2.3.3 *Field Settlement*

Field observations on the settlement of *Pomatoceros triqueter* were carried out using the 'turtle' raft belonging to International Marine Coatings Ltd., moored in the river Yealm, South Devon (see Figs. 2.2.1 and 2.2.2). Four settlement panels (25 cm x 25 cm), one each of rough perspex, smooth perspex, wood and tufnel were fixed to each of the waterline, 'turn' and bottom facets of the raft. Observations were

carried out over the period March 1979 to February 1980, at monthly intervals, when the conditions of temperature and salinity, over 1M depth, at the raft site were recorded, during both high and low tides. The number of *Pomatoceros* settling in each month was recorded. Tubes were marked with waterproof ink to facilitate the counting of new individuals. At this time also, any other growth on the settlement panels was removed so that 'free space' was always available to settling *Pomatoceros* larvae.

2.3.4 *Laboratory Growth*

In the laboratory, settlement of *Pomatoceros* was not achieved on any substrate other than slate, which was not suitable for subsequent growth studies which were to involve frequent re-weighing procedures. It was found that in the field *Pomatoceros triqueter* readily settled on perspex, both roughened and smooth (T. Black, personal communication). Consequently, roughened perspex slides (7 cm x 2.5 cm) were used in a spat collection array in the river Yealm, South Devon. The roughened perspex slides were weighed prior to exposure and secured to a panel suspended from a paints test raft in the Yealm estuary. A major spat-fall occurred in mid-April, 1979. Slides fouled with *Pomatoceros* were removed as soon as the first (calcareous) tube stages were widely established. In the laboratory, each slide was cleaned, and all growth other than the smallest tube-stage *Pomatoceros* individual removed.

A number of experiments were carried out to investigate the effects of temperature, salinity, calcium level, daylength and ration on early 'tubegrowth' in *Pomatoceros*. The index of growth used was the increase in frash weight with time.

2.3.4.1 *The Effect of Temperature, Salinity and Calcium.*

Groups of twenty newly settled *Pomatoceros* from the spat collection array were acclimatized in the laboratory, over 5 days to one of each of the following temperature and salinity combinations:

(i) At 20°C., 34°/oo., 26°/oo., 18°/oo and 10°/oo.

(ii) At 15°C., 34°/oo., 26°/oo., 18°/oo and 10°/oo.

(iii) At 10°C., 34°/oo., 26°/oo., 18°/oo and 10°/oo.

(iv) At 6°C., 34°/oo., 26°/oo., 18°/oo and 10°/oo.

Following the period of acclimatization, each slide was carefully cleaned to remove all traces of algae, blotted dry and re-weighed. The fresh weight of the young worms plus their calcareous tubes was then recorded. Hence the starting weight (in all subsequent 'tubegrowth' experiments) was that of worms approximately 7 days old, and formed the baseline for growth rate observations.

Groups of 10 worms from each temperature and salinity category were transferred to another series of tanks at their respective temperature and salinity combinations. In this second experimental series, in each set of conditions, the environmental concentration of calcium was increased by the addition of appropriate quantities of $CaCl_2$. *Pomatoceros* held at 34°/oo salinity, across the temperature range, were exposed to hypercalcinated conditions (490 mg/L); whilst in the lower salinities, the concentration of calcium was raised to that of full salinity sea water, at 430 mg/L.

Young *Pomatoceros* in each tank were secured to a nylon coated wire mesh frame. The slides were wired at an inclined angle so that the anterior end of the tube opened downwards. At weekly intervals throughout

the 15 week experimental period (or until 50% or more of a tank population had expired), the tubes and slides were cleaned and re-weighed. The worms were transferred to fresh sea water at each temperature/salinity/calcium combination, three times weekly. Each tank was well aerated and maintained under a 12 hour daylength period. Feeding levels were constant at 15 mls/tank/day of *Isochrysis galbana*, at a concentration of 4,000 to 5,000 cells per ml.

2.3.4.2 *The Effect of Light (Daylength Period).*

The effect of varying daylength upon the rate of 'tubegrowth' in *Pomatoceros* was investigated at 4 levels:

(i) 12 hour daylength (4×10^{-3} lux, control).

(ii) 8 hour daylength (4×10^{-3} lux)

(iii) 4 hour daylength (4×10^{-3} lux)

(iv) Continuous darkness.

Each experimental tank contained 10 newly settled individuals on perspex slides, and was maintained at 20^{o}C./34^{o}/oo salinity. The sea water was well aerated, and ration remained at 15 mls/tank/day of *Isochrysis galbana* (concentration, 4,000 to 5,000 cells per ml.). The sea water in each tank was changed twice weekly, and the experimental period was restricted to 8 weeks due to the loss of the *Isochrysis* cultures. Prior to weekly fresh weight determinations, the slides were again cleaned and blot dried.

2.3.5 *Field Growth*

Field growth data was obtained at monthly intervals over a twelve month period from March 1978 to February 1979. Young tube-stage

Pomatoceros were obtained from the spat collection array fixed to a paints trial raft in the river Yealm, South Devon. In the laboratory, the slides were cleaned of all but the youngest (smallest) individual and re-weighed in order to determine the starting weight of each animal. The young worms were randomly allocated to one of 7 groups of twenty individuals. In each group the slides were wired to the inside of small galvanized cages, so that the anterior end of the tube opened downwards. Each cage was then shackled to the riser cable of two buoys.

One buoy (F buoy) was moored in Plymouth Sound. Three cages of 20 animals each were attached to the cable of this buoy. One cage was set at a depth of 1m below the surface, another at 5m and the third at 10m. The second buoy was situated in the mouth of the estuary of the river Tamar, Plymouth (N1 buoy). An additional cage of *Pomatoceros* was attached here, at a depth of 20m, giving the range 1m, 5m, 10m and 20m.

At monthly intervals the cages were retrieved, and the slides transported to the laboratory for cleaning and re-weighing of each animal, before being replaced usually within 24 hours. At the time of cage retrieval measurements of temperature, salinity, light and dissolved oxygen were taken at each depth at both buoy stations.

2.3.6 *Light Microscopic Studies*

Metatrochophore larvae of *Pomatoceros triqueter* were fixed, embedded in HEMA resin and dry sections cut as previously described in the light microscopy of *Tubularia larynx* (see 2.2.4). A range of histochemical methods were employed (using 2 um thick sections) to investigate the nature of the various gland cell masses of the metatrochophore, and are listed in Table 2.3.2. Staining schedules followed Pearse (1968) and no modifications were necessary.

2.3.7 *Electron Microscopy*

For T.E.M. the best results were observed when metatrochophore larvae were fixed in 3% glutaraldehyde in 0.2m Sorensen's phosphate buffer containing 0.25m sucrose solution (pH 7.2), for 2 hours at $4^{o}C$. After rinsing in the buffer and sucrose solution, post fixation in 1% osmium tetroxide in 0.2m phosphate buffer and sucrose for 1 hr. at $4^{o}C$. was carried out. Embedding, sectioning and examination of material followed the same procedures as described in the T.E.M. of *Tubularia* actinula larvae (see 2.2.5.1).

Scanning electron microscopical studies of both metatrochophore larvae and newly settled (tube-stage) *Pomatoceros* were carried out. Settled individuals were carefully prised from slate 'slides' together with a small piece of slate using a scalpel. Fixation methods were the same as previously described for T.E.M. (above). After gradual dehydration through a series of ethanols specimens were critically point dried and mounted on S.E.M. stubs with double-sided sticky tape, gold-coated (15 nm) and examined in a Jeol JSM 35 Scanning Electron Microscope operated at 25 Kv. Some of the newly settled specimens were selected for X-ray analysis using the Jeol JSM 35 operated at 25 Kv (spot size c. 10 nm) together with a Link Systems (Basingstoke, England) X-ray microprobe analysis unit.

Figure 2.1.1

U.K. Sites Distribution.


20+
21
22
-24
25
-27
18
+19
32
28
+29
30
+31
33
34
16
+
17
35
-38
14+15
9-13
5-8
3+4
1+2

Table 2.1.2

U.K. Sites

Site		Position
1+2	Culdrose	50° 10'N;5° 03'W
3+4	Fowey	50° 06'N;4° 26'W
5-8	Portland	50° 28'N;2° 40'W
9-13	Solent	50° 40'N;0°59'W
14+15	Cardigan Bay	52° 11'N;4°48'W
16+17	Isle of Man	53° 21'N;4° 40'W
18+19	Raasay Sound	57° 21'N;6° 7'W
20+21	Loch Ewe	57° 51°N;5°40°W
22-24	Sound of Harris	56° 58'N;6°57'W
25-27	St. Kilda	56° 48'N;8°34'W
28+29	North Sea No.1.	56° 20'N;1°00'W
30+31	May Island	56° 10'N;2°16'W
32	Moray Firth	57° 41'N;4° 08'W
33	Tees Fairway	54°41'N;4° 08'W
34	Herd Sands	54° 32'N;0°41'W
35-38	Flamborough	53° 55'N;0° 03'W

Figure 2.1.3.

Norway Sites Distribution.

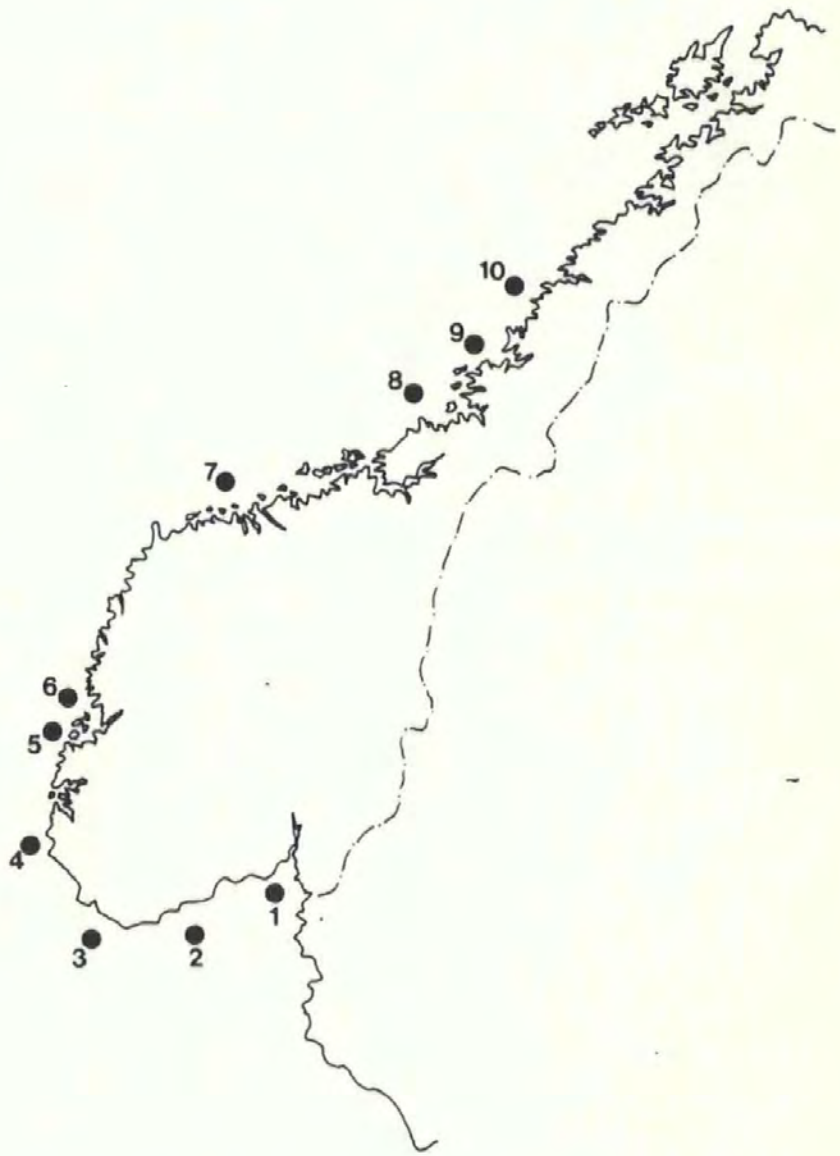

10
9
8
7
6
5
4
3
2
1

Table 2.1.4.

Norway Sites

Site		Position
1	Sandfjord	58° 59'N;10° 19°E
2	Farsund	58° 01'N;6° 56'E
3	Kristiansand	58° 12'N;7°58'E
4	Stavangar	58° 46'N;5° 25'E
5	Bergen 1 (Blømo)	60° 29'N;4° 50'E
6	Bergen 2 (Alvø)	60° 36'N;4° 58'E
7	Vigra	62° 58'N;6° 06'E
8	Vikna	64° 28'N;10° 15'E
9	Leka	65° 10'N;11° 31'E
10	Vega	65° 36'N;11° 34'E

Figure 2.1.5

Buoy sampling sites.

Sketch of buoy site structures, A(U.K. buoys), B (Norway buoys) and showing depth zones, in metres, n.b. riser cable was sampled at regular depths to the sea bed.

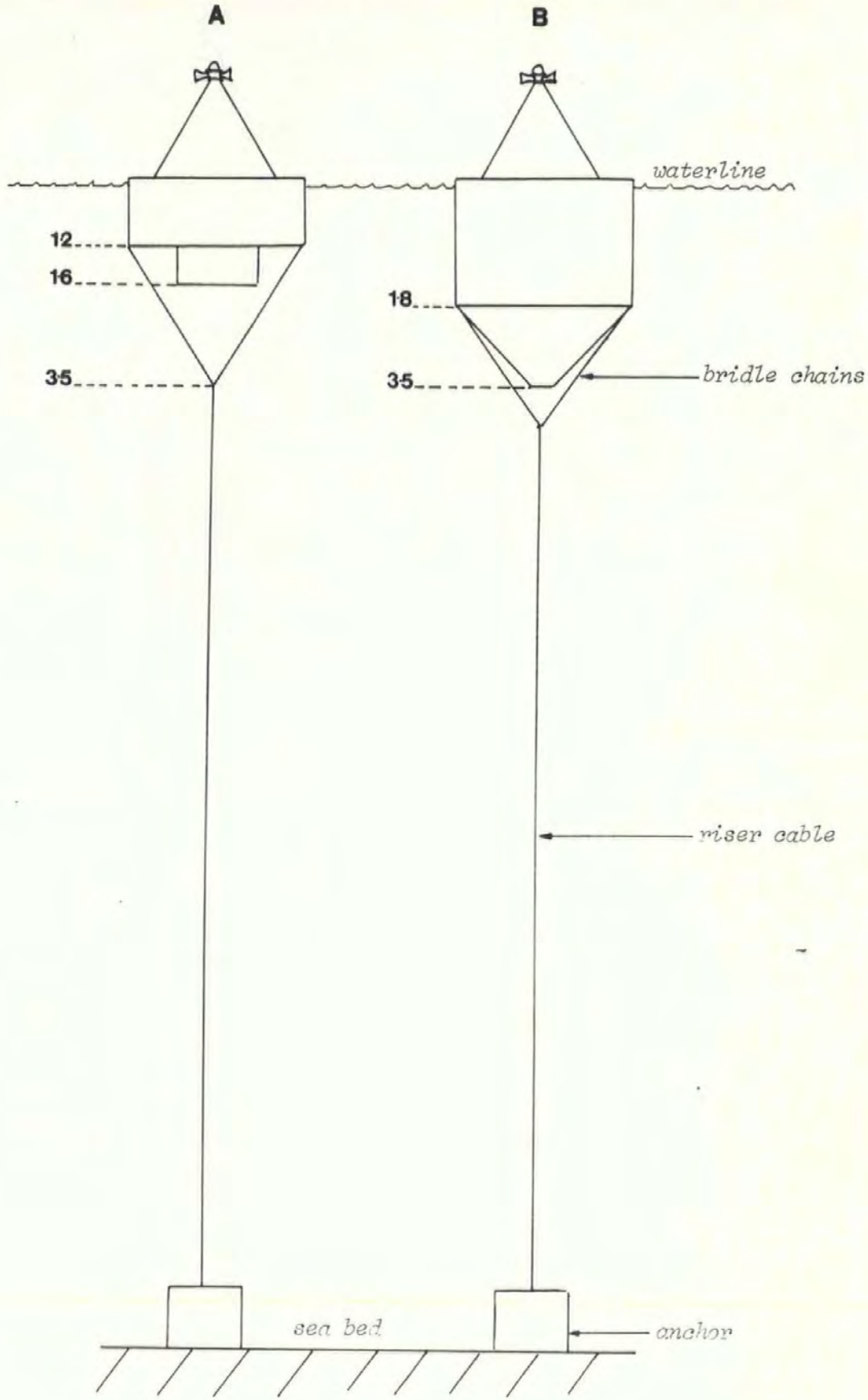

A
B
waterline
12
16
18
35
35
bridle chains
riser cable
sea bed
anchor

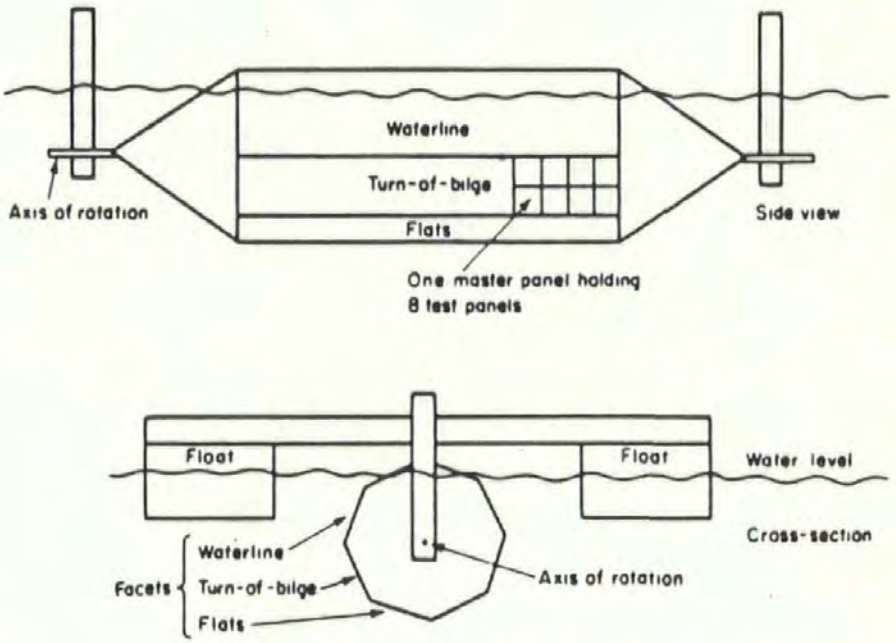
Figure 2.2.1.

Tubularia field settlement

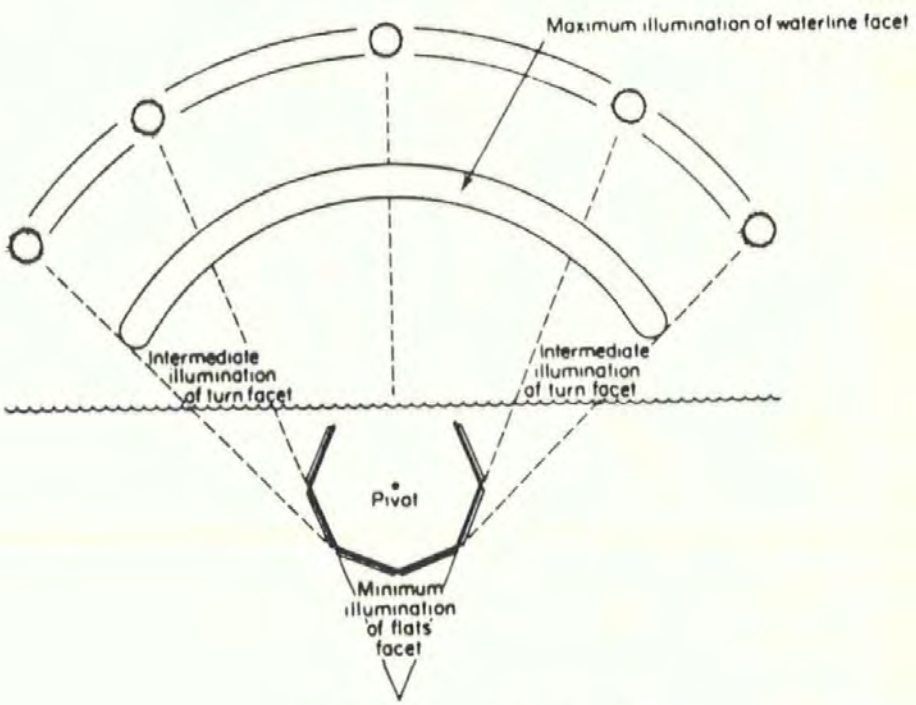
Sketch of turtle raft (from Lovegrove, T., 1978).

(a) Layout of raft

(b) Principle of turtle action.



a. Schematic layout of a Turtle raft.



b. Principle of Turtle action.

Table 2.2.2.

Settlement in Tubularia larynx.

Histochemical methods.

(a) Carbohydrates

Proteins

Lipids

Chitin.

Table 2.2.2.

Chitin	Groups identified	Reference
Alkaline tetrazolium	protein/SS and SH groups	Pearse (1968)
Mallory's triple stain	mucosubstances	Gurr (1962)
(a) Carbohydrates		
Periodic acid-Schiff (PAS)	MPS, vic-glycol groups	Pearse (1968)
Lead tetra-acetate-Schiff	MPS	Pearse (1968)
Alican blue pH 1.0	Selective staining	
pH 2.5	for acidic MPS	Pearse (1968)
Alcian blue: critical electrolyte concentration		
(b) Proteins		
Mercury bromophenol blue	Protein amino groups	Pearse (1968)
Diazotization-coupling	Tyrosine	Glenner and Lillie (1952)
Millons (Baker's modification)	Tyrosine	Pearse (1968)
Sakaguchi (Baker's modification)	Arginine	Pearse (1968)

Table 2.2.3.

Settlement in Tubularia larynx.

Histochemical methods

(a) Whole mount methods.

Table 2.2.3

Test	Groups identified	Reference
Chromaffin reaction	phenolic compound	
Iodaffin reaction	" "	
Gibb's reaction	phenolic compound with a free position para to one of the hydroxyl groups	Knight (1972)
Alkaline diazonium coupling	phenolic compound with free ortho or para position	
Vulpians ferric	an orthodihydroxyphenol	
Molybdate method	" "	
Argentaffin	Strong reducing agent	
Catechol oxidase	Phenol oxidase	Johri and Smyth (1956)
Rubeanic acid	Copper	Pearse (1968)

Figure 2.3.1.

Pomatoceros Laboratory Settlement.

Settlement Tank.

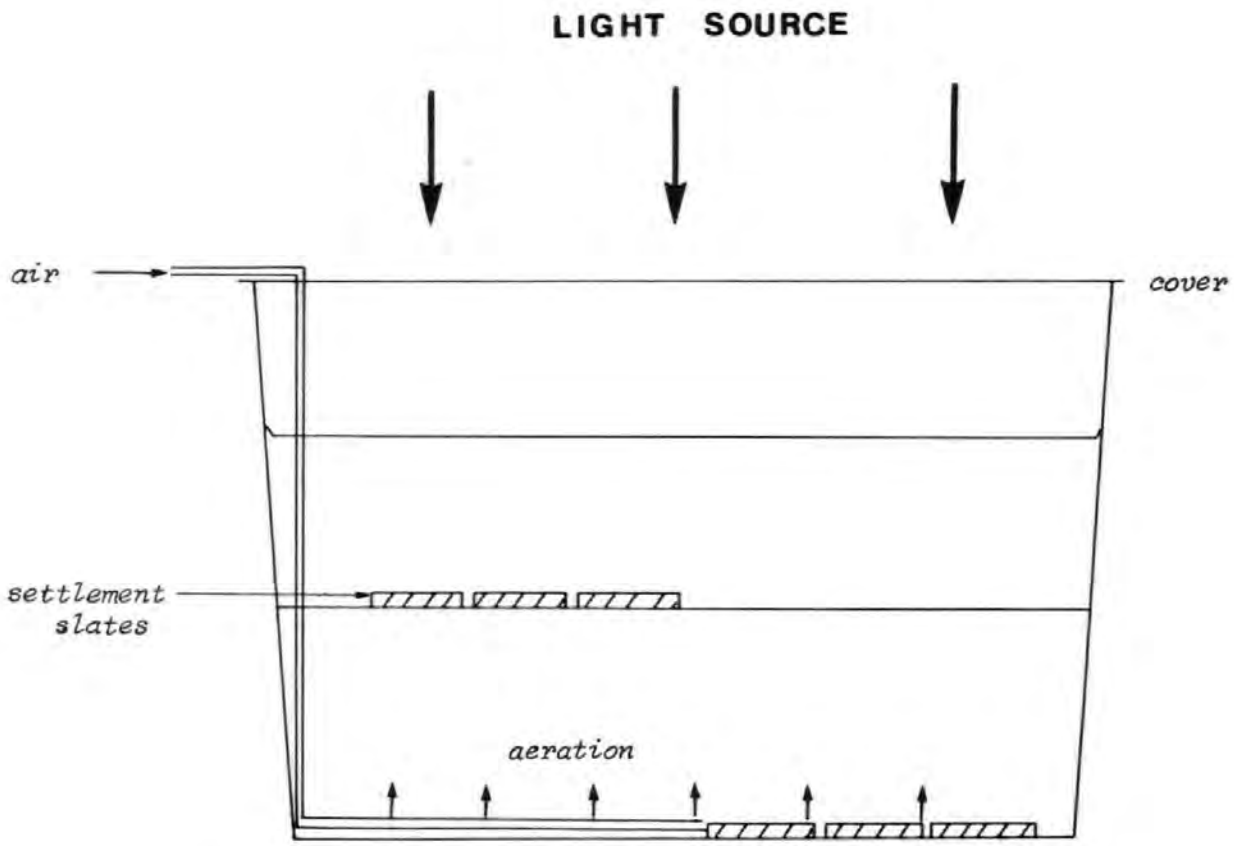

LIGHT SOURCE
air
cover
settlement
slates
aeration

Table 2.3.2.

Settlement in Pomatoceros triqueter.

Histochemical methods.

Table 2.3.2

(a) Carbohydrates

Test	Groups Identified	Reference
Periodic acid-Schiff	MPS, vic-glycol groups	Pearse (1968)
Lead tetra-acetate-Schiff	" " "	Pearse (1968)
Alcian Blue pH 1.0		
" " pH 2.5	Selective staining	Pearse (1968)
Alcian Blue: critical electrolyte concentration	for acidic MPS	
Periodic acid/low iron diamine/AB 2.5 Low iron diamine/AB PA - HID - AB HID - AB	Differentiation of neutral and acidic MPS	Spicer (1965)
Alcian Blue-Alcian Yellow (AB - AY)	Differentiation of sulphated and non-sulphated MPS	Ravetto (1964)

(b) Proteins

Mercury Bromophonol Blue	Proteins, amino acids	Pearse (1968)
Millons (Baker's modification)	Tyrosine containing proteins	Pearse (1968)

(c) Lipids

Sudan Black	Lipids	Pearse (1968)

(d) Calcium

Alizarin Red S	Calcium deposits	Dahl (1952)
Calcium Red	" "	McGee-Russell (1955)

CHAPTER 3

Fouling Species Distribution

The type of fouling community encountered was found to vary from site to site. However, besides these geographical variations, the vertical distribution of fouling organisms was found to be generally similar. Although the depth of penetration and abundance of different algal and faunal groups varied from site to site, the general pattern of the vertical distribution of fouling organisms was as follows.

The 'splash-zone' was often colonized by slime-like growths of filamentous chlorophytes (e.g. species of *Codium*, *Enteromorpha* and *Cladophora*). Occasionally, brown algae such as *Phaeosaccion collinsii* and species of *Ectocarpus*, and some filamentous red algae were also found in this zone. Overall, the macro-algae were most abundant in the first 3 to 4 metres. The chlorophyta were dominant from the 'splash-zone' to 0.5 m depth, whilst below this zone the phaeophyta and rhodophyta were more abundant. Most algae were absent from the underside of the buoys at 1.2 m, but often some large plants such as species of *Laminaria*, *Porphyra* or *Ulva* were attached to the rim of the base. Representatives of the green, brown and red algae penetrated as deep as 10 - 12 m, but generally the weight of algal fouling decreased with depth.

The general pattern of animal fouling on the buoys was one of moderate intensity in the first metre. Often, a number of amphipods and pycnogonids were associated with the algal growths occupying the 'splash-zone'. *Balanus* species frequently colonized the sides of the buoys to a depth of 1.2 m. Typically, mussel fouling was often found below the zone of green algae and either formed dense assemblages or

occurred in discontinuous 'patchy' bands interspersed with *Balanus* species and the algal community. Mussel fouling was also frequently abundant on the underside of the buoy. At 1.2 m, the underside of the buoy, animal fouling was usually at its greatest, both in diversity and abundance. Here, the soft-bodied forms such as sponges, anemones, hydroids and tunicates were well represented, and starfish were frequently found. In addition, tubeworms (predominantly, *Pomatoceros triqueter)* often occupied large areas of this zone. Animal fouling extended through the water column to the sea bed. Mud-tubes of the semi-sessile amphipod *Jassa falcata* were sometimes found covering much of the surface of the cables and although usually with a patchy vertical distribution, *Jassa* was commonly observed. The anchor, or sinker, often supported a dense assemblage of encrusting animal organisms which was typically markedly different from the faunal assemblages of the buoy and cables. On the mooring chains, or cables, the termination of fouling at the lower limit was sharply defined, due to the scouring action of the cable on the sea-bed during tidal fluctuations and storms.

3.1 *U. K. Sites*

A total of 383 species of animals and macro-algae have been identified from the marine fouling communities of offshore sites (buoy stations) around the U.K. 149 species of algae were found representing the Chlorophyta, Phaeophyta and the Rhodophyta. The 234 animal species classified were included in 10 phyla: Porifera, Coelenterata, Platyhelminthes, Nemertea, Annelida, Arthropoda, Mollusca, Polyzoa, Echinodermata and Urochordata. The species-sites list appears in Appendix 3.

Site affinities based on the log transformed biomass of the species

(reduced data) were defined using the Bray-Curtis measure of similarity and group-average sorting (figure 3.1.1). At this general level of classification 8 groups of sites were defined. (Table 3.1.2). Site group D is further divided into 5 sub-clusters or sub-groups. A principal components analysis, using the same similarity matrix as above gave the cluster diagram in figure 3.1.3, which showed essentially the same result as the dendrogram. The first two components, however, accounted for only 78.6% of the total variability. The general pattern of fouling community structure in the site groupings revealed by the hierarchical clustering techniques, and the vertical distribution of the major component groups are illustrated in figures 3.1.4 to 3.1.15. These groupings were confirmed using Twinspan (Hill, 1979), which also identified a number of characteristic species (or 'positive preferentials') and indicator species for each site group. When buoy stations were sampled over successive years the species composition of the fouling growths was often very similar, resulting in distinctive communities which were characteristic of particular sites and site groups.

(i) *Site group A. (S.W., English Channel sites)*

Algae from the Chlorophyta, Phaeophyta, and Rhodophyta were predominant in the first metre, and the Chlorophyta typically penetrated to a depth of 14 m. Also typical of the fouling communities of this site group was a large weight of animal fouling which was present through the water column and extended to the sea bed. Annelids, almost entirely *Pomatoceros triqueter*, formed a large part of the animal fouling. Arthropods and the Mollusca were also abundant at these sites. Notably, the Ascophoran polyzoan, *Watersipora complanata* (previous

northerly limit, Scilly Isles, Hayward, 1980) was found encrusting the anchor of the Culdrose buoy in 1981. Classification with Twinspan (Hill, 1979) identified the molluscs, *Hiatella arctica, Musculus discors* and *Modiolus barbatus* as indicator species, whilst for this division *Codium bursa, Chondrus crispus, Plocamium cartilagineum, Lepas anatifera, Bugula fulva* and *Bugula neritina* were the characteristic species or 'positive preferentials'.

(ii) *Site Group B (S., English Channel sites)*

At these sites, the intensity of algal growth was similar to that observed amongst the buoy stations of site group A. The Chlorophyta were dominant in the first metre and penetrated to a depth of 15m. Typically, the animal fouling was made up principally of Mollusca, whilst the fouling of both the Annelida and Arthropoda was reduced in intensity. The Coelenterata were represented from 1.2 m to 20 m depth. Classification with Twinspan (Hill, 1979) identified the hydroids *Plumularia halecoides* and *P. setacea,* and the annelid *Lanice conchilega. Antithamnion floccosum, Plocamium cartilagineum* and *Balanus crenatus* were the characteristic species or 'positive preferentials'.

(iii) *Site group C (South, English Channel sites)*

Typically, the fouling communities of these sites were usually less productive, and low levels of abundance in the Phaeophyta, Porifera, Coelenterata. Polyzoa, Echinodermata and Urochordata, were recorded. The Arthropoda, principally *Balanus crenatus,* were a prominent component of the fouling communities from 1.2 m to 16 m, whilst the Mollusca were dominant from 10 m to 16 m depth. Classification with Twinspan (Hill, 1979), identified the molluscs, *Acanthochitona crinatus* and *Crepidula fornicata* as indicator species, and *Chondrus crispus,*

Plocamium cartilagineum and *Balanus crenatus* were the characteristic species or 'positive preferentials'.

(iv) *Site group D : sub- cluster (1) (Western sites)*

Typical of this site group was the heavy mussel growths which were observed from 0.5 m to 14 m depth. The Chlorophyta were dominant in the first 0.5 m depth and extended to 10 m; whilst the Phaeophyta and Rhodophyta were less well represented. Classification with Twinspan (1979), identified the coelenterate *Sagartia sphyrodeta* as an indicator species, whilst *Sagartia troglyoides, Balanus crenatus* and *Mytilus edulis* were listed as the characteristic species or 'positive preferentials'.

(v) *Site group D : Sub-clusters (2) and (3) (North-western sites)*

These north-western sites were typified by moderate to heavy algal fouling and a comparatively reduced intensity of animal fouling. In both sub-clusters the Chlorophyta, Phaeophyta and Rhodophyta were equally well represented, and in sub-cluster 2, the Chlorophyta extended to 12 m depth, whilst the brown algae extended to 14 m. Classification with Twinspan (Hill, 1979) identified *Callithamnion arbuscula* and *Sacchorhiza polyschides* as indicator species in sub-cluster 2, whilst *Laminaria digitata, Balanus balanoides, Balanus crenatus* and *Scuparia chelata* were all characteristic species, or 'positive preferentials'. In sub-cluster 3, the indicator species were *Sacchorhiza polyschides* and *Crisia eburnea* together with the characteristic species or 'positive preferentials' of *Obelia geniculata, Balanus balanoides* and *Balanus crenatus*.

(vi) *Site group D ; sub-clusters (4) and (5) (North-eastern and East Coast sites)*

Buoy stations in both these site group sub-divisions were much less heavily fouled with algal growths, although large plants of *Laminaria saccharina* were observed. The red algae were particularly sparsely represented. The Chlorophyta were confined to the first 1.2 m and 1.6 m in sub-clusters 4 and 5 respectively. Of the animal fouling the Coelenterata, chiefly hydroids, formed a relatively large component in each case and extended from 1.2 m to 18 m and the sea-bed. Tubiculous annelids, mainly *Hydroides elegans* had colonised the riser cable and were also found encrusting the anchor at 20 m at the sites of sub-cluster 5. Classification with Twinspan (Hill, 1979) identified the hydroid, *Syncoryne eximia,* and the tubeworm, *Hydroides elegans* as indicator species, and *Petalonia fascia, Punctaria lattifolium* and *Tubularia larynx* were the characteristic species or 'positive preferentials'. From the east coast sites of sub-cluster 5, *Laminaria saccharina, Phyllitis fascia* and *P. zosterofolia* were identified as indicator species, whilst *Petalonia fascia, Syncoryne eximia, Tubularia larynx* and *Hydroides elegans* were the characteristic species or 'positive preferentials'.

(vii) *Site group E (North-eastern stations)*

The Chlorophyta were dominant in the first 1.2 m, but were restricted to the first 1.6 m depth. The brown and red algal growths were of moderate intensity and 'patchy' in distribution. The fouling communities of these sites were typified by heavy coelenterate fouling, extending from 1.2 m to the sea-bed. In addition, the Annelida (principally the tubeworms, *Hydroides* and *Pomatoceros)* were a

prominent component of the animal fouling of these sites. Classification with Twinspan (Hill, 1979), identified the coelenterates *Hormathia digitata* and *Tubularia larynx* as indicator species, and *Petalonia fascia, Syncoryne eximia* and *Pomatoceros triqueter* were the characteristic species or 'positive preferentials'.

(viii) *Site group F (North-western sites).*

These shallow-water sites were characterised by intense algal fouling. The Chlorophyta were dominant in the first 0.5 m, but the brown and red algae were more conspicuous deeper. Both the Chlorophyta and the Rhodophyta extended from the 'splash-zone' to 7.5 m depth. The Arthropoda (mainly barnacles) and the Mollusca (mostly *Mytilus)* constituted much of the animal fouling. Typically, the Urochordata also formed a large part of the animal fouling and colonial tunicates covered much of the underside of the buoy at 1.2 m depth. Classification with Twinspan (Hill, 1979) identified *Bryopsis plumosa,* and the colonial ascidians, *Botryllus leachi* and *B. nigrum* as indicator species. *Ceramium rubrum, Dilsea carnosa, Balanus crenatus, Botryllus schlosseri* and *Ciona intestinalis* were the characteristic species or 'positive preferentials'.

(ix) *Site Group G. (North-western sites)*

Intense algal fouling was also typical of the fouling communities at St. Kilda. The Chlorophyta were again dominant from the 'splash-zone' to 0.5 m depth, whilst below this zone, the brown and red algae extended to 10 m depth. A feature of the animal fouling of these communities was an abundant Arthropod component (principally *Lepas anatifera* 1.2 m to 5 m) which was most obvious at 1.2 m and extended to

18m depth. Classification with Twinspan (Hill, 1979), identified *Lepas anatifera* and *Botryllus nigrum* as indicator species, whilst *Hiatella arctica*, and the polyzoans *Scuparia ambigua* and *S. chelata* were the characteristic species or 'positive preferentials'.

(x) *Site group H. (Southern, English Channel, sites)*

Typically, the buoys of site group H, were only moderately fouled and most major fouling groups were equally represented. Algal growths were dominant in the first 5 m depth, and the green algae extended to 10 m depth, whilst *Porphyra* plants were recorded at 12 m depth. Much of the animal fouling consisted of Annelida and Mollusca. The Coelenterata (mainly Anthozoa) were also a conspicuous component of the animal fouling. Classification with Twinspan (Hill, 1979) identified *Asperoccocus bullosus*, *Actinia equina* and *Metridium senile* as indicator species, and *Plumularia halecoides*, *Acanthochitona crinatus* and *Crepidula fornicata* were the characteristic species, or 'positive preferentials'.

A number of 'principal fouling organisms' common to most U.K. sites and often contributing a large proportion of the total biomass of each site were recorded. These species included the following green, brown and red algae (frequency > 80% in U.K. sites): *Enteromorpha intestinalis*, *E. linza*, *Ulva lactuca*, *Laminaria saccharina*, *Ceramium rubrum*, *Polysiphonia nigrescens* and *Porphyra umbilicalis*. Animal species (found at every U.K. site) were: *Tubularia larynx*, *Pomatoceros triqueter*, *Balanus crenatus*, *Jassa falcata* and *Mytilus edulis;* and often extended from the surface waters to the sea bed.

Figure 3.1.16 illustrates the vertical distribution pattern of the fouling growth from a buoy station in site group A. This is a cluster

analysis of the species/depth associations of the fouling community recorded from Culdrose No.1 RTB (1980 collection). Four major species groups were identified (Table 3.1.17). The first group (species 1 to 12) were almost entirely algae and occurred almost exclusively in the uppermost zone from the waterline to 1.6 m depth. The second group (species 3 to 34) of mixed algal and faunal organisms, consisted of species with a patchy or scattered vertical distribution, but all of which occurred within the first 12 m depth. A third, small group of animal species (species 27 to 38) consisted of *Tubularia larynx*, *Mytilus edulis*, *Balanus crenatus* and *Pomatoceros triqueter*. These organisms occupied large parts of the buoy and cables throughout the water column from 0.5 m to 40 m and the sea bed, and were part of the ubiquitous 'pool' species or 'principal fouling species' identified from the U.K. sites. A fourth division (species 39 to 46) was another group of animal organisms (of Polyzoa, Echinodermata and Urochordata) and restricted almost exclusively to the deepest water and the sea-bed (anchor) at 60 m depth.

A notable feature of most of the fouling communities studied was a marked decrease in the abundance, and also the depth distribution, of cirripede arthropods whenever the hydroid canopy (usually almost entirely *Tubularia larynx)* was extensive. However, at those sites where the hydroid cover was not so abundant, dense assemblages of barnacles occupying much of the water column, were often recorded. Figure 3.1.18 illustrates the vertical distribution of hydroid colonies and *Balanus* species at Culdrose RTB 1980 (site-group A, western English Channel) and North Sea RTB 1980 (site group E, north-east). At the Culdrose site (1980 collection) hydroid fouling, although extending from 0.5 m to 20 m depth, was light, and barnacles

(mainly *Balanus crenatus)* occupied almost all the water column reaching a maximum intensity (about 27 g/100 cm^2) at 3.5 m depth. By comparison, at the North Sea site (1980 collection) much denser hydroid growths (almost all *Tubularia larynx)* covered much of the buoy and cables, and barnacle colonization and growth was limited. At this site, barnacle fouling was restricted to 3.5 m depth, and maximum biomass values of only 2 g/100 cm^2 were recorded.

Samples of the fouling communities found at the U.K. sites, indicated the frequency and distribution of both the hydroid *Tubularia larynx*, and the tubeworm, *Pomatoceros triqueter*. Of the 38 sets of samples collected and analysed, *T. larynx* was found in all but 2, and the hydroid was recorded from every site during the period of study. Figure 3.1.19 illustrates the mean biomass/depth profile of total coelenterate fouling and the proportion of *Tubularia larynx* for each of the U.K. site groups demonstrated by cluster analysis. In most of the site groups, *T. larynx* formed a large proportion of the overall coelenterate fouling. The proportion and intensity of *Tubularia* fouling was greatest amongst the fouling communities of east coast and North Sea sites (site groups D, sub-clusters 4 and 5, and E). At these stations *Tubularia larynx* often extended from the first metre depth to the sea-bed. The intensity of coelenterate fouling was a much more moderate feature of the fouling communities of site group A in the western English Channel. However, *Tubularia larynx* still comprised a major proportion of the overall coelenterate fouling and was recorded from 1.2 m depth to 20 m. In the fouling communities of the buoy stations in the remaining site groups, much lower levels of overall coelenterate growth were recorded and the intensity of *Tubularia larynx* fouling was reduced. The tubeworm, *Pomatoceros triqueter*, was also

found at all the U.K. sites and contributed the bulk of Annelid fouling at most stations. Many sites were moderately to heavily fouled with *Pomatoceros*, often from the surface waters to the sea-bed. The buoy stations of site groups B and C (southern, English Channel) and F (north-western, shallow-water) were relatively lightly fouled with *Pomatoceros* (Figure 3.1.20); whereas the heaviest growths of the tube worm were recorded from the fouling communities collected from buoys off the east coast (site group D, 5) in the North Sea (site group E) and in the western English Channel (site group A). The vertical distribution pattern of *Pomatoceros* was variable. Sometimes it was sporadic throughout the water column forming discrete, dense 'patches' of tubeworm (site group G) or the intensity of fouling remained relatively constant with depth (site group D3). Notably the underside of the buoys (at 1.2m) were often particularly well colonized and supported large aggregations of tubeworms. *Pomatoceros* tubes were also frequently found encrusting the anchor on the sea-bed.

3.2 *Norway sites*

A total of 178 species of fouling organisms (74 macro-algae and 104 animal species) were identified from the marine growths collected from the offshore buoys around Norway. The species-sites list appears in Appendix 3. As was the case for samples collected from U.K. sites, some specimens were identified to generic level only. Site affinities based on the log transformed biomass of the species (reduced data) were defined using the Bray-Curtis index of similarity and Wards (error sum of squares) sorting (Figure 3.2.1). A principal components analysis using the same similarity matrix gave similar results (Figure 3.2.2). Three site groups were shown:

Group 1 consisted of stations 1, 3 and 6 (Sandford, Kristiansand and Bergen 2).

Group 2 consisted of stations 2, 4, 5 and 7 (Farsund, Stavanger, Bergen 1, Vigra).

Group 3 consisted of stations 8, 9 and 10 (Vikna, Leka, Vega).

The vertical distribution pattern of the major fouling groups in site groups 1, 2 and 3 is illustrated in figures 3.2.3 and 3.2.5. In each site group algal growths were dominant in the surface waters. Site groups 1 and 2 were the most similar. Buoy stations of site group 1 were heavily fouled with algal growth (green, brown and red algae) from the 'splash-zone' to about 5 m depth. The Chlorophyta extended to 12 m depth. In both site groups 1 and 2 the bulk of the animal fouling (which occupied much of the cables throughout the water column) consisted of annelids (mainly *Pomatoceros triqueter)* arthropods (mainly Balanus species) and molluscs (mainly *Mytilus edulis)*. The buoy stations of site group 3 were typically less heavily fouled. Brown algae were dominant from 0.5 m to 5 m depth and the Chlorophyta extended to 10 m. Coelenterate fouling consisted almost entirely of *Tubularia larynx* which colonised the buoy and cable from 0.5 m to 20 m depth. A number of fouling species, (both flora and fauna) were found at each site. *Cladophora arcta, C. rupestris, Enteromorpha compressa, E. intestinalis, Ceramium rubrum, C. strictum, Laminaria saccharina, Hydroides elegans, Pomatoceros triqueter, Balanus crenatas,* and *Mytilus edulis.*

A feature of the fouling assemblages from the offshore sites of Norway was a relatively large polyzoan component. Typically the polyzoa occurred through a large part of the water column from 1.2 m

to 20 m depth. Seventeen species of polyzoa were identified from the 10 sites sampled and a distinct geographical distribution was recorded. Figure 3.2.6 illustrates the distribution of the polyzoa among the buoy sites, *Esharella* species (*abyssicola, immersa, klugei* and *lacqueata)* were typical of the south and south-western sites (1, 2, 3 and 4). These species were absent from the north-western and western sites (7, 8, 9 and 10) which were characterised by the presence of *Porelloides laevis* and *P. struma* (see figure 3.2.6). Other species with a north western distribution were: *Electra monostachys, Lagenipora pygmaea, Membranipora membranaceae* and *Tegella unicornis.* Two species were found only in the fouling communities from western sites: *Chartella barleeii* and *Haplopoma planum;* whilst *Electra pilosa* was found in each site group.

The frequency and abundance of both *Tubularia larynx* and *Pomatoceros triqueter* in each site group, and their vertical distribution are illustrated in figure 3.2.7. The coelenterate fouling recorded from buoy stations in site groups 2 and 3 was almost entirely *Tubularia larynx,* and extended from 0.5 m to 20 m depth. A number of other hydroids, including species of *Obelia* contributed the bulk of the coelenterate fouling from site group 1. At these stations, *Tubularia larynx* contributed less than 30% of the overal coelenterate fouling, and was found between 1.6 m and 16 m depth. *Pomatoceros triqueter* formed the major component of the annelid fouling in each of the site groups. In both site groups 1 and 2, annelids contributed a large part of the overall animal fouling and were recorded from 0.5 m to 40 m depth. In site group 3, where the fouling growths were less abundant, the weight of annelid fouling (almost all *Pomatoceros triqueter)* was much reduced and restricted to between 1.2 m and 14 m depth.

3.3 *Discussion*

A marked geographical variation in the fouling assemblages associated with offshore buoys around the U.K. was observed. Simple hierarchical agglomerative clustering techniques defined a number of site groupings characterized by particular types of fouling communities. These fouling growths could be broadly divided into East coast and North-Eastern communities, Channel communities and North-Western communities. A general trend in fouling community structure was a West to East and a North to South, compression of the depth of penetration of algal species and decrease in the overall fouling community productivity. However, although variations of species composition and differences in the relative abundance of fouling organisms were observed, many frequently occurring species were recorded including a number of animal and algal species which were found at almost all the sites during successive years throughout the study period. These 'principal fouling' species included many of the organisms believed by Meadows (1969) to be part of a "pool" of potentially important fouling species with a widespread geographical distribution around the northern coasts of Britain. In the present study these 'pool' or 'principal fouling' species usually contributed a large part of the overall abundance of the fouling communities around the whole of the U.K., and were often the dominant growths of much of the buoy surfaces and cables throughout the water column.

The offshore buoys utilised as sites for the collection of marine growths in this study may be seen as transitional between coastal stations (such as piers and harbours) and the deep water oil and gas platforms of the North Sea. As a consequence, the type of fouling

community which develops at these sites will be influenced not only by the factors which govern the development of inshore epibenthic communities (see Crisp, 1976), but will also be influenced by factors such as distance from the shore and depth (Freeman, 1977; Oldfield, 1980). The net effect of depth was generally a gradual reduction in the intensity of the fouling growth and an alteration of the dominant species, and was similar to the vertical distribution pattern recorded in the fouling communities of North Sea oil platforms (Freeman, 1977; Oldfield, 1980; Kingsbury, 1981). Just as rocky shores show zonation of species, a similar situation was observed in the fouling communities of the buoys in this study, where algal growths were dominant in the surface zones but were displaced by mixed animal species with depth. For instance, in the fouling community of the Culdrose site (1980 collection of site group A, see figure 3.1.16 and Table 3.1.17), most of the algal growth was restricted to the first 1.6 m depth where *Enteromorpha* and *Codium* dominated. Here, only sponges (*Sycon* and two encrusting species) and light barnacle fouling occurred. Many animal species occupied the underside of the buoy at 1.2 m depth, and a mixed-species population of algae (mainly *Cladophora, Ulva* and some large plants of *Laminaria)* and animal organisms extended to 12 m depth. The animal fouling contributed the bulk of the growth, and consisted of dense shelled forms (mostly barnacles and bivalve molluscs) and a few soft-bodied forms (mostly hydroids and anemones). Below this zone the remainder of the cable to the sea bed was occupied by a small group of animal organisms only. These species (*Balanus crenatus, Mytilus edulis, Tubularia larynx* and *Pomatoceros triqueter)* were not exclusive to this zone, but were 'principal fouling' species and were found throughout the water column. The anchor supported a discrete community of polyzoans,

echinoderms and tunicates. Although this general trend of animal and algal fouling was observed at each site the extent of each zone was variable (see figures 3.1.4 to 3.1.15) and the vertical distribution of the fouling organisms (particularly the depth penetration of algae) may partly be attributable to light conditions. These findings agree with earlier reports of the influence of light on the zonation of fouling communities on buoys (e.g. Fraser, 1938; Zevina, 1972) whilst earlier, Milne (1940) had found that buoys situated in turbid waters supported reduced algal growths. The buoy stations of site group C may similarly be exposed to turbid conditions in waters close to the large urban centres of Portsmouth and Southampton, which may partly explain both the restricted algal growths and the reduced overall abundance of those communities.

Most of the buoy stations were located in depths of between 20 m and 40 m (up to maximum depths of 60 m) and were laid during the spring and early summer months, i.e. when most invertebrate species and algae are recruited (Houghton, 1978; Oldfield, 1980), although some organisms may settle during the winter months (Houghton, 1978) and colonization may occur all the year round. At the time of sample collection abundant fouling growths of mixed species composition were usually found at the majority of sites, and the number of species and diversity of the fouling communities recorded, indicate that most buoys were rapidly colonised by shore and sub-littoral populations of algae and invertebrates. Amongst the sites where the heaviest fouling growths were recorded were the deepest buoy stations of site groups A and B (South-West and South English Channel) and site group E (North Sea) at 60 m depth. All buoy stations would appear, therefore, to fall within Oldfield's (1980) category of coastal sites, although he reported that

structures beyond the 40 m depth contour were not rapidly fouled by coastal organisms. Settlement from bottom living species may also occur. The buoy station, North Sea RTB (of site group E) was situated almost 60 miles from the coast, and was heavily colonised over successive years by *Tubularia larynx*. Whereas algal spores and developing invertebrate larvae from shore populations drift through normal sea currents in coastal waters and may colonize offshore structures (Freeman, 1977; Houghton, 1978; Oldfield, 1980), many organisms which settle soon after release, e.g. the larvae of some ascidians (van Duyl et al., 1981; Olsen, 1982) as well as the actinulae of *Tubularia larynx* (Pyefinch and Downing, 1949) may not be able to settle on structures situated too far from the coast. Settlement from bottom living colonies of *Tubularia larynx* then, appears to be the source of rapid recolonization of the North Sea buoy station by the hydroid, and agrees with previous observations of *Tubularia* colonization of oil rigs in the North Sea (Leitch, 1980; Ralph and Troake, 1980; Hardy, 1981). This view is further supported by reports of the widespread occurrence of *T. larynx* over the sea bed of the North Sea (Ralph and Troake, 1980; Goodman and Ralph, 1981). Other fouling organisms which are capable of rapid colonization, e.g. Sponges (Ayling, 1980; Curtis et al., 1982) and polyzoans (Houghton, 1978; Oldfield, 1980) may also colonize the buoy sites from bottom living communities, and may partly explain the clear geographical variation in the type of fouling assemblage found amongst the buoy stations.

Whether colonisation of the buoys occurred from shore and sub-littoral populations of algae and invertebrates, or from bottom living communities, or both, most buoy stations were heavily fouled within between 10 to 14 months exposure. Although the general trend of fouling

at each site was a gradual reduction in the intensity of fouling with increasing depth, the underside of the buoys, at 1.2 m depth, often supported the highest biomass levels. These findings confirm the observation of Merrill and Edwards (1975) who observed a similar vertical distribution of fouling growth on offshore buoys of the eastern seaboard of North America. The shaded, underside of the buoys was usually colonised by a large number of animal species and few algae were recorded. Settling invertebrate larvae are known to favour dark habitats such as rock overhangs and under surfaces (see Crisp, 1976) and the undersides of experimental panels (e.g. settling tubeworm larvae, McDougall, 1943; Bosence, 1979). Similarly, gravity and light responses may account for the intensity of settlement of many invertebrate species on the darkened underside of the buoys in the present study. The susceptibility of the buoys to rapid fouling together with the limited available space on the buoy surfaces and cable chains, suggests that the earliest arriving species may pre-empt unoccupied surfaces and deny substrate to subsequent fouling groups. For instance, the heavy fouling by filamentous algae observed in the surface zones of the buoy stations may preclude settlement of other groups by direct competition for attachment space, as frequently observed on the upper surfaces of experimental panels (e.g. Schmidt, 1983ab). In addition, algal fronds have been shown to act as a barrier to the settlement of invertebrate (barnacle) larvae (Hatton, 1938; Southward, 1956; Luckens, 1970). In this way early foulers may come to dominate large parts of the buoy and cables, and influence the recruitment of later species. These assumptions coincide with the views of Oldfield (1980) that the first organisms to colonise new surfaces may dictate the type of fouling community which develops. The density of settlement of many of the

'principal fouling species' in the present study, (including *Tubularia larynx* and *Pomatoceros triqueter*), indicates that these organisms may be amongst the first species to colonise the newly-laid buoys. The intensity of settlement observed in these species (which often occupy large parts of the buoy and cables throughout the water column) may also result in the rapid colonisation of unoccupied surfaces, and if so would appear to confirm the views of Karlson and Jackson (1981) and Scheltema, et. al. (1981).

A possible interference effect by the hydroid *Tubularia larynx* on the settlement of barnacles was observed, (see figure 3.1.18) Where dense growths of *Tubularia* were found (particularly at North Sea sites) barnacle settlement was limited and usually restricted to zones free of *Tubularia*. However, when the buoys and cables were only lightly fouled with *Tubularia*, barnacles were abundant and often found throughout the water column. These findings seem to confirm the earlier observations of McDougall (1943) who noted large numbers of *Balanus improvisus* on surfaces kept free of *T. crocea*, and few barnacles in areas dominated by the hydroid. Standing (1976) has also reported a similar interference effect, in *Obelia*, on barnacle recruitment and concluded that the hydroid canopy was a physical barrier to cyprid settlement. Further, Standing (1976) and subsequently Dean and Hurd (1980), Dean (1981) and Schmidt (1983b) reported increased ascidian settlement beneath the hydroid canopy, but this was not observed in the present study.

Throughout the period of study the buoys at each of the sites had been exposed for only between 10 and 14 months. However, most of the fouling communities observed appeared to be close to a climax stage. By comparison, Freeman (1977) and Oldfield (1980) found that at the

deeper water oil and gas rigs of the North Sea, the typical climax community ("mussel-dominated, tending to be superceded by tubeworms, barnacles and hydroids at lower depths") of these structures was developed over 3 to 4 years or longer. This difference in the time to reach maximum development of the fouling community, may result from the rapid colonisation by coastal (and bottom living) organisms of the intermediate depth buoys used in the present study, where both the 'hard' and 'soft' categories of Freeman's (1977) classification quickly became established. The hydroid, *Tubularia larynx* (of the 'soft' fouling component) was found at almost every site during successive years, and was classified as one of the 'principal fouling' species corresponding to Meadows' (1969) 'pool' species of important fouling organisms. Similarly, as part of the 'hard' fouling category, *Pomatoceros triqueter* (another 'principal fouling' species) contributed almost all the tubeworm fouling at each site.

The analysis of fouling communities from offshore buoys around Norway also revealed a number of 'principal fouling' species or 'pool' species, which were common to every site and often contributed a large part of the total abundance at each site. Although there were geographical variations in the species composition of these fouling communities, there was no opportunity to sample the sites over a number of years as was possible at the U.K. buoy stations. However, even with this limited data many of the assumptions and views discussed in relation to the U.K. sites may also be valid in the fouling communities of the Norwegian buoys. Notably, discrete polyzoan communities with a distinct geographical distribution were found at the Norwegian sites, most of which were subject to moderate to intensive fouling with *Tubularia larynx* and *Pomatoceros triqueter*.

Overall, the main potential of this part of the study has been to provide the first major overview of offshore fouling communities around the whole of the U.K. and Norway. A marked geographical variation in fouling community structure was demonstrated at both U.K. and Norwegian sites, and a number of common 'principal fouling' species or 'pool' species identified. In addition, the frequency and abundance of both *Tubularia larynx* and *Pomatoceros triqueter*, and their importance as fouling organisms has been shown. Although far from complete, and in particular lacking any seasonal information (Milne, 1940), the cataloguing of the component species and description of the vertical and geographical distribution of these fouling communities has provided a useful data-base for further study.

Figure 3.1.1

Dendrogram resulting from the Q-type classification analysis of U.K. sites data (reduced) from species biomass (g/100 cm^2) information in Appendix 3. Scale on the ordinate is the Bray-Curtis coefficient, transformed to log e.

Eight major site groupings, A to H, were identified.

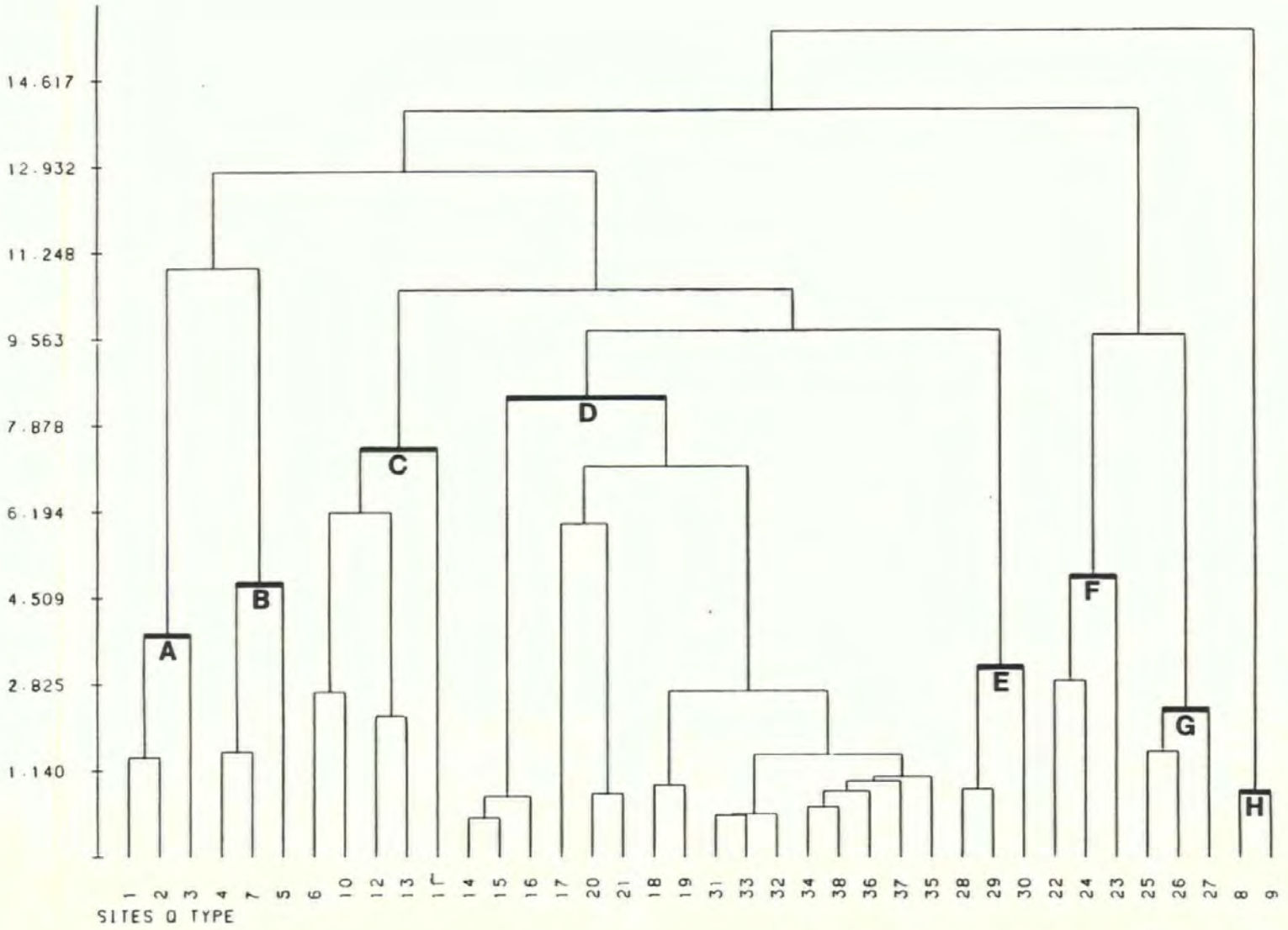
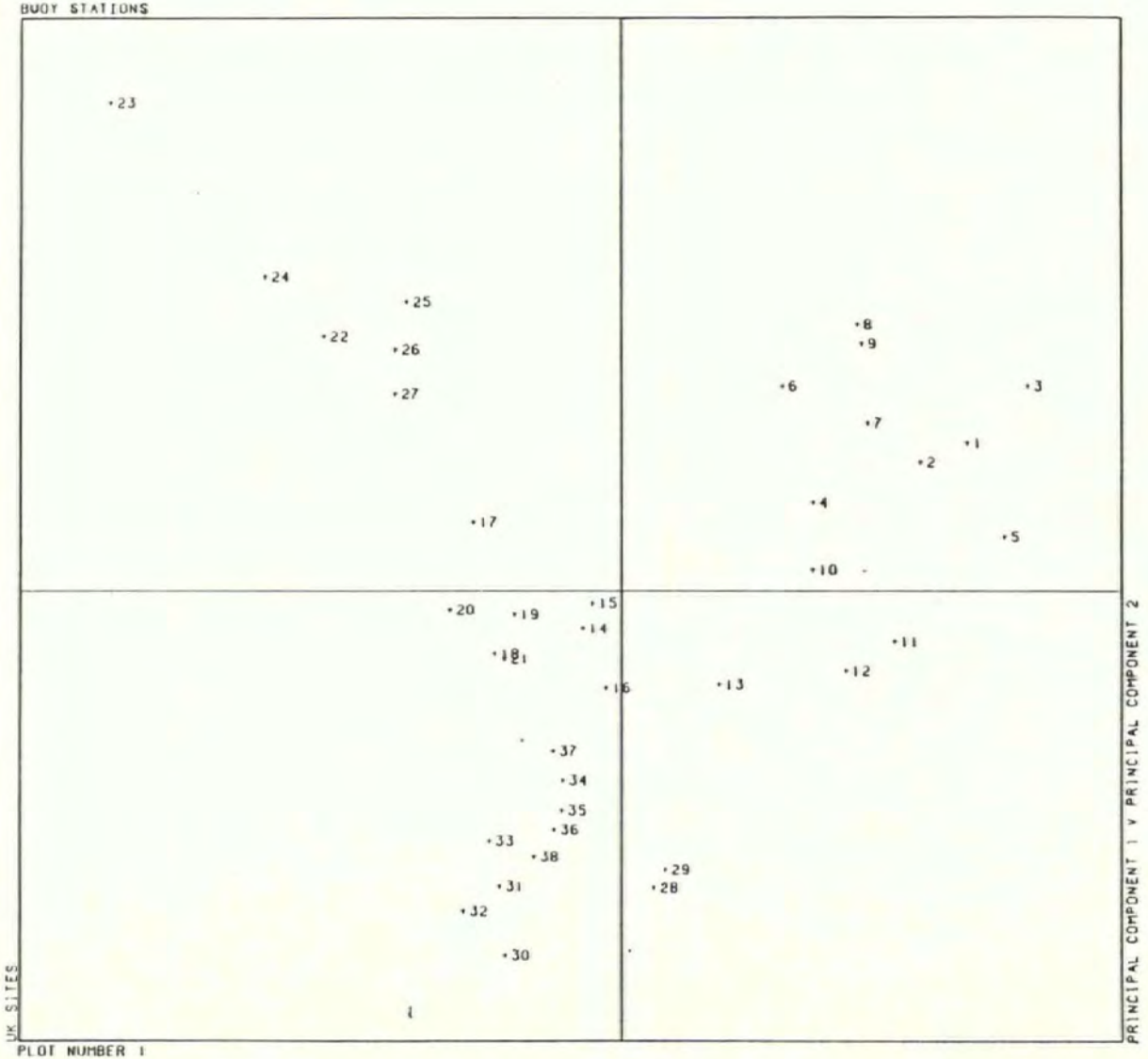

14.617
12.932
11.248
9.563
7.878
6.194
4.509
2.825
1.140
A
B
C
D
E
F
G
H
1 2 3 4 7 5 6 10 12 13 11 14 15 16 17 20 21 18 19 31 33 32 34 38 36 37 35 28 29 30 22 24 23 25 26 27 8 9
SITES O TYPE

Figure 3.1.2

Scatter diagram resulting from the Q-type classification analysis of U.K. sites data (reduced) from species biomass ($g/100\ cm^2$) data in Appendix 3.


BUOY STATIONS
23
24
25
22
26
27
17
8
9
6
3
7
1
2
4
5
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20
19
15
14
18
11
12
13
16
37
34
35
36
33
38
29
31
28
32
30
UK SITES
PLOT NUMBER 1
PRINCIPAL COMPONENT 1 V PRINCIPAL COMPONENT 2

Table 3.1.3.

U.K. Site groups, cluster analysis

Site Group

A (1, 2 and 3), S.W.

Culdrose, 1980

Culdrose, 1981

Fowey, 1978

B (4, 7 and 5), S. and S.W.

Fowey, 1980

Portland, 1978

Portland, 1980.

C (6, 10, 11, 12 and 13), S.

Portland, 1979

Solent, 1979

Solent, 1980a

Solent, 1980b

Solent, 1981

D, sub- cluster 1 (14, 15 and 16) W.

Cardigan Bay, 1979

Cardigan Bay, 1980

Isle of Man, 1979

D, sub-cluster 2 (17, 20, 21) N. and W.

Isle of Man, 1980

Loch Ewe, 1980

Loch Ewe, 1981

Site group

D, sub-cluster 3 (18 and 19) N.

Raasay, 1978

Raasay, 1980

D, sub-cluster 4 (31, 32 and 33), N.E.

May Island, 1980

Moray Firth, 1983

Herd Sands, 1980.

D, sub-cluster 5 (34, 38, 36, 37, 35) E.

Tees, 1980

Flamborough, 1982

Flamborough, 1979

Flamborough, 1980

Flamborough, 1978

E (28, 29 and 30), N.E.

North Sea No.1, 1979

North Sea No.1, 1980

May Island, 1979.

F (22, 24 and 23) N.W.

Sound of Harris, 1978

Sound of Harris, 1981

Sound of Harris, 1979

G (25, 26 and 27), N.W.

St. Kilda, 1978

St. Kilda, 1979

St. Kilda, 1981

Site group

H (8 and 9), S.

Portland, 1982

Solent, 1978

Figures 3.1.4 to 3.1.15.

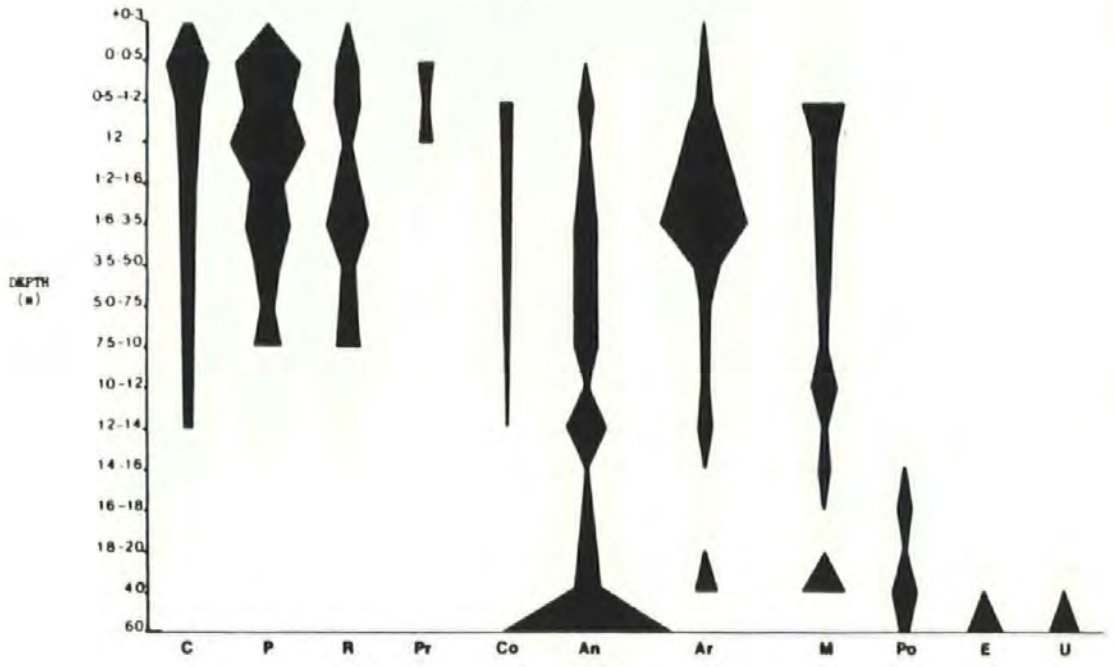
U.K. Site groups.

The vertical distribution of the major fouling groups (algae and animal phyla) at each of the U.K. buoy site groupings A to H, defined by cluster analysis. 'Kites' represent mean biomass values at each site group.

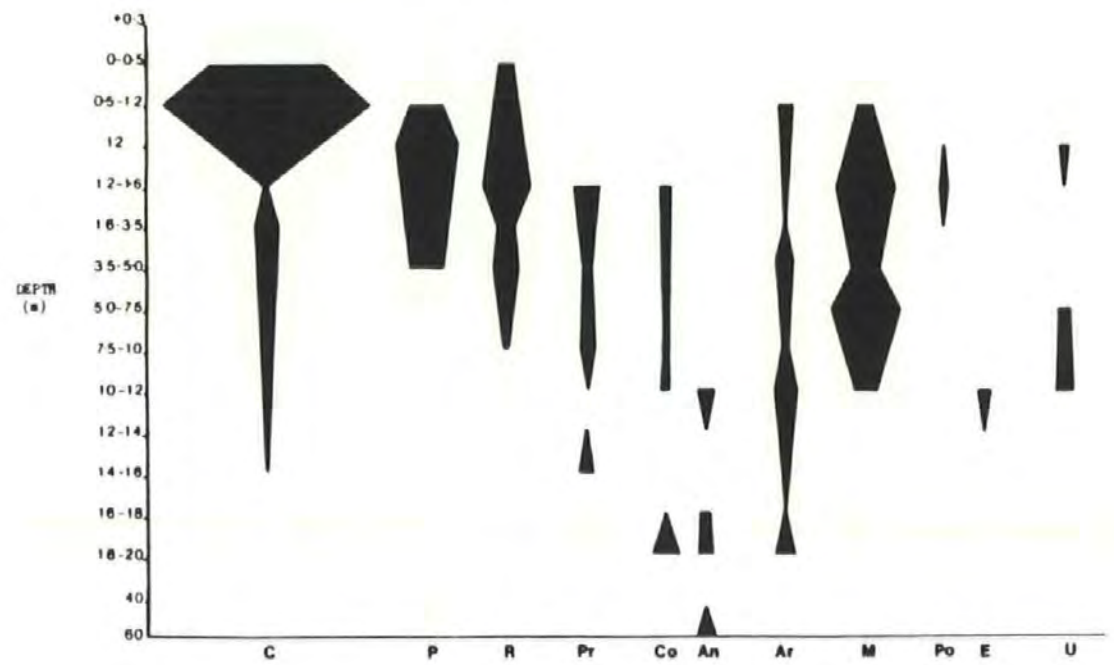
1. Algae
   - C = Chlorophyta
   - P = Phaeophyta
   - R = Rhodophyta

2. Fauna
   - Pr = Porifera
   - Co = Coelenterata
   - An = Annelida
   - Ar = Arthropoda
   - M = Mollusca
   - Po = Polyzoa
   - E = Echinodermata
   - U = Urochordata

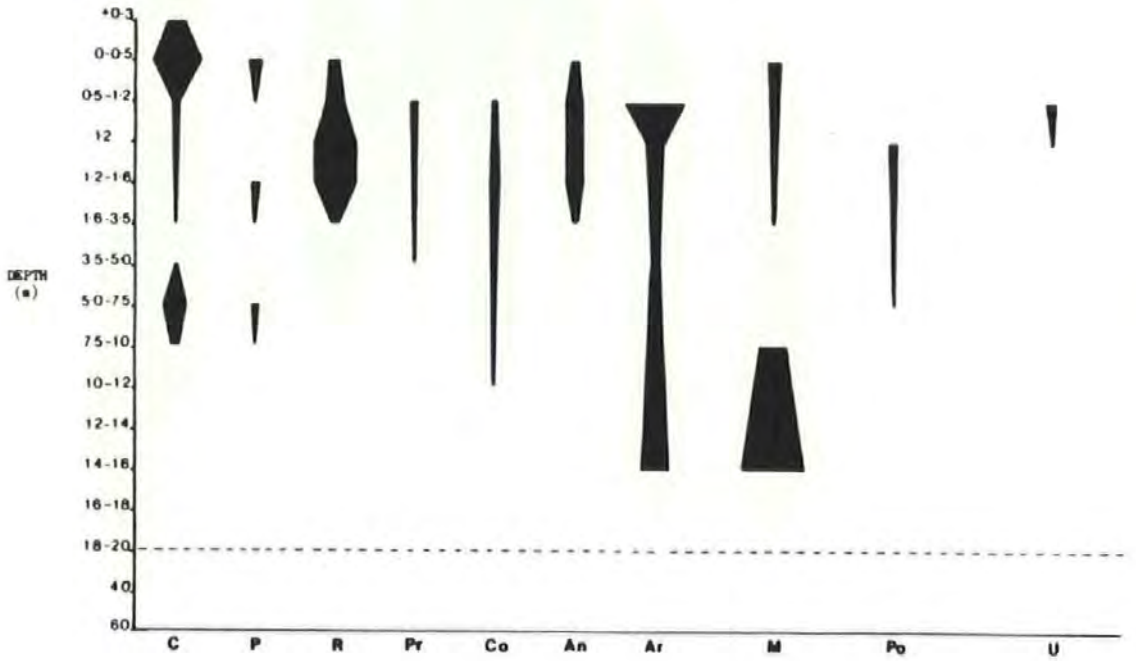
SITE GROUP A



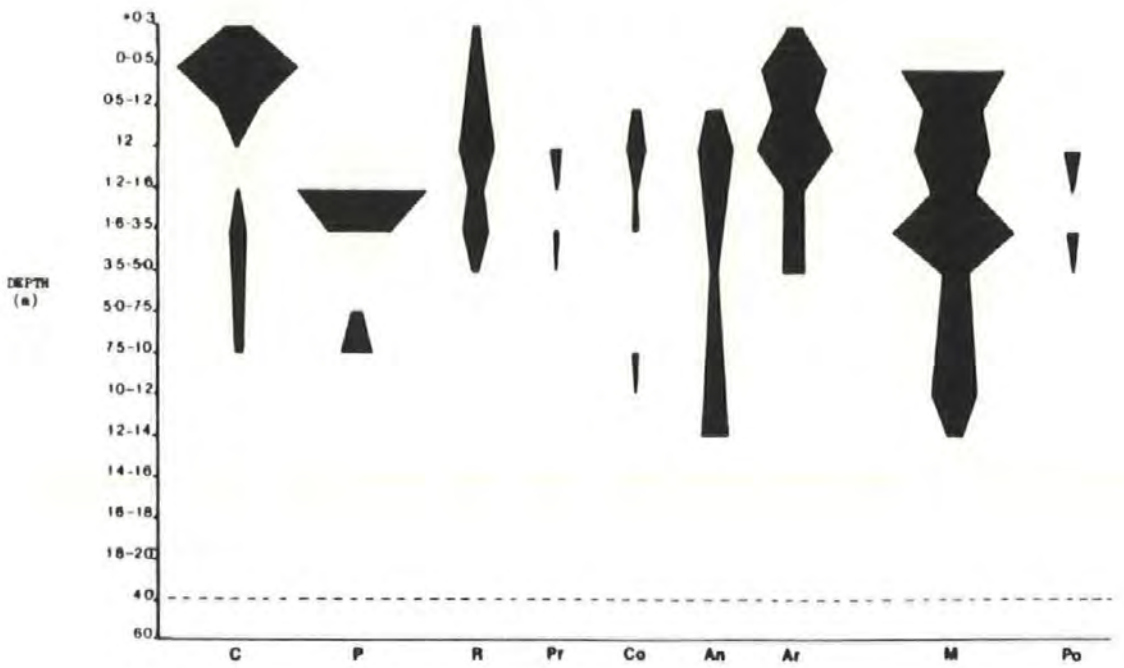
SITE GROUP B



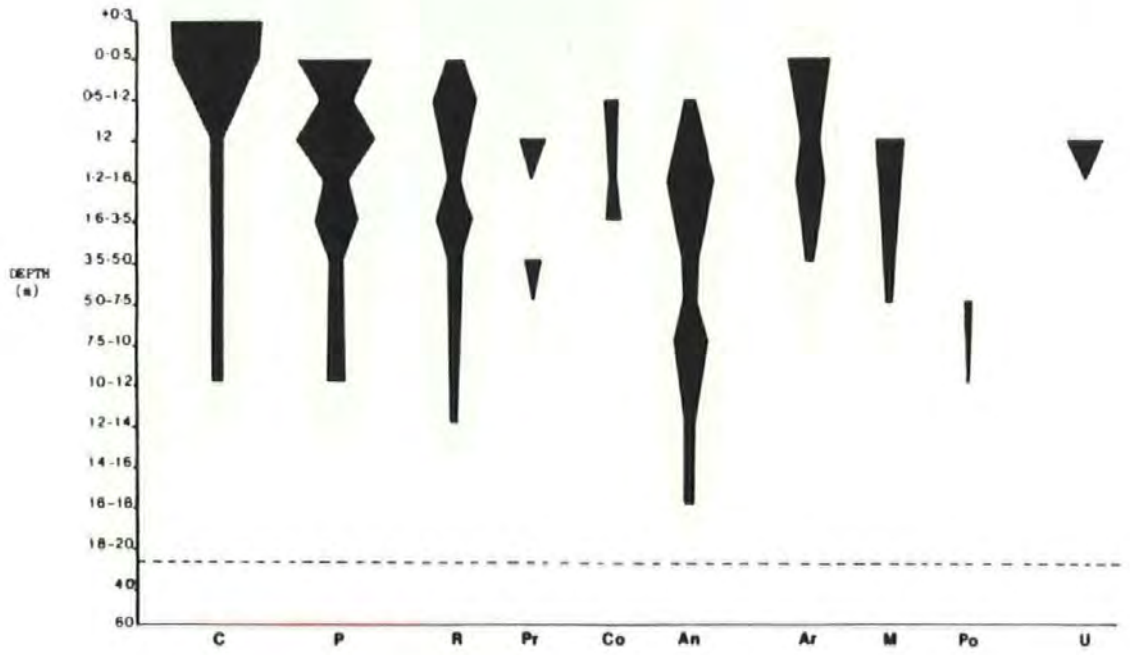
SITE GROUP C



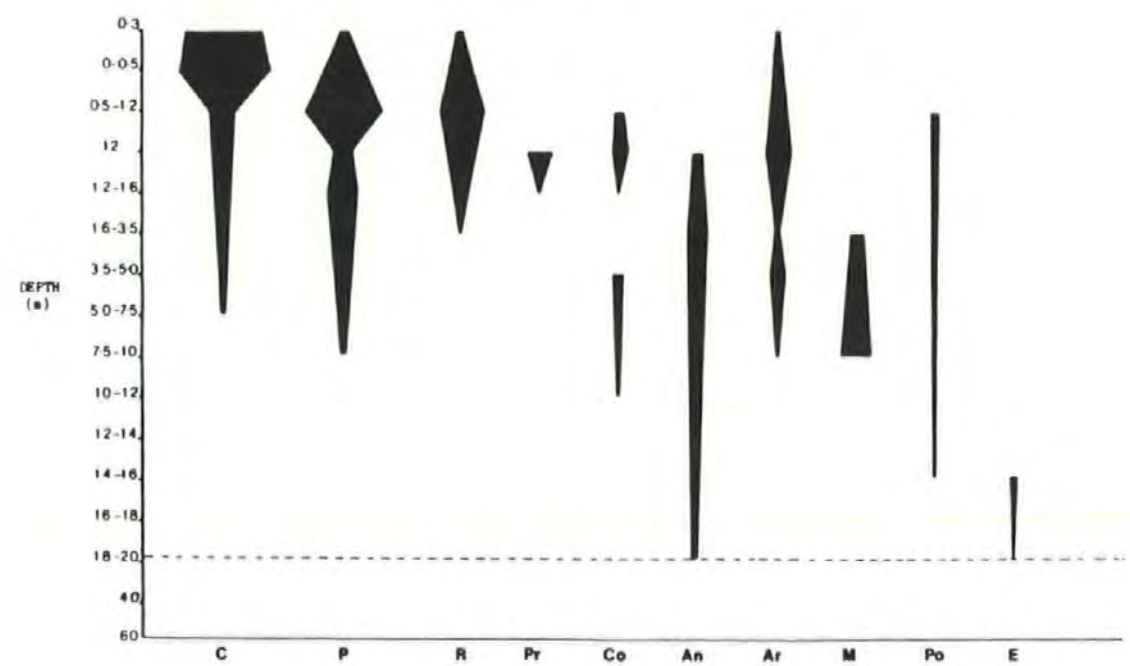
SITE GROUP D (sub - cluster I)



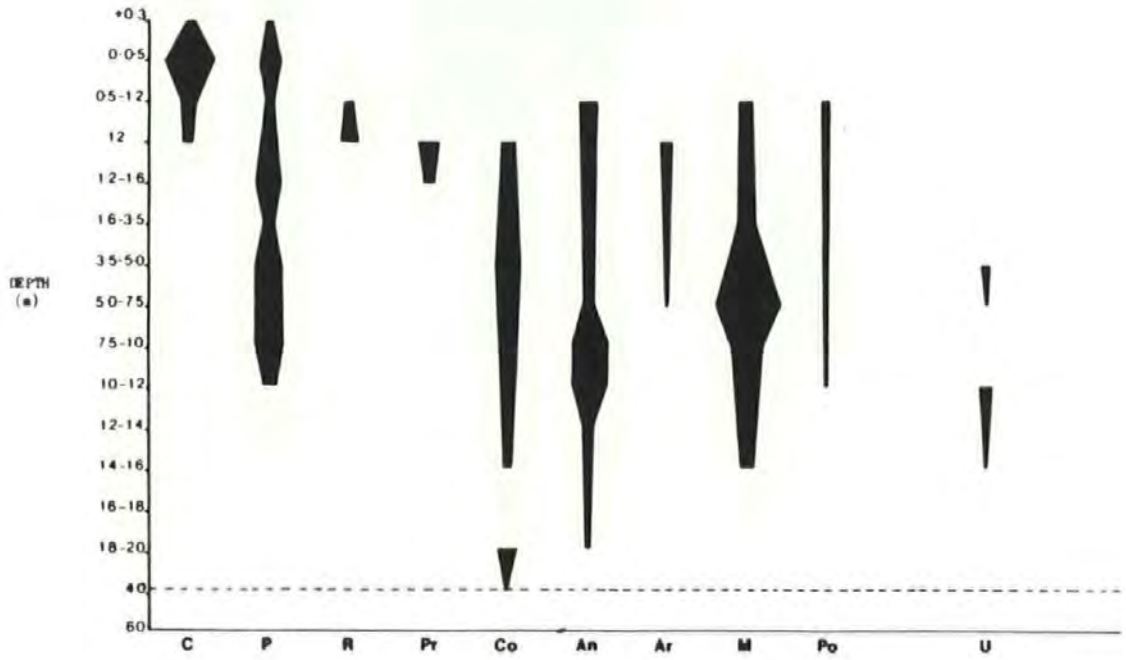
SITE GROUP D (sub - cluster 2)



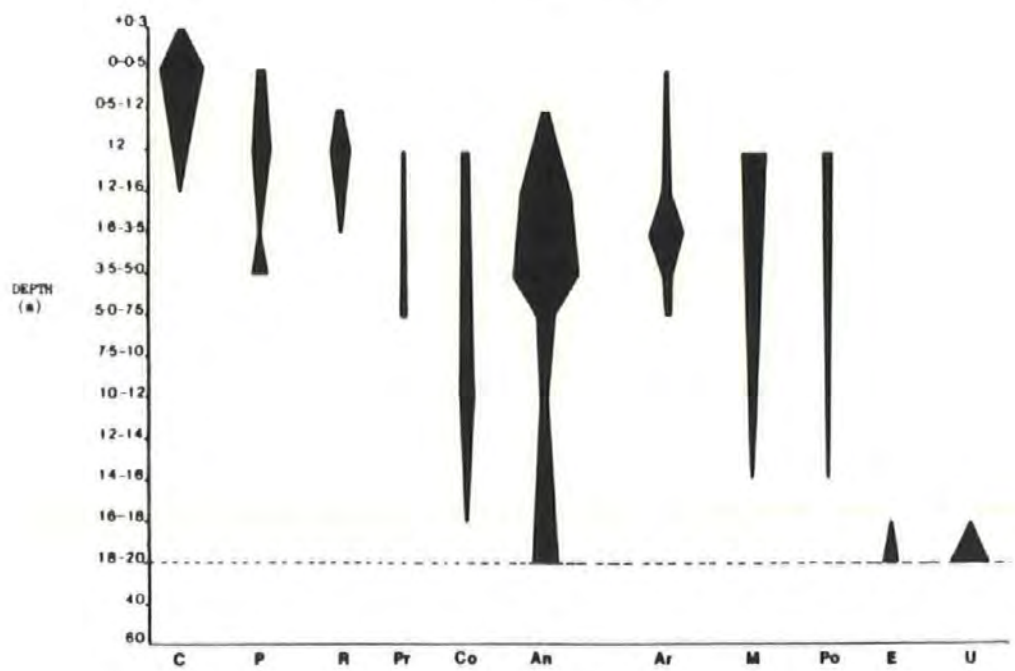
SITE GROUP D (sub - cluster 3)



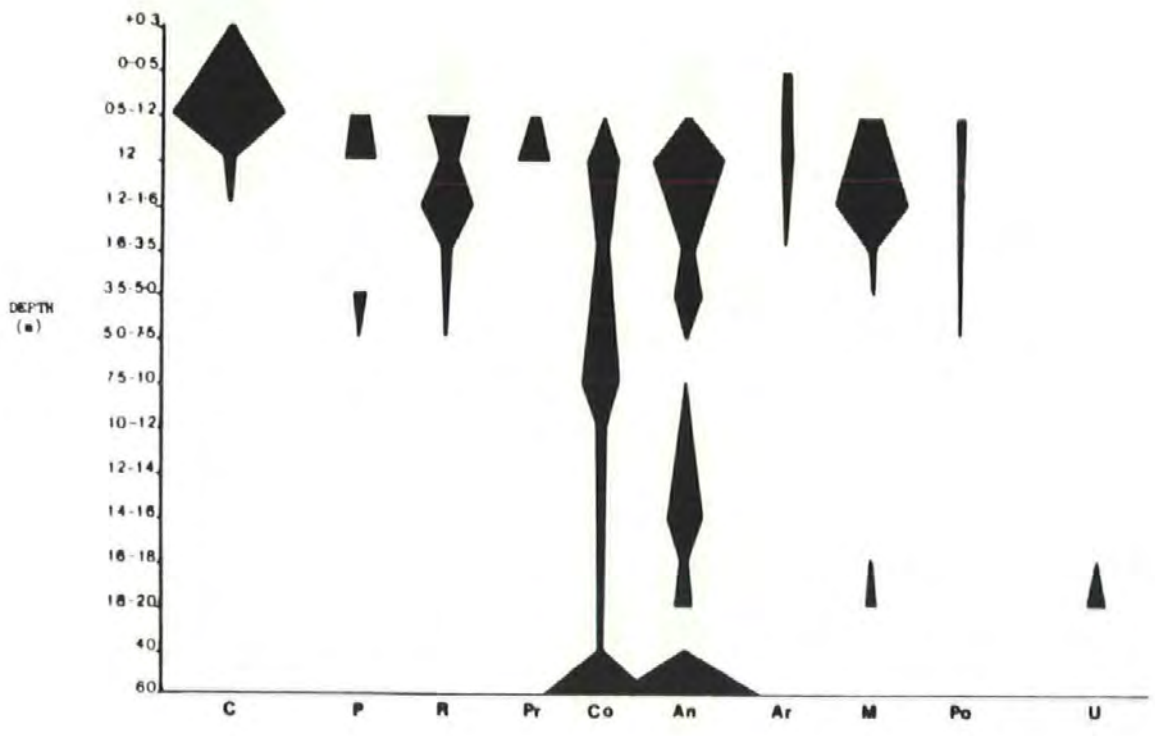
SITE GROUP D (sub - cluster 4)

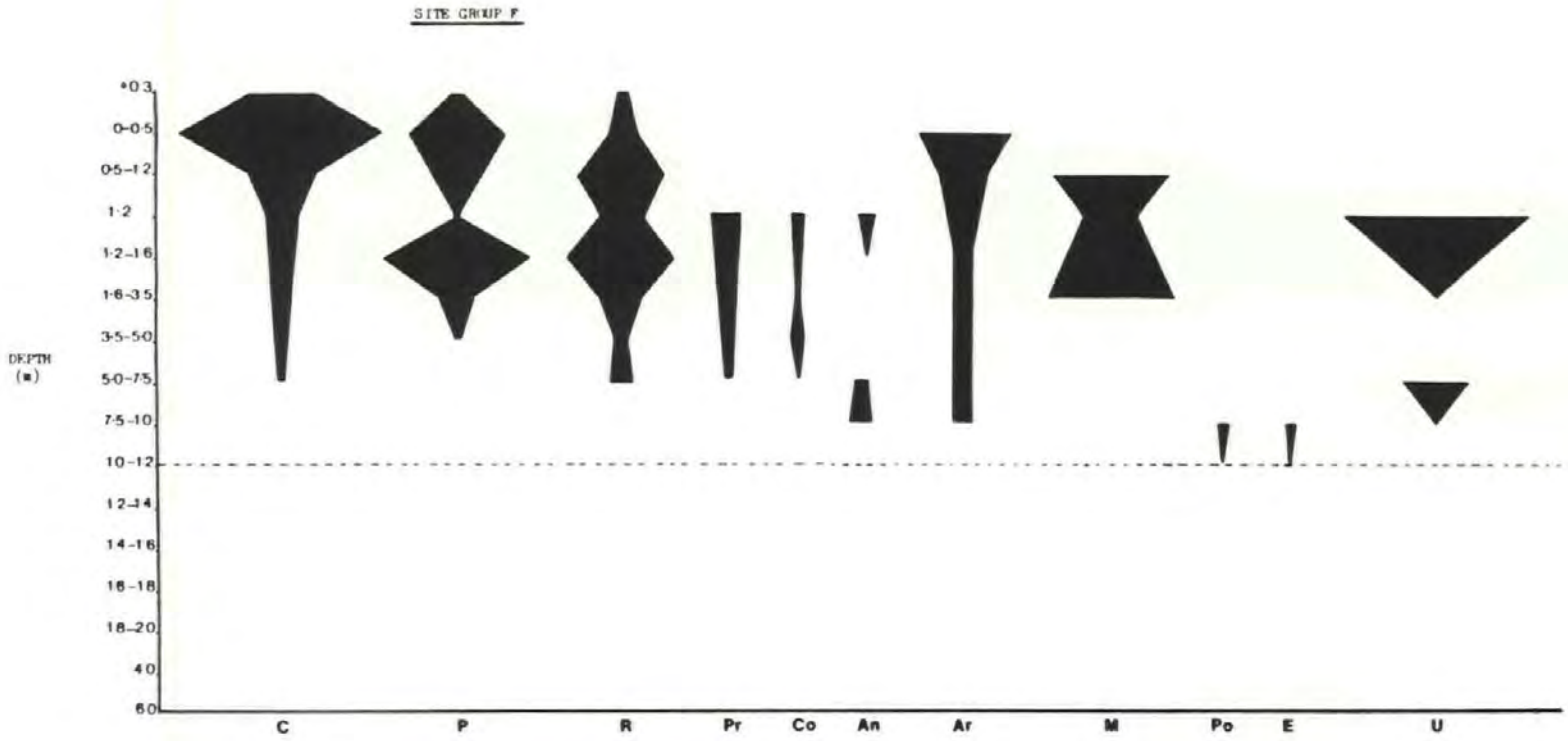


SITE GROUP D (sub - cluster 5)

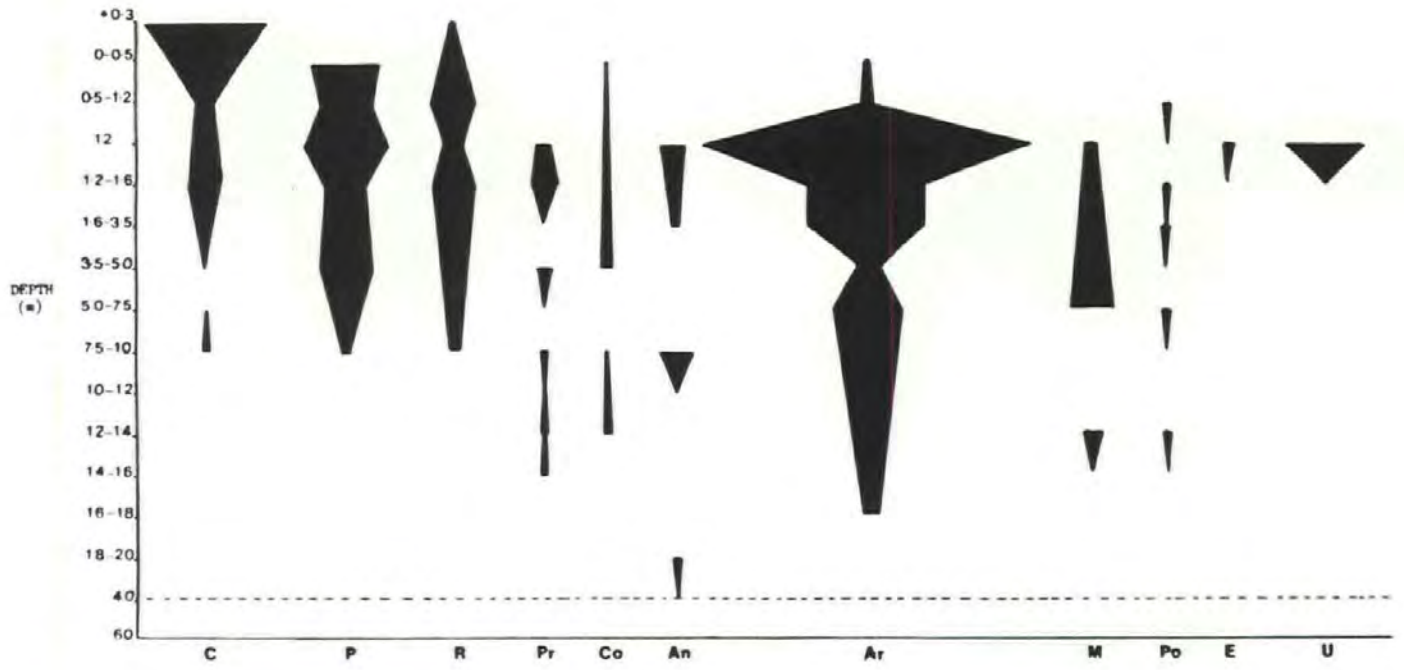


SITE GROUP E


DEPTH (m)
+0.3
0-0.5
0.5-1.2
1.2
1.2-1.6
1.6-3.5
3.5-5.0
5.0-7.5
7.5-10
10-12
12-14
14-16
16-18
18-20
40
60
C
P
R
Pr
Co
An
Ar
M
Po
U


SITE GROUP F
DEPTH (m)
*0·3
0·0·5
0·5–1·2
1·2
1·2–1·6
1·6–3·5
3·5–5·0
5·0–7·5
7·5–10
10–12
12–14
14–16
16–18
18–20
40
60
C
P
R
Pr
Co
An
Ar
M
Po
E
U

SITE GROUP G


+0·3
0-05
05-12
12
12-16
16-35
35-50
50-75
75-10
10-12
12-14
14-16
16-18
18-20
40
60
DEPTH (m)
C
P
R
Pr
Co
An
Ar
M
Po
E
U

SITE GROUP H

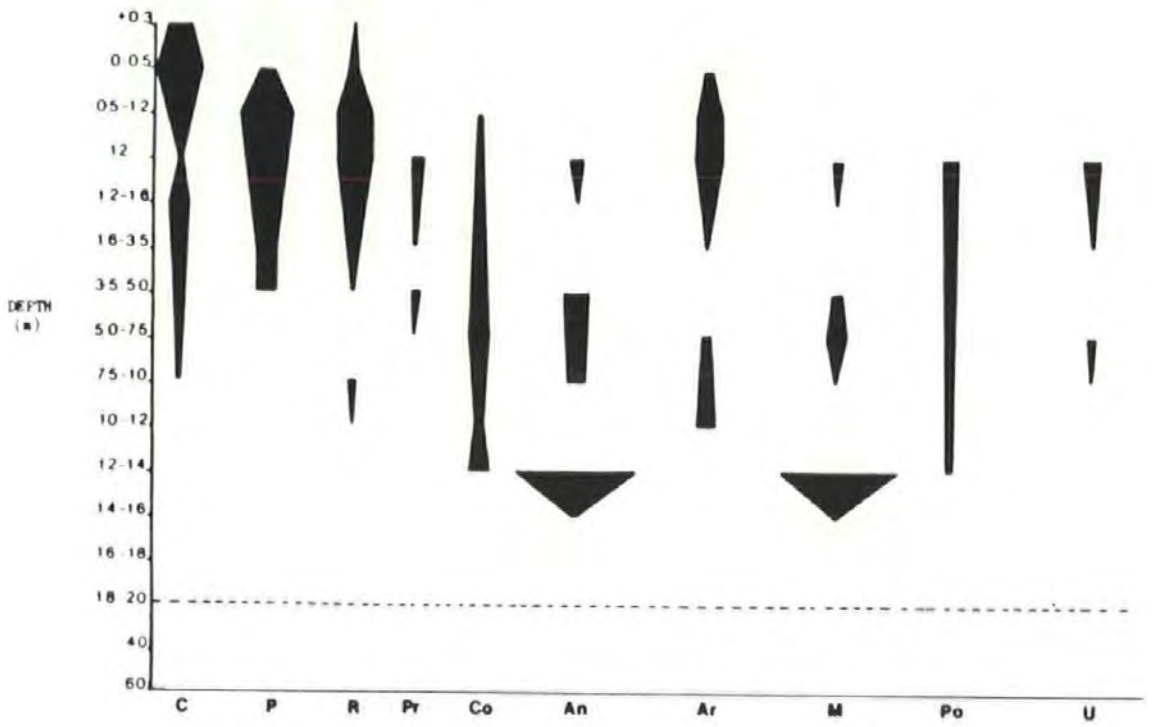

DEPTH (m)
+0 3
0 05
05-12
12
12-16
16-35
35 50
50-75
75-10
10 12
12-14
14-16
16 18
18 20
40
60
C
P
R
Pr
Co
An
Ar
M
Po
U

Figure 3.1.16.

Dendrogram resulting from the R type analysis of species (reduced) data (see Table 3.1.17) from Culdrose RTB, 1980 collection. Scale on the ordinate is the Bray-Curtis coefficient, transformed to log e.

Four major species groupings, 1 to 4, with respect to depth, were identified.

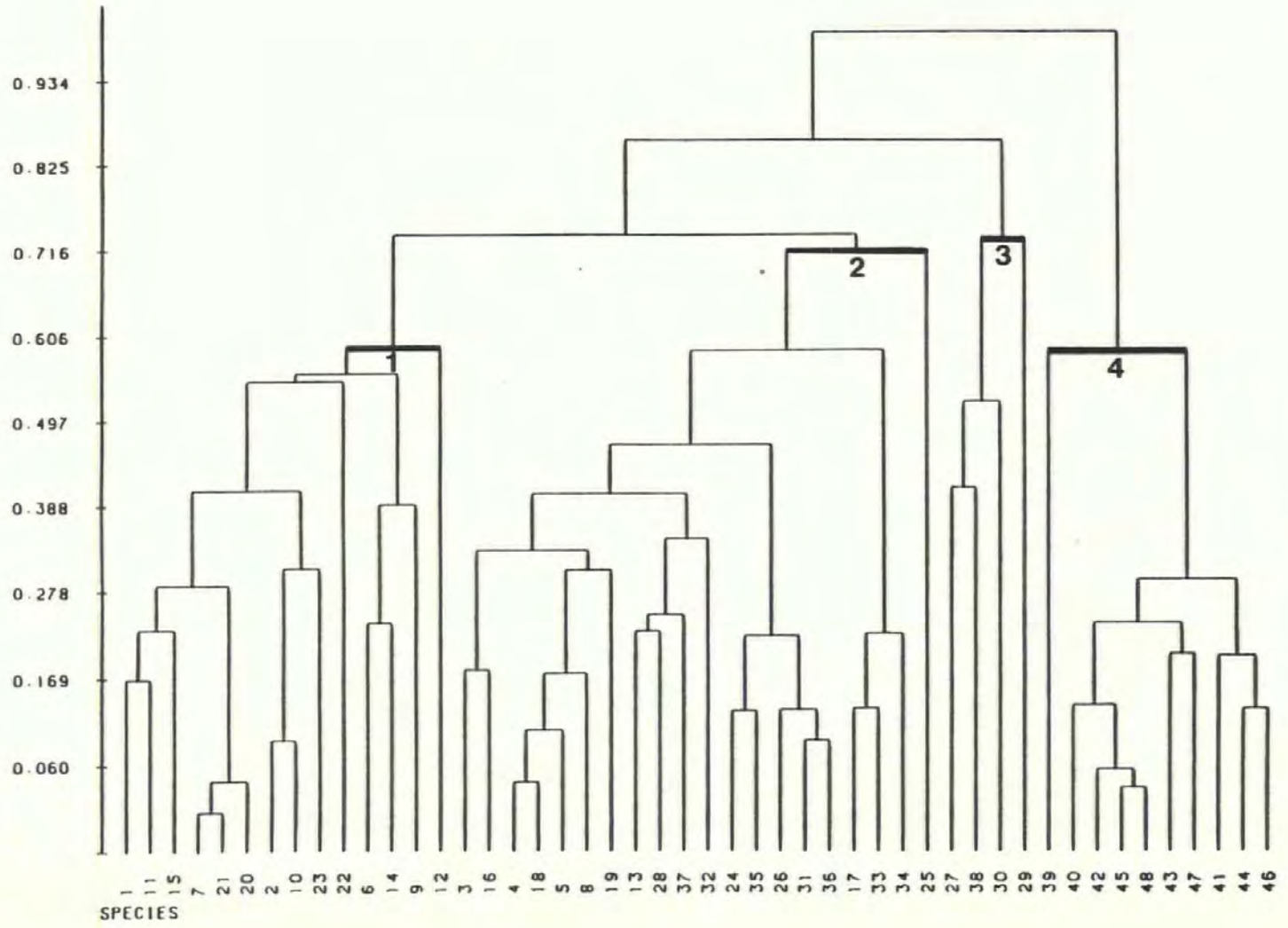

0.934
0.825
0.716
0.606
0.497
0.388
0.278
0.169
0.060
1
2
3
4
1 11 15 7 21 20 2 10 23 22 6 14 9 12 3 16 4 18 5 8 19 13 28 37 32 24 35 26 31 36 17 33 34 25 27 38 30 29 39 40 42 45 48 43 47 41 44 46
SPECIES

Table 3.1.17

Vertical distribution of Fouling Organisms

at Site 1 (Culdrose, 1980).

SPECIES GROUP I.

1. *Codium adherens*	10. *Cystosira nodicaulis*
11. *Asperococcus bullosus*	23. *Sycon coronatum*
15. *Brongniartella byssoides*	22. *Sycon ciliatum*
7. *Enteromorpha intestinalis*	6. *Enteromorpha linza*
21. *Porphyra umbilicalis*	14. *Striaria attenuata*
20. *Polysiphonia nigrescens*	9. *Ascophyllum nodosum*
2. *Codium bursa*	12. *Laminaria digitata*

SPECIES GROUP II.

3. *Codium tomentosum*	31. *Balanus balanus*
16. *Ceramium rubrum*	24. *Actinia equina*
4. *Cladophora arcta*	35. *Hiatella arctica*
18. *Plocamium cartilagineum*	26. *Plumularia setacea*
5. *Cladophora rupestris*	30. *Balanus balanoides*
8. *Ulva lactuca*	36. *Modiolus barbatus*
19. *Polysiphonia elongata*	17. *Chrondrus crispus*
13. *Laminaria saccharina*	33. *Lepas anatifera*
28. *Nereis pelagica*	34. *Facelina longicornis*
37. *Musculus discors*	25. *Plumularia halecoides*

SPECIES GROUP III.

27. *Tubularia larynx*	32. *Balanus crenatus*
38. *Mytilus edulis*	29. *Pomatoceros triqueter*

SPECIES GROUP IV

39. *Bugula fulva*	43. *Ophiothrix fragilis*
40. *Bugula neritina*	47. *Ciona intestinalis*
42. *Asterias rubens*	41. *Bugula turbinata*
45. *Psammechinus miliaris*	44. *Paracentrotus lividus*
48. *Polycarpa rustica*	46. *Ascidella aspersa.*

Figure 3.1.18.

The vertical distribution of the hydroid canopy (solid circles, mainly *Tubularia larynx*), and barnacle settlement at Culdrose RTB, 1980 (A) and North Sea No. 1 RTB, 1980, (B).

● = hydroid canopy

○ = Balanus species

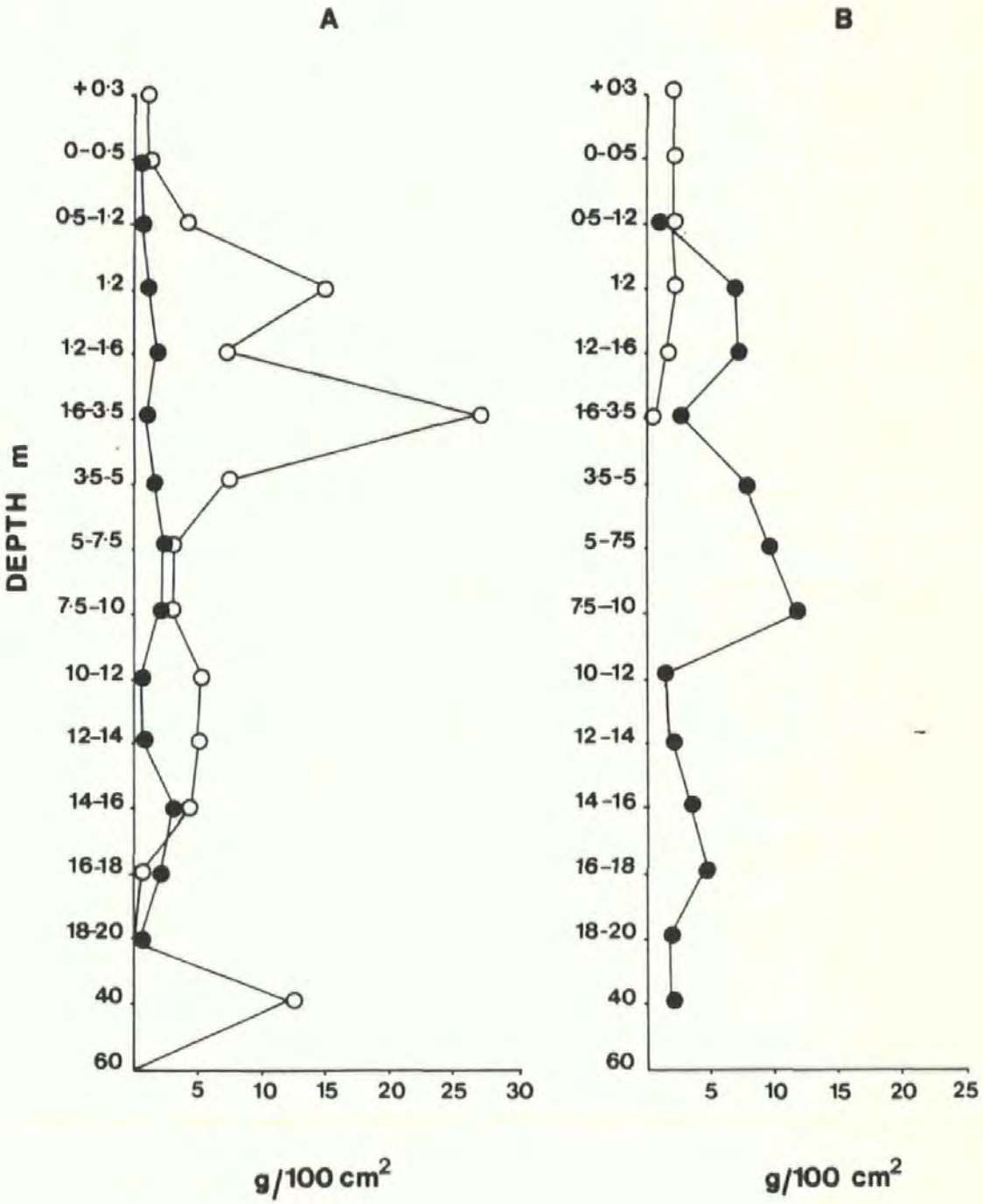

A
B
+0·3
0-0·5
0·5-1·2
1·2
1·2-1·6
1·6-3·5
3·5-5
5-7·5
7·5-10
10-12
12-14
14-16
16-18
18-20
40
60
DEPTH m
5
10
15
20
25
30
g/100 cm²
+03
0-05
0·5-1·2
1·2
1·2-1·6
1·6-3·5
3·5-5
5-7·5
7·5-10
10-12
12-14
14-16
16-18
18-20
40
60
5
10
15
20
25
g/100 cm²

Figure 3.1.19.

The abundance and frequency of *Tubularia larynx* at U.K. site groups A to H.

Vertical axis = depth in metres

horizontal axis = biomass in g/100 cm^2

▨ *Tubularia* fouling

□ Total hydroid fouling

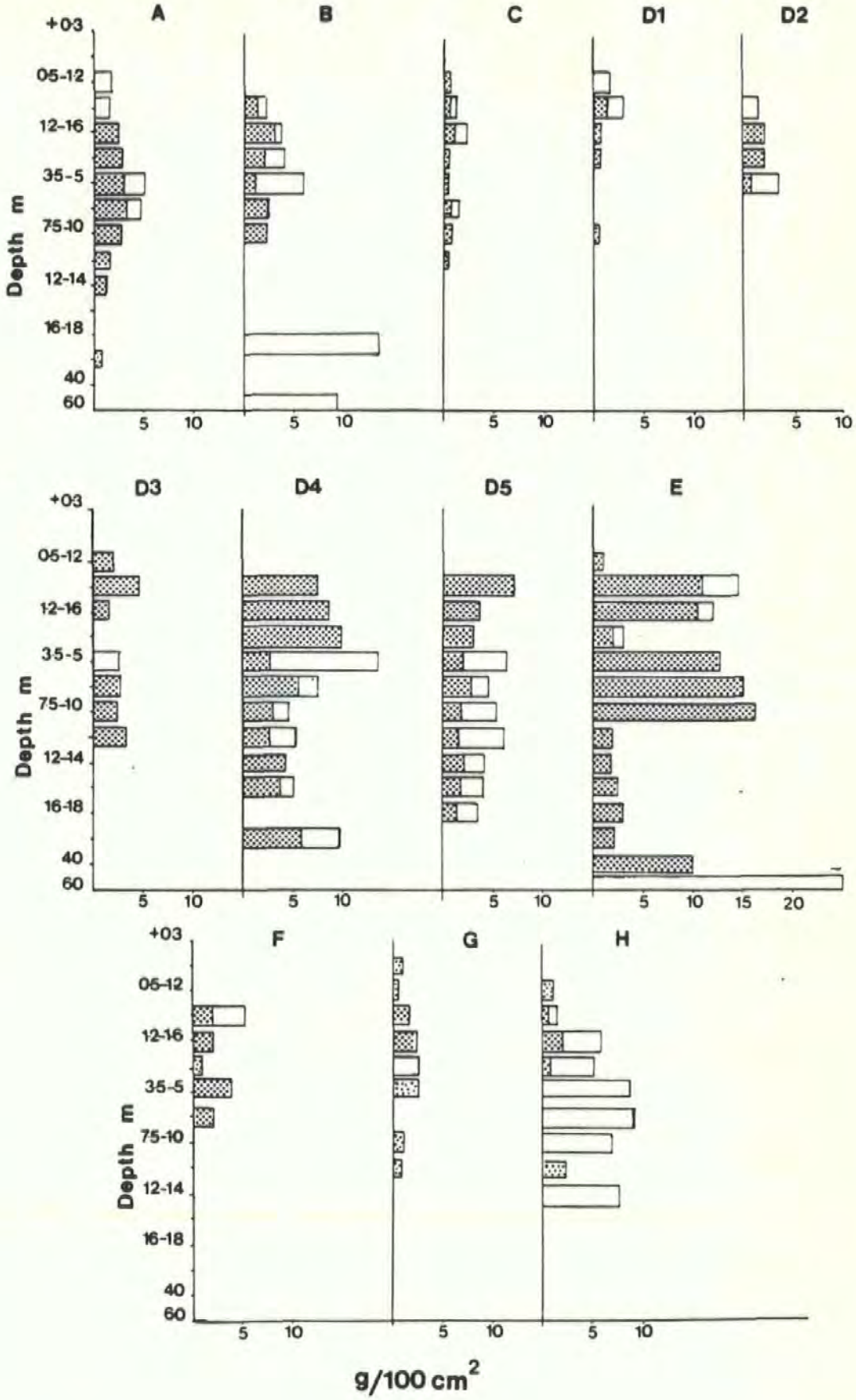

A
B
C
D1
D2
D3
D4
D5
E
F
G
H
+03
05-12
12-16
35-5
75-10
12-14
16-18
40
60
Depth m
5
10
15
20
g/100 cm²

Figure 3.1.20.

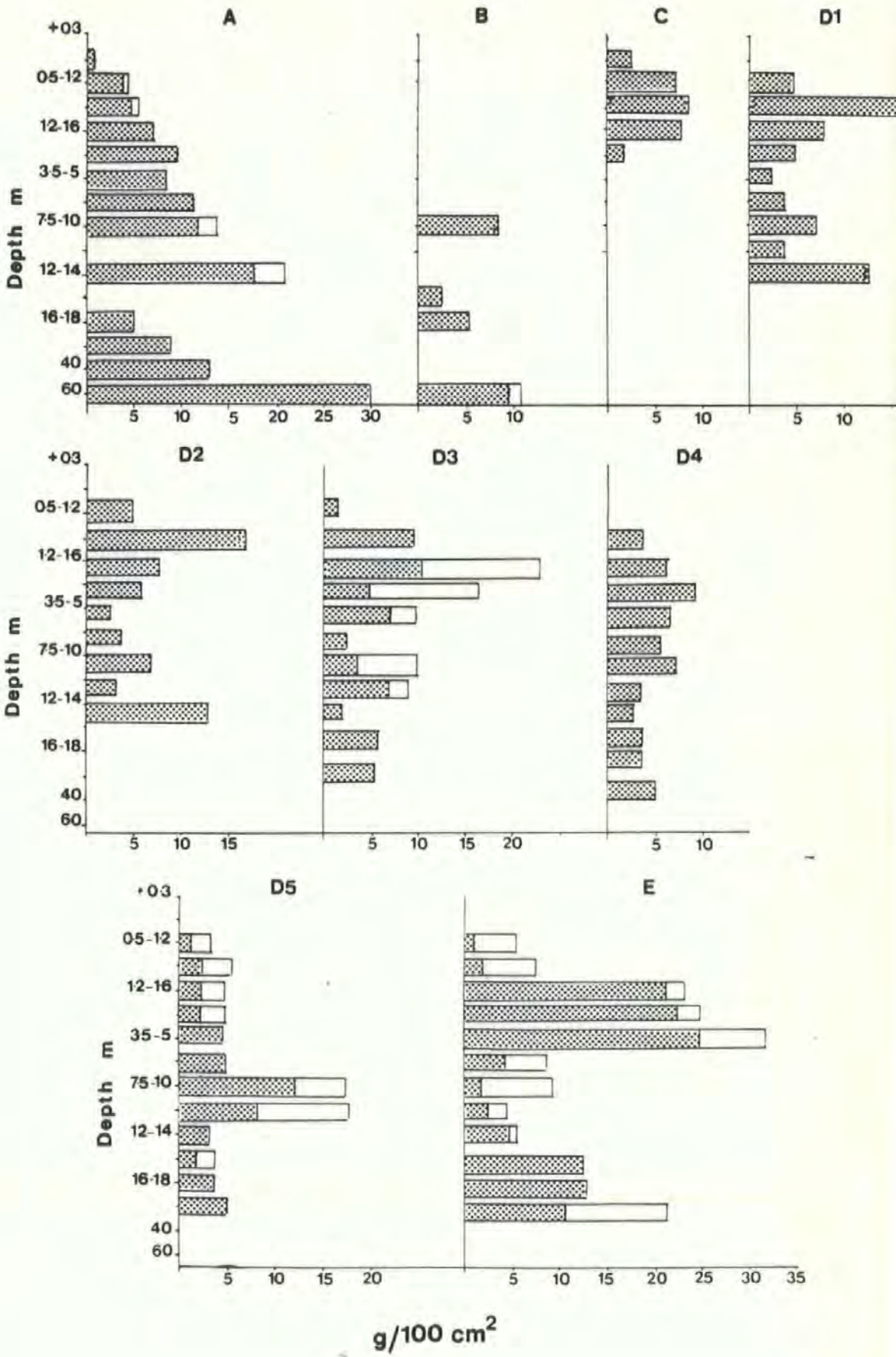
The abundance and frequency of *Pomatoceros triqueter* at U.K. site groups A to H.

Vertical axis = depth in metres

horizontal axis = biomass in g/100 cm^2

▩ *Pomatoceros* fouling

□ Total annelid fouling


A
B
C
D1
D2
D3
D4
D5
E
Depth m
+03
05-12
12-16
35-5
75-10
12-14
16-18
40
60
g/100 cm²

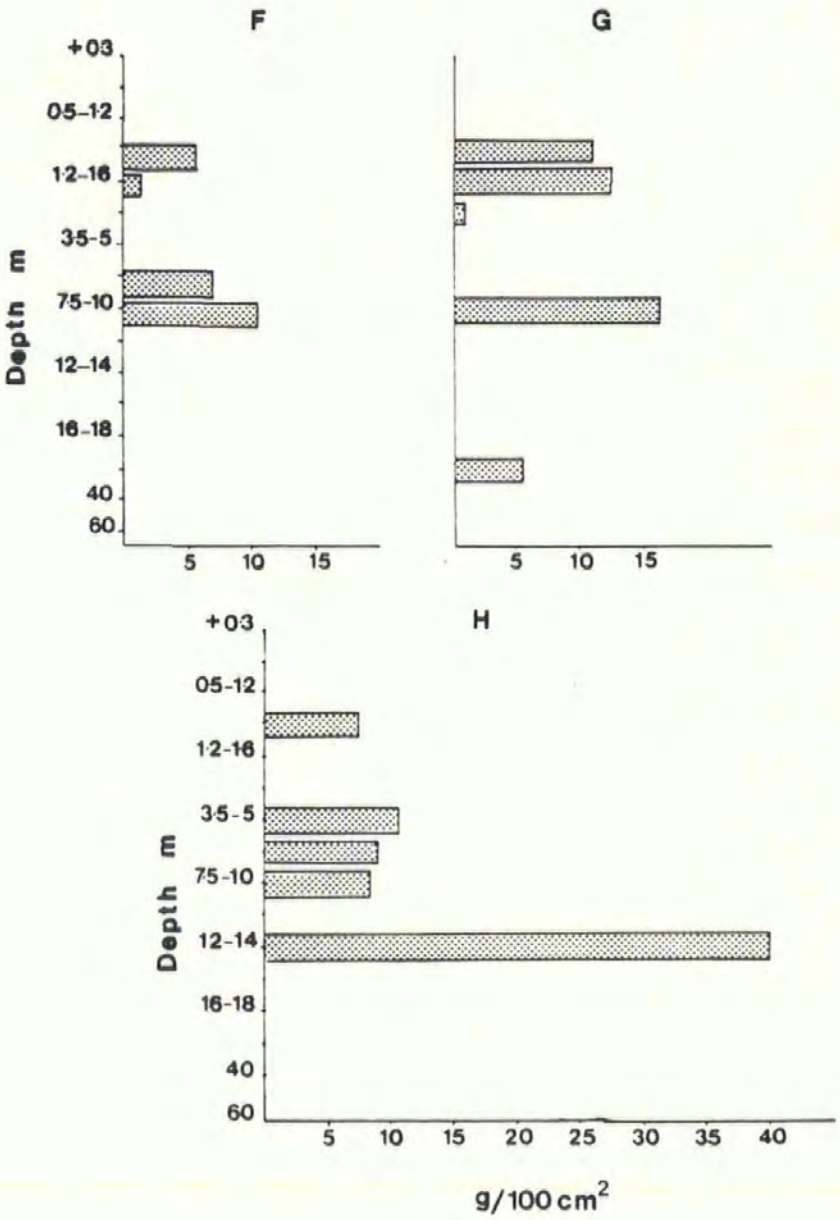

F
G
H
+03
05-12
12-16
35-5
75-10
12-14
16-18
40
60
Depth m
5
10
15
20
25
30
35
40
g/100 cm²

Figure 3.2.1

Dendogram resulting from the Q-type classification analysis of Norway Sites data (reduced) from species biomass ($g/100\ cm^2$) data in Appendix 3. Scale on the ordinate is the Bray-Curtis coefficient, transformed to log e.

Three major Site Groups, 1 to 3, were identified.

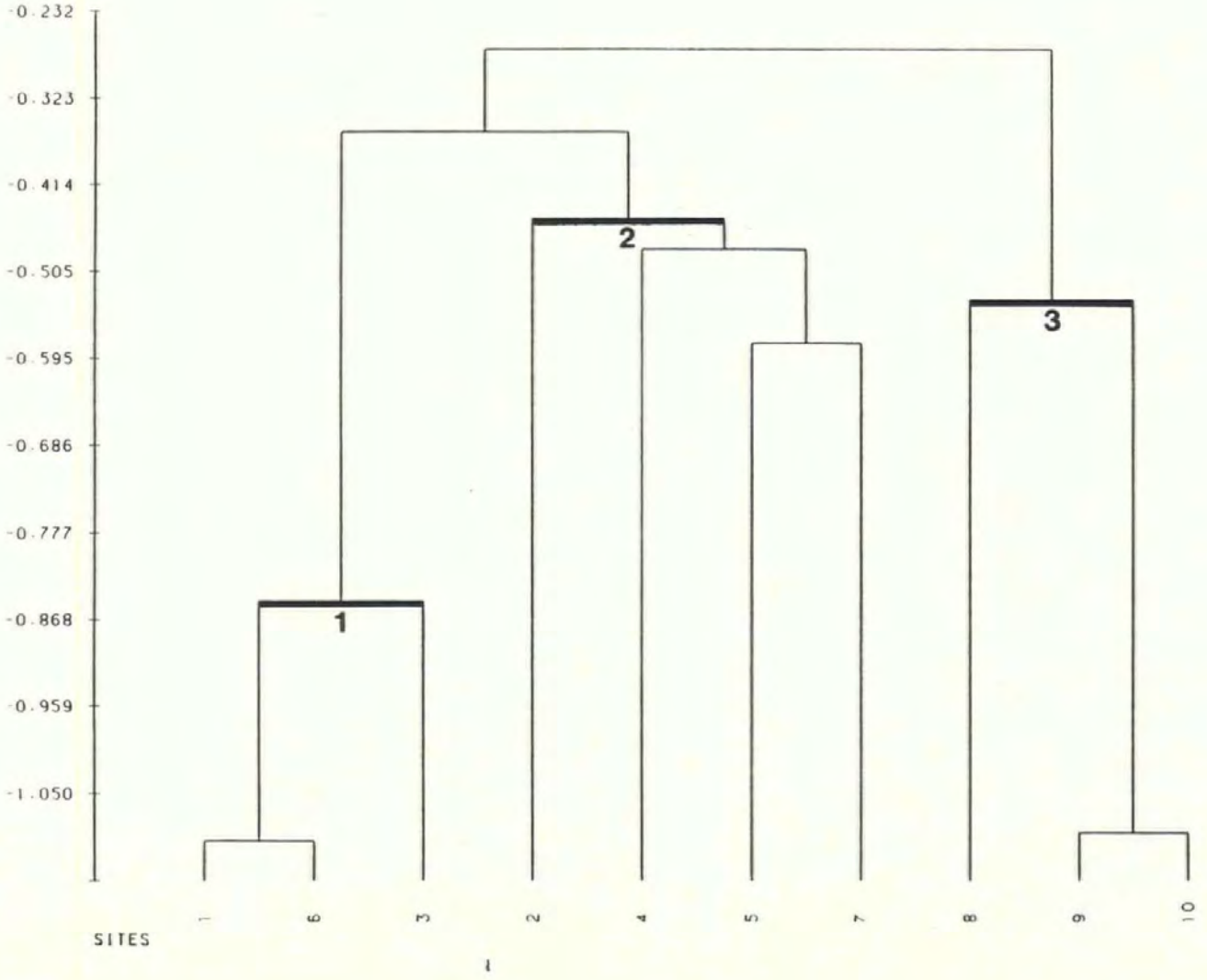
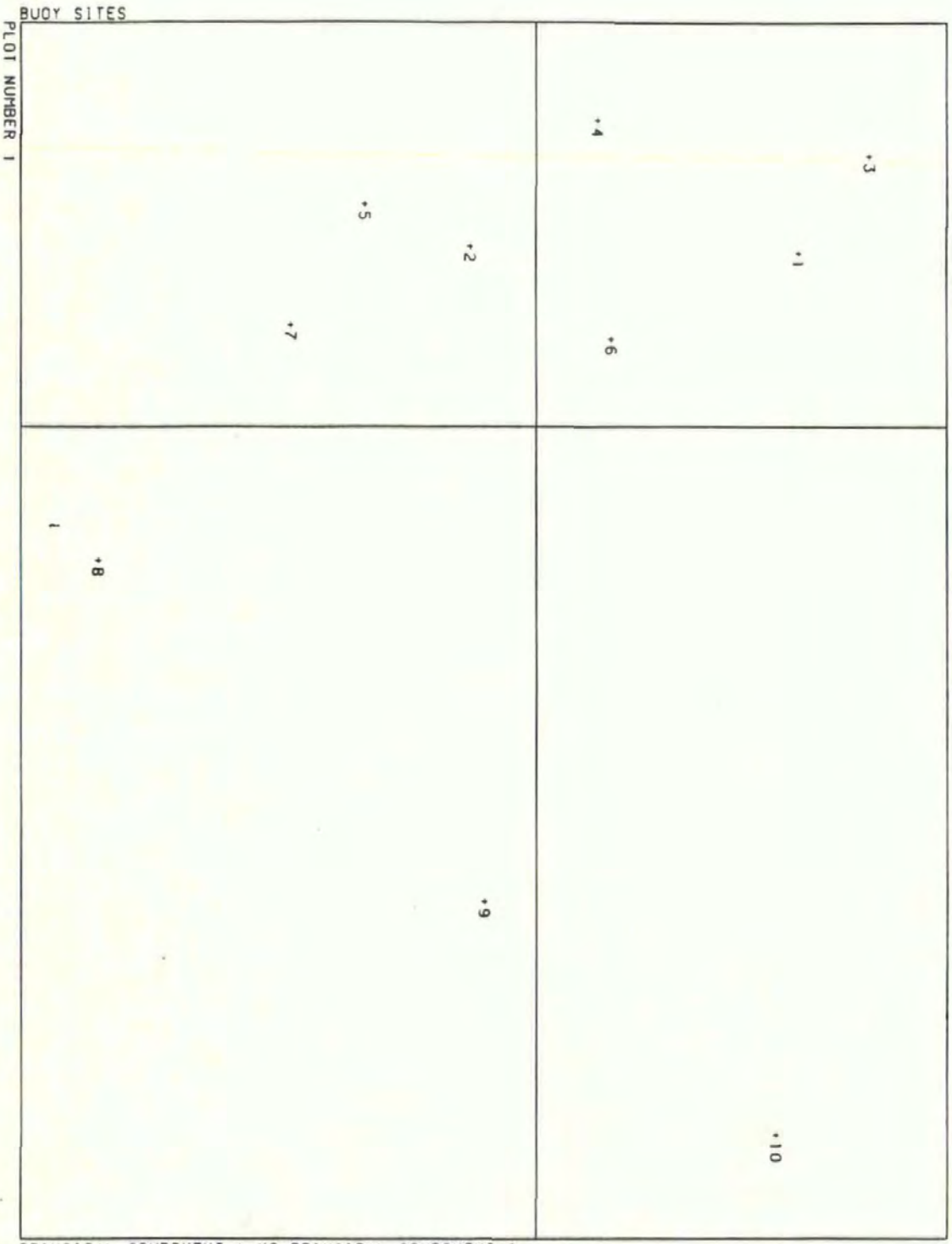

-0.232
-0.323
-0.414
-0.505
-0.595
-0.686
-0.777
-0.868
-0.959
-1.050
1
2
3
1
6
3
2
4
5
7
8
9
10
SITES

Figure 3.2.2.

Scatter diagram resulting from the Q-type analysis of Norway Sites data (reduced) from species biomass ($g/100\ cm^2$) data in Appendix 3.


BUOY SITES
PLOT NUMBER 1
1
2
3
4
5
6
7
8
9
10
PRINCIPAL COMPONENT 1 VS PRINCIPAL COMPONENT 2

Figures 3.2.3 to 3.2.5.

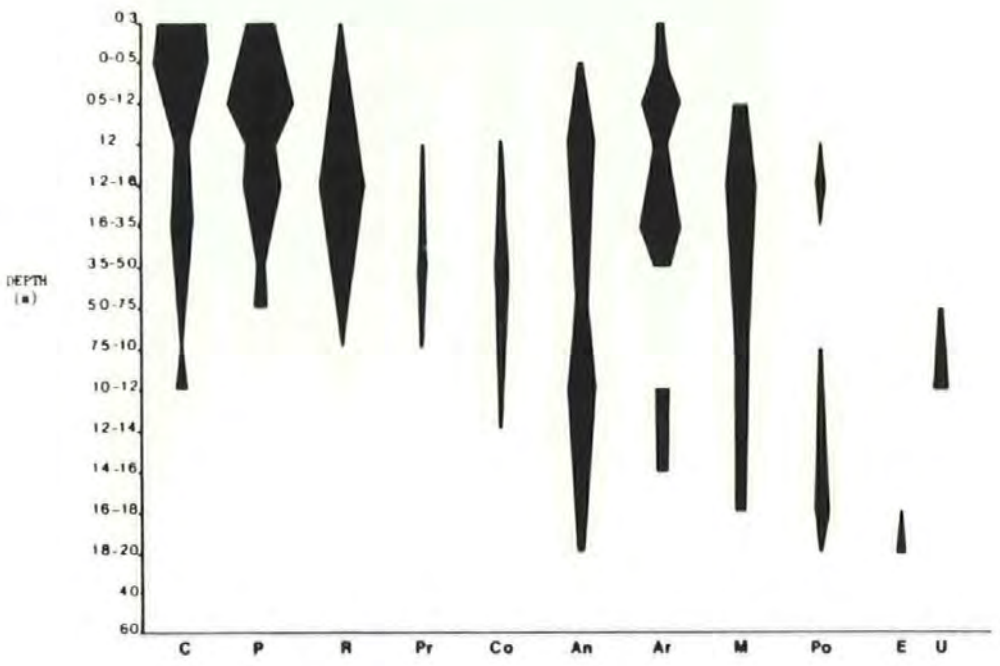
Norway site groups.

The vertical distribution of the major fouling groups (algae, and animal phyla) at each of the Norway buoy site groupings, 1 to 3, defined by cluster analysis. 'Kites' represent mean biomass values at each site group.

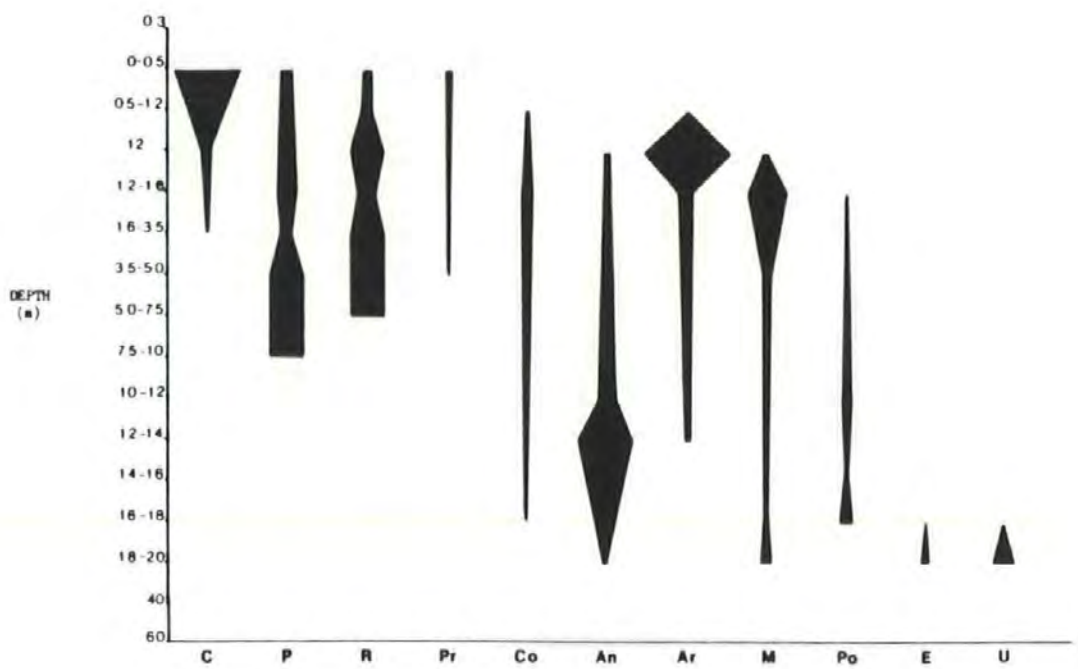
1. Algae
   - C = Chlorophyta
   - P = Phaeophyta
   - R = Rhodophyta

2. Fauna
   - Pr = Porifera
   - Co = Coelenterata
   - An = Annelid
   - Ar = Arthropoda
   - M = Mollusca
   - Po = Polyzoa
   - E = Echinodermata
   - U = Urochordata

SITE GROUP 1



SITE GROUP 2



SITE GROUP 3

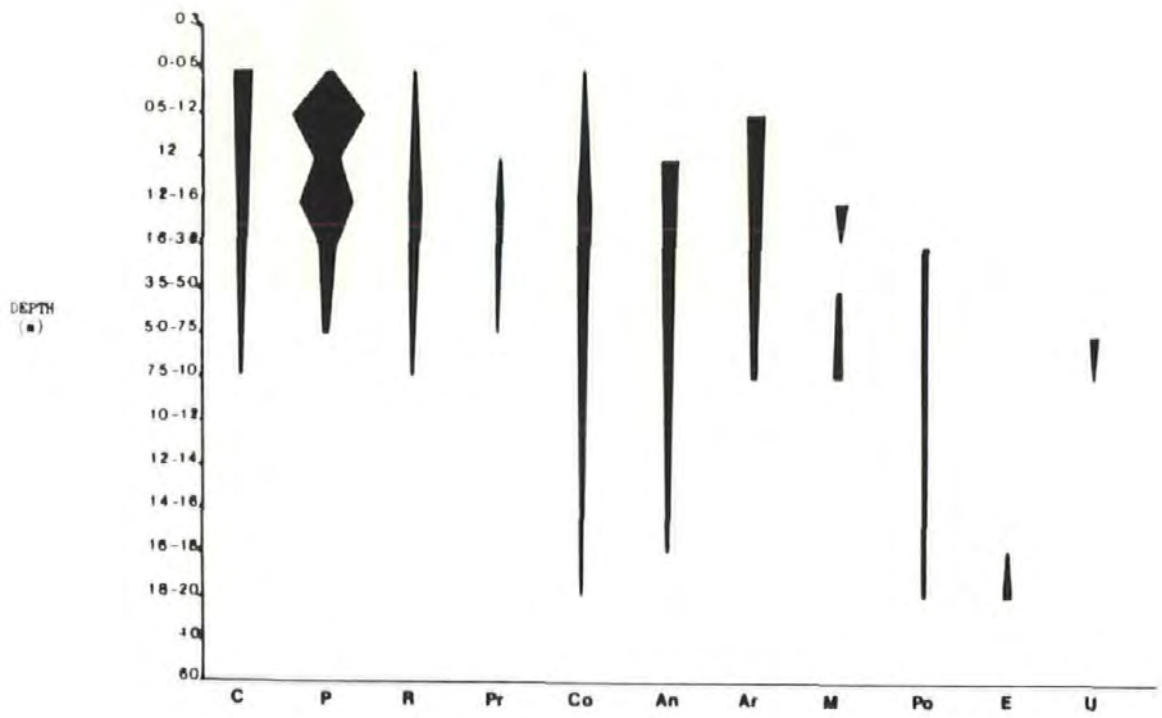
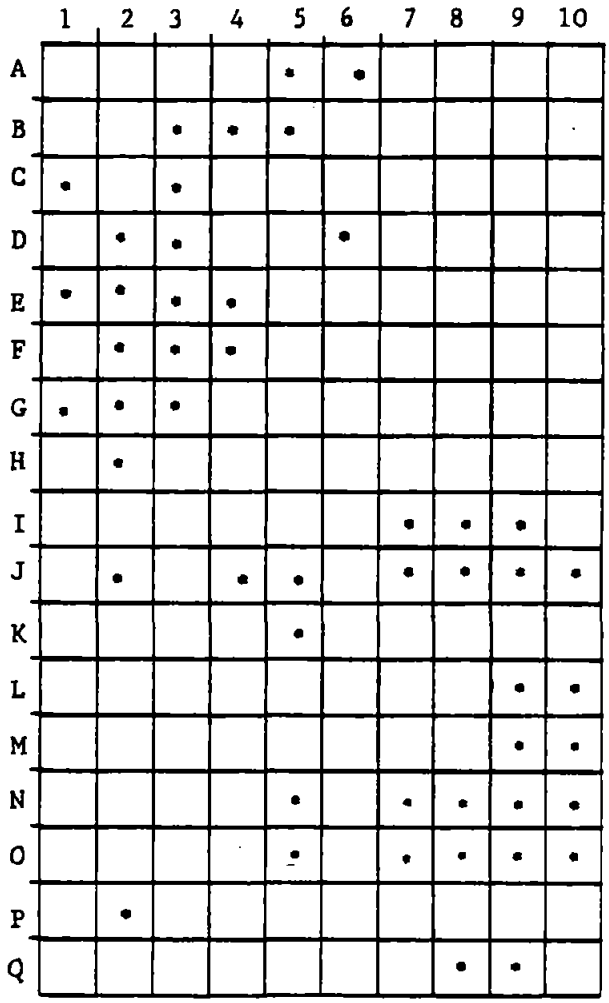

DEPTH
(m)
0-3
0-05
05-12
12
12-16
16-35
35-50
50-75
75-10
10-12
12-14
14-16
16-18
18-20
40
60
C
P
R
Pr
Co
An
Ar
M
Po
E
U

Figure 3.2.6.

Norway fouling species distribution. The distribution of 17 species of *Polyzoa* (A to Q) at Norway buoy sites 1 to 10.



A *Chartella Barleei*

B *Cryptosula ramosa*

C *Eschara Securifrons*

D *Escharella abyssicola*

E *Escharella immersa*

F *Escharella klugei*

G *Escharella lacqueata*

H *Electra crustulentra*

I *Electra monostachys*

J *Electra pilosa*

K *Haplopoma planum*

L *Lagenipora pygmaea*

M *Membranipora membranaceum*

N *Porelloides laevis*

O *Porelloides struma*

P *Stomachetosella sinuosa*

Q *Tegella unicornis*

Figure 3.2.7.

The abundance and frequency of both *Tubularia larynx* and *Pomatoceros triqueter* at Norway site groups 1, 2 and 3.

Vertical axis = depth in metres

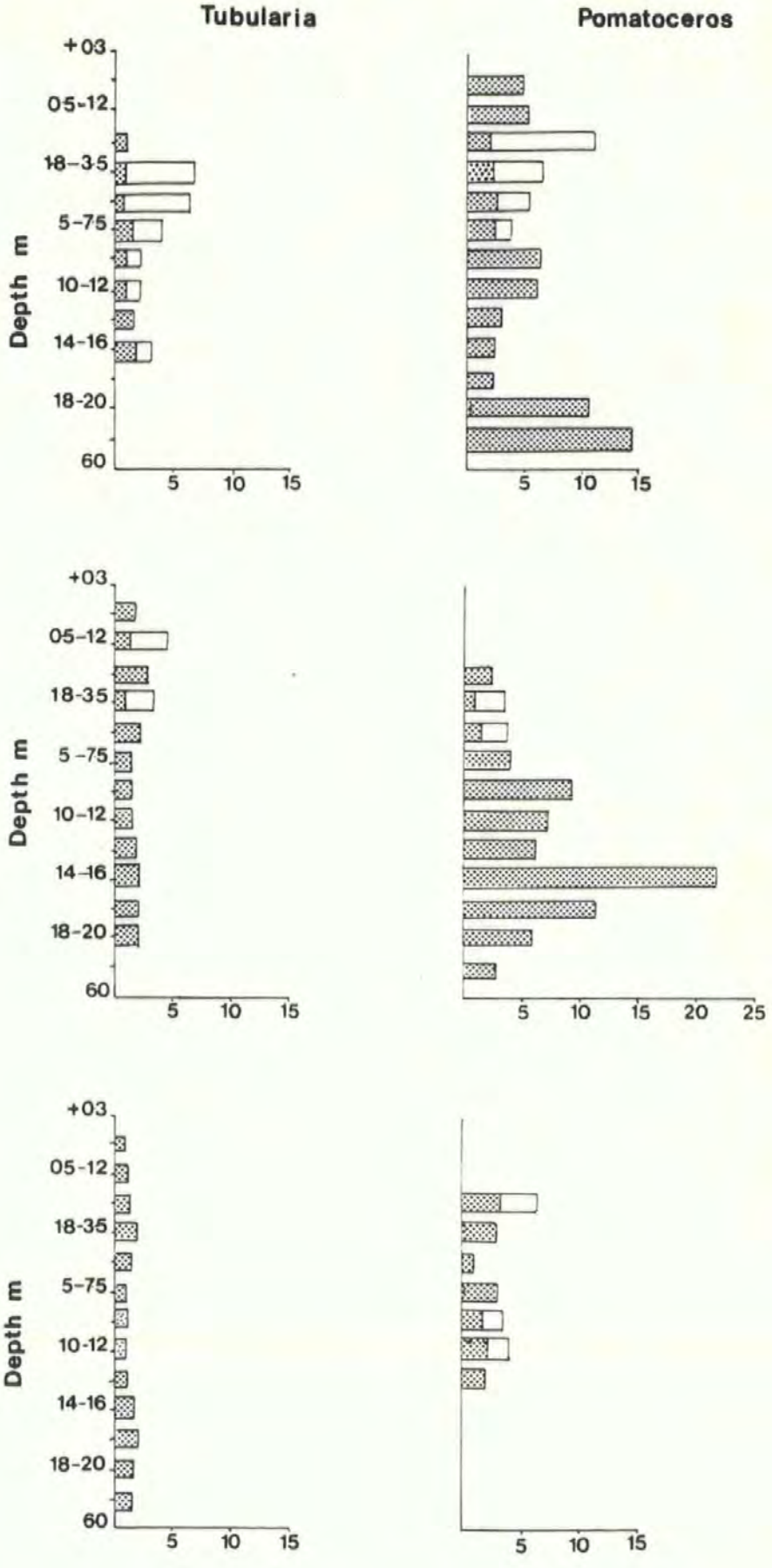
horizontal axis = biomass in g/100 cm^2

▩ *Tubularia* fouling

▩ *Pomatoceros* fouling

□ Total hydroid fouling

□ Total annelid fouling


Tubularia
Pomatoceros
Depth m
+03
05-12
18-35
5-75
10-12
14-16
18-20
60
5
10
15
20
25

CHAPTER 4

Larval Settlement in *Tubularia larynx* (Ellis and Solander.)

4.1 *The Mechanism of substrate attachment and settlement.*

The newly liberated actinula larva was ovoid in shape and between 650 um and 700 um in length, excluding the tentacles. The mouth was surrounded by up to 6 or 7 short, oral tentacles at one pole, and behind it were a number (usually 8 to 12) of long aboral tentacles which radiated stiffly outwards. Pyefinch and Downing (1949) fully described the actinula larva in *Tubularia*, and their observations are confirmed in the present study.

In still water no free movement over the substratum was observed. The actinulae attached to a number of surfaces, including perspex settlement 'slides', the surfaces of the settlement tanks, glass pipettes, plastic air-line and even aerator stones. This initial attachment was effected by the tips of the aboral tentacles and was temporary in nature. Larvae attached in this way could be dislodged by jets of water from a pasteur pipette in most instances, but were able to reattach, often to the next object encountered.

Observations on a number of the earliest permanently settling individuals showed that within 2 to 4 hours after liberation, the tentacles which had effected this initial 'temporary attachment' had become detached from the substrate. At this stage, the young *Tubularia* polyp, with aboral tentacles raised, assumes the feeding posture. During this period, the aboral pole became securely attached to the substrate and the larvae could not be dislodged by even strong jets of water. Contact of the aboral tentacles to the substrate was broken as the result of limited, spasmodic flexing movements of the hydrocaulus

together with polyp elongation. These movements were seen to stretch and relax the aboral tentacles, in turn, until they became detached from the substrate. The sequence of events involved in the attachment and settlement of the actinula larva, in *Tubularia*, to the substrate is illustrated in figure 4.1.1.

'Temporary attachments' by the aboral tentacles were effected by means of the nematocysts contained in the swollen tips of each tentacle. Upon contact with a hard surface many of the nematocysts were discharged and their threads served to anchor the tentacles to the substratum (Plates 4.1.2, 4.1.3 and 4.1.4). In most cases almost all the aboral tentacles are involved in the 'temporary attachments' (Plate 4.1.5). The actinula larva is then orientated in such a way as to bring the aboral pole into contact with the substratum (Plates 4.1.5 and 4.1.6). At this stage the aboral pole is seen as a circular, raised button of tissue (Plate 4.1.7), which consists of deeply columnar ectodermal cells (Plate 4.1.8).

Following contact of the aboral pole with the substratum (Plate 4.1.9) this 'button' of tissue began to protrude (Plate 4.1.10), and a bulge of tissue or stolon bud was formed beneath the aboral pole (Plate 4.1.11). These protrusions seemed to be adhesive in nature, and often many mucus-like threads were seen between the aboral pole and the substrate (Plate 4.1.11). Such outgrowths appeared to rupture the membranous sheath of perisarc bounding the larva and grow outwards from the aboral pole as a bulbous mass (Plates 4.1.12, 4.1.13, 4.1.14 and 4.1.15). More than one stolon bud may be formed, however, (Plate 4.1.16) and a number of protrusions may radiate from the aboral pole. At this stage the actinula is securely fixed to the substrate, and the 'temporary attachment' provided by the aboral tentacles is broken.

The surface of the stolon bud was smooth in contrast to the 'grooved' and sculptured nature of the perisarc of the hydrocaulus and aboral pole. In addition, the leading edges of stolon buds appeared to make a 'sticky' contact with the substratum and gave the overall impression of being adhesive in nature (see Plates 4.1.12 and 4.1.13). Subsequent elongation of stolon buds gave rise to 'primary' stolons which grew rapidly across the substratum (Plates 4.1.17, 4.1.18 and 4.1.19). The tip of each 'primary' stolon (the leading 60-70 um) remained smooth in appearance, however, the older parts, in contrast, had taken on the sculptured appearance of the perisarc of the hydrocaulus, and became striated and 'grooved'.

The perisarcal sheath (see Plate 4.1.8) investing the young *Tubularia* polyp was continuous in the stolons, and formed a particularly thick layer at the tip of actively growing stolons (Plate 4.1.20). The ectoderm at the tip of the primary stolons was thickened, and resembled the tissue of the aboral pole in the actinula larva (see Plate 4.1.8). Composed of columnar cells with a large central nucleus (Plate 4.1.21), the ectodermal cells contained large numbers of intensely-staining granules, densely packed at the apical surface (Plate 4.1.22).

In addition, the zone of growth (the columnar ectodermal cells) in primary stolons may change direction, so that older portions of stolon give rise to new protrusions of actively growing stolon (Plate 4.1.23). By such stolonic growth, the newly settled *Tubularia* individual was firmly attached to the substrate. (Plates 4.1.24 and 4.1.25), and the typical ramifying network of basal stolons rapidly produced.

This sequence of events from free actinula to settled polyp was not always accomplished, however, and a number of larvae underwent elongation and stolon bud production whilst the aboral pole was not yet

in contact with the substratum (Plate 4.1.26). The initial protrusion of the stolon bud may be due to a reorganization of body tissues in the actinula (Plate 4.1.27) and the active tissue again appears to be the 'button' of columnar ectodermal cells of the aboral pole. Here, the ectodermal cells, with their characteristic densely-staining granules packing the apical surface of the cells, formed the tip of the stolon bud. (Plate 4.1.27.)

Transmission electron microscopical studies revealed the nature of the intensely-staining granules of the columnar ectodermal cells of the aboral pole of the actinula, and in the stolon tip. Two types of granules or vesicles occupying the apical half of the ectodermal cells of the aboral pole were identified. Type A vesicles were more numerous and striking in appearance. They were spherical (sometimes irregular), membrane bound and varied in diameter up to 0.6 um. They contained broad bands of electron dense lamellae in parallel stacks or otherwise arranged in various patterns (Plates 4.1.29, 4.1.30 and 4.1.31). The background matrix was more moderately electron dense and often highly granular. There was, however, a notable degree of variation in the electron density of Type A vesicles. Type B vesicles were more uniformly moderately electron dense (Plates 4.1.29 and 4.1.30). These bodies were spherical or rod-shaped, more usually being 0.1 um or less in diameter, but often vesicles of varying diameter up to 0.6 um were found. They were typically homogeneous in content, moderately granular and membrane bound.

The cytoplasm of the apical half of the cells contained a large number of spherical and rod-shaped mitochondria, and was distinctly vesicular (see Plate 4.1.28). Typically large empty vesicles were seen in the cytoplasm towards the apical surface. Both Type A and

Type B vesicles were densely packed close to the apical surface of the ectodermal cells, adjacent to the membranous perisarcal sheath, and often rows of Type B vesicles were seen at the junction of the cell surface with the perisarc.

Occasionally, large aggregations of Type A vesicles were seen in the mid cell region (Plate 4.1.32). These multivesicular bodies were often closely associated with granular endoplasmic reticulum and numerous rod-shaped mitochondria, and may be the site of manufacture of Type A vesicles (Plate 4.1.33).

The proximal half of the cell was characterized by a large nucleus in close association with deep profiles of granular endoplasmic reticulum (Plate 4.1.34), but contained fewer of both Type A and Type B vesicles. Occasionally, large multivesicular bodies were found close to the nucleus. Also typical of this region of the cell were a number of large 'cored' vesicles (Plate 4.1.34). These membrane bound structures were spherical, often irregular, and contained a granular matrix of varying electron density. Although similar in appearance to Type A vesicles, these structures lacked the organization of electron dense lamellae forming parallel bands or other patterns, characteristic of Type A vesicles.

The ectodermal cells of the tip of the primary stolons in newly settled *Tubularia* polyps were very similar in appearance to those described at the aboral pole of the actinula. However, the nucleus was often more centrally situated and the cytoplasm typically highly vacuolated especially at the apical surface (Plate 4.1.35). The apical surface of the cells also contained large numbers of Type A vesicles, although fewer Type B vesicles were seen. There appeared to be a release of products at the cell surface. These products, of intense to moderate

electron density, were laid down on the inner surface of the perisarcal sheath in a series of layers (Plate 4.1.36).

4.2 *Histochemistry*

The results of light microscope histochemical tests on resin and wax sections are listed in Table 4.2.1. The ectodermal cells and the perisarc of the aboral pole in the free actinula larva, the newly settled polyp and in primary stolons, all reacted positively to both the Periodic acid-Schiff test and the lead tetra-acetate method for carbohydrates. However, no significant reactions were observed with Alcian blue methods, indicating the presence of non-acidic mucopolysaccharides in the ectodermal cells and perisarc of each test material.

Aboral pole (free actinula larva)

A weak over-all reaction, seen as a pale bluish product in both the ectoderm and the perisarc was recorded with the alkaline tetrazolium test, whilst the perisarcal sheath was faintly stained with Mallory's triple stain. All the protein tests, except Sakaguchi's method for arginine, were moderately positive in the ectoderm but did not react with the perisarc. Some sections stained with mercury bromophenol blue showed an intense blue reaction, localised in granules in the ectodermal cells.

Aboral pole (newly settled polyp)

A similar reaction to the alkaline tetrazolium was recorded from the bulbous protrusion of the 'stolon bud' securing the actinula larva to the substrate. An overall pale blue reaction was again seen in the ectodermal cells. Exterior to the 'stolon bud' the diffuse mass of

secretory material stained more intensely blue with alkaline tetrazolium, possibly indicating the presence of chitin. This material also stained blue with Mallory's triple stain, although the reaction product was 'patchy'. With the exception of Sakaguchi's test for arginine, the protein tests were again positive. In the ectodermal cells of the 'stolon bud' mercury bromophenol blue reacted intensely with some strongly coloured granules being observed near the apical surface. Faint reactions to the protein tests (except Sakaguchi's method) were also observed in the diffuse secretory material exterior to the ectodermal cells.

When exposed to a 10% sodium hypochlorite solution in sea water, young, permanently attached, *Tubularia* polyps were detached from the substratum and 'dissolved' within 8 hours.

Primary Stolon Tip

A pale blue reaction to the alkaline tetrazolium method was seen in the mass of secretory material at the tip of the primary stolon. The intensity of staining did not appreciably decrease away from the tip, and the ectodermal cells were only faintly stained. In Mallory's trichrome stain the mass of material at the tip of the primary stolon stained more intensely blue, whilst the perisarc of the remainder of the stolon reacted less strongly and formed a pale blue reaction product. Positive staining reactions to mercury bromophenol blue and Millons (Baker's modification) were recorded in both the ectodermal cells and the secretory material at the tip of the primary stolon. Reactions to the diazo-coupling method were less intense, whilst Sakaguchi's test was again negative. When exposed to 10% sodium hypochlorite solution in sea water, young *Tubularia* polyps were again

detached from the substratum and 'dissolved' within 8 to 10 hours. Reactions to the alkaline tetrazolium method were often diffuse and difficult to interpret. Reaction colours observed were probably due to the presence of SS and SH protein groups in the chitin (Pearse, 1968).

Tests for Phenolic tanning

In addition a range of whole mount histochemical methods to demonstrate the components of a phenolic tanning mechanism were carried out. The results are summarized in Table 4.2.2.

The aboral pole of the free actinula larva was positive to each of the tests for a phenolic compound, although the reaction was often weak, but notably intensely purple/black to the Iodaffin test. These results indicated the presence of an orthodihydroxyphenol in the ectodermal cells of the aboral pole. The ectodermal cells also contained an orthodiphenol oxidase as indicated by the amber (with dopamine) and black (with catechol) reaction products obtained with the catechol oxidase method; no darkening occurred with tyrosine, indicating that darkening was not caused by oxidation of endogenous orthomonohydroxyphenols

The ectodermal cells of the aboral pole, the primary stolon and the hydrocaulus in the newly settled polyp, all reacted positively to the tests for a phenolic compound. A much fainter reaction was seen in most of the perisarc except at the primary stolon tip where the bulbous mass of secretory material was negative to each of the methods. The catechol oxidase test for phenol oxidase activity produced strong positive reactions with dopamine and catechol in the ectoderm and, to a lesser degree, the perisarc in the young polyp (Plate 4.2.3). When *Tubularia* polyps were incubated with tyrosine, no reaction was observed. In addition, no phenol oxidase activity could be demonstrated using this technique in the secretory material at the tip of the primary stolon.

Throughout these experiments no obvious 'tanning cells' were observed in either the actinula larvae or in the newly settled polyp. Similarly, the presence of copper (Rubeanic acid test) in the perisarc or ectodermal cells could not be demonstrated.

4.3 *The Duration of the Larval Period*

In this part of the study, an investigation of the duration of the larval or 'pelagic' period, i.e. from release of the actinulae larva to successful settlement attachment, was carried out. There were 5 replicate sets.

Newly released actinula larvae introduced to the settlement tanks exhibited no site selection behaviours and were held in the water column only by circulatory movements of the water, originating from aeration. 'Temporary attachments' were frequently observed, and actinula larvae readily attached to and settled on all surfaces in the experimental tanks.

Within 2 hours the mean larval settlement was 6%. In experiment 5, 16 young polyps were permanently attached after 2 hours. Within 4 hours the mean settlement was 30%, and the maximum rate of settlement was achieved after 8 hours, during which time, on average 71% of each tank population had accomplished settlement (figure 4.3.1). Larvae continued to settle, although at a much reduced rate, up to 20 hours after liberation. In the 5 experimental tanks a mean settlement of over 90% was recorded within the first 20 hours. (Table 4.3.2.) At this time few 'pelagic' larvae were seen. Throughout the experiment care was taken to count the white, almost transparent settled polyps as accurately as possible, and aeration was minimal during the period of counting. Larvae were adjudged to be permanently settled when the aboral pole was securely attached to the substrate, with the aboral

tentacles raised in the feeding posture.

4.4 *Laboratory Substrate Preference*

The laboratory study of substrate preference in *Tubularia* actinula larvae was also carried out with 5 replicates. As previously described, actinula larvae readily settled on all surfaces in the experimental tanks, including aerator stones and air-line.

In each experiment only an average of 27% of each tank population settled on the experimental surfaces at all. No preferences between experimental substrates were observed and the actinula larvae settled in almost equal numbers on each material (Figure 4.4.1). In experiment 4, only 21% of the larvae which had achieved settlement after 24 hours (88% settlement rate) colonized the experimental plates, the remaining 67 polyps inhabited all the other surfaces in the experimental tank. (Table 4.4.2.)

4.5 *Field Studies*

Field observations were carried out on the 'turtle' raft moored in the river Yealm, S. Devon. In this part of the study difficulties were encountered in determining how much of the estimated cover of *Tubularia* was due to settlement, and how much was due to vegetative growth of established colonies. Although, initially, the small size of new colonies enabled them to be readily identified, during periods of rapid colonial growth they could not be easily distinguished from established colonies. However, the work was included in this section since it gave some indication of the seasonal pattern of *Tubularia* settlement, and revealed the influence of light conditions and type of substrate. Only new colonies and actively growing colonies possessing hydranths were included in the estimation of % cover. Dormant colonies

of stolonic growth only, where the hydranths had been lost, were omitted from the twice monthly count. Table 4.5.1 lists the mean monthly temperature and salinity, for high and low tides, at the raft site.

The settlement and growth of *Tubularia larynx* in the river Yealm, followed a seasonal cycle (Table 4.5.2). Most of the panels were rapidly colonized soon after exposure, and at the end of April a mean cover from all the experimental panels of 14% was recorded. The peak rate of colonisation and growth was observed at the end of May. At this time 28% of the experimental surfaces bore growths of *Tubularia*. Most colonies died back during the summer months of June, July and August, and many hydranths were lost. Following this period of minimum settlement and growth, there was rapid increase in the mean % cover to 16% of the panel surfaces, at the end of September. The reduction in % cover *Tubularia* during the summer months may have been influenced also by nudibranch mollusc predation, although few individuals (e.g. *Facelina*) were found.

4.5.1 *The Effect of Light*

A marked difference was observed in the rate of settlement and growth of *Tubularia larynx* on panels in light zone C and light zones A and B (Figure 4.5.3.). In each month, the greatest mean % cover was recorded from the panels in light zone C. In this zone, the rate of settlement and growth was often double that recorded from panels in light zones A or B. By mid-April the mean cover recorded from the 4 experimental panels in light zone C was 18.5%, whilst corresponding figures of 4% and 9.5% were recorded from panels in light zones A and B respectively. The maximum mean cover observed was 44% and was

recorded from the panels in light zone C in mid-June. The summer decline in the cover of *Tubularia* was seen in each light zone. By the end of September, the rate of settlement and growth was again increasing rapidly. At this time the mean cover recorded from the experimental panels in each light zone was 9% (A), 14% (B) and 25% (C). A one-way analysis of variance showed the effect of light to be highly significant at P = 0.001. (Table 4.5.4).

4.5.2 *The Effect of Substrate*

Over the 6 months period of observations the same seasonal pattern of settlement and growth in *Tubularia* was recorded from the 4 test substrates (Figure 4.5.5). Each of the experimental surfaces was rapidly colonised, and by mid-April the mean cover (of each substrate in all light zones) was 12.5% on wood and tufnel panels, 10% on smooth perspex and 6.5% on rough perspex. By the May peak in the rate of settlement and growth in *Tubularia*, the mean cover varied from 33% (wood panels), to 28% (tufnel panels) and 25% (both rough and smooth perspex panels). This marked similarity in the % cover of *Tubularia* on each type of substrate was evident throughout the experimental period, and % cover figures of 16% (wood and smooth perspex panels) to 15% (tufnel panels) and 14% (rough perspex panels) were recorded at the end of September. A one-way analysis of variance test showed that the effect of substrate was not significant in the settlement and growth of *Tubularia larynx* (Table 4.5.6).

4.6 *Discussion*

The newly liberated actinula larvae of *Tubularia larynx* were capable of very rapid substrate attachment. In the laboratory, most

larvae became permanently settled within 8 hours. These findings largely confirmed the earlier reports of Ciamician (in Pyefinch and Downing, 1949), Pyefinch and Downing, (1949), Pyefinch, (1950) and Hawes, (1958) although most of these workers reported a rather longer 'free' larval period than was observed in the present study. Some actinula larvae did not successfully settle until up to 20 hours after liberation. Hawes (1958) believed that a delay in settlement following liberation "of some hours" was necessary for the cnidoblasts in the tips of the aboral tentacles to develop into nematocysts capable of effecting 'temporary attachments', during which time the larvae would be "widely dispersed" by water currents. Clearly the actinula larva is capable of rapid settlement and the duration of the larval period in *Tubularia larynx*, is short-lived. In comparison, for instance, the planula larvae of other hydrozoans (e.g. Williams, 1976) may persist for several days. Laboratory observations also showed that nearly all the actinula larvae in each experiment eventually succeeded in becoming attached, confirming the earlier work of Pyefinch and Downing (1949) who found that under "normal environmental conditions" in the laboratory 90% settlement in *Tubularia* was common.

The mechanism of settlement of the actinula larva in *Tubularia larynx* was divided into two distinct phases. The first was a 'temporary attachment' effected by the nematocysts contained in the swollen tips of the aboral tentacles. This primary or 'temporary attachment' was succeeded by the secondary or 'settlement attachment', where the actinula was securely attached to the substratum by means of the aboral pole.

Prior to settlement no site selection behaviours were observed, confirming the observations of Hawes (1958). Earlier studies (Hincks,

1868 ; Allman, 1871; Pyefinch and Downing, 1949), suggested that the actinula larva performed limited site selection activities immediately before settlement. However, it is unlikely that the 'temporary attachments' frequently observed in the present study represent testing of the substrate, although they could be confused with the "crawling" behaviour reported by Pyefinch and Downing (1949). During the 'free' larval period, the actinulae showed no direct response to either light conditions or substrate. The lack of any co-ordinated larval behavioural activities in *Tubularia*, is in sharp contrast to the often prolonged patterns of site-selection behaviour reported in the planula larvae of many other coelenterates (Duerden, 1902; Williams, 1965, 1976; Nishihira, 1967; Harrigan, 1972; Lewis, 1974; Siebert, 1974; Cargo, 1979). This contrast in the level of activity may be a result of the advanced stage of development of *Tubularia* larvae when released from the gonophore. Upon liberation the actinula larva in *Tubularia* can be regarded as a young polyp, and, as previously observed by Hawes (1958), needs undergo no striking metamorphosis to produce the settled juvenile.

In the laboratory, the actinula larvae of *Tubularia larynx* readily settled on a number of substrates and showed no preference for any particular material. These observations substantiate previous reports of the ability of actinulae to attach to varied surfaces, including even smooth glass (Pyefinch and Downing, 1949; Mackie, 1966). Similarly, field observations suggested that *Tubularia* larvae also failed to display any substrate preference under more natural conditions. In fact, the four test materials used in this study represented a wide range of substrate texture and quality, and yet all were equally acceptable as sites ultimately suitable for the growth of the organism. The results confirmed earlier field observations of

larval settlement in *Tubularia* by Barnes and Powell (1950) and Pyefinch (1950). However, Fry (1975) reported that actinula larvae settled preferentially on slime films. His findings were not investigated here, although laboratory observations showed the presence of a slime film was certainly not necessary for settlement to occur, and large numbers of actinulae readily settled on newly immersed, clean surfaces.

The settlement of *Tubularia larynx* may also be influenced by light. The settlement of *Tubularia* on the 'turtle' raft in the river Yealm, was greatest in the zone where the least incident light was recorded, confirming earlier field reports (McDougall, 1943; Fry, 1975), that the denser growths of *Tubularia* occurred in shaded conditions. Similarly, Hawes (1958) reported that *Tubularia* readily settled in large pipes and conduits away from incident light. More recently, a number of studies of fouling from oil and gas platforms in the North Sea indicate that *Tubularia larynx* settlement occurs more frequently and intensely at depths below 10 m, in zones of reduced ambient light (Freeman, 1977; Ralph, Goodman and Boyle, 1979; Leitch, 1980; Goldie, 1981; Goodman and Ralph, 1981; Kingsbury, 1981). In addition, Schmidt (1983a,b) reported that *Tubularia larynx* settled abundantly on the lower sides of experimental panels in Langstone harbour, Portsmouth, but occurred sparsely on the upper surfaces. These findings suggest *Tubularia* larvae may be photo-negative immediately prior to settlement, as demonstrated in a number of other hydrozoan (planula) larvae (see Chia and Bickell, 1977). However, in the present study field observations from the 'turtle' raft, also showed that some young *Tubularia* colonies became established in both the illuminated and intermediate light zones of the raft. During this experiment all other fouling organisms were regularly removed from the test panels and, in the partial absence of competition, settlement was not

restricted to the dark zone. In addition, laboratory observations revealed that *Tubularia* actinulae readily settled under fully illuminated conditions. Earlier, Stubbings and Houghton (1964) postulated that algal growths found on the upper, illuminated sides of experimental panels may prevent or reduce the settlement of actinula larvae in surface waters. Their observations are substantiated in this study and the vertical distribution of *Tubularia* may not be directly due to the effects of light intensity on larval settlement.

The division of the settlement process in *Tubularia larynx* into a primary or 'temporary attachment' phase, and a secondary or 'settlement attachment' phase, agrees with the accepted sequence of events recorded in the settlement of other coelenterate larvae (see Chia and Bickell, 1977). 'Temporary attachments' were effected by the discharge of the nematocysts carried in the swollen tips of the aboral tentacles, and were previously described in *Tubularia* by Pyefinch and Downing (1949) and Hawes (1958). The role of mechano-sensitive nematocysts in 'temporary attachments' was also reported in other hydrozoan larvae, including *Hydra* (Ewer, 1947) and *Hydractinia echinata* (Teitelbaum, 1966; Muller, Wicker and Eiben, 1974). However, the wide range of substrates settled on by *Tubularia*, precludes the existence of a 'contact-chemical' response as identified in the planula larvae of the symbiotic hydroid, *Proboscidactyla flavicirrata* (Donaldson, 1974).

Succeeding this phase, the secondary or 'settlement attachment' was shown to be effected by means of a massive secretion of unpolymerized, 'sticky' chitin from the glandular ectoderm of the aboral pole, which was subsequently hardened by a phenolic tanning process.

The role of a thickened, glandular epithelium in the aboral pole, in the 'settlement attachment' of coelenterate planula larvae has been

well documented (see Chia and Bickell, 1977). Many studies have variously identified the contents of these gland cells as complex mucopolysaccharides, and implicated a mucoid secretion in the 'settlement attachment' of planula larvae. In the present study, light microscopical investigations revealed that the columnar ectodermal cells in the aboral pole of the *Tubularia* actinula were characteristically densely packed with a large number of intensely staining granules. These observations confirmed earlier reports in *Tubularia* by Lowe (1926) and Pyefinch and Downing (1949). Histochemical methods showed the ectoderm (and perisarc) of the aboral pole contained a non-acidic mucopolysaccharide complex. The membranous perisarcal sheath was stained lightly blue with Mallory's trichrome, indicating its chitinous nature, agreeing with the observations of Pyefinch and Downing (1949). The ectodermal cells also contained protein groups, possibly localised into granules. Reactions of moderate intensity to Millons and the Diazo-coupling method indicated the presence of tyrosine residues. Sagakuchi's method for arginine was negative.

The mucopolysaccharide and protein groups of the ectodermal cells may form the structural components of the chitinous secretion from the aboral pole of *Tubularia* actinula larvae. Upon 'settlement attachment' the secretion of chitin initially took the form of a 'stolon bud' which after subsequent elongation and growth gave rise to a 'primary stolon'. The growing tip of the 'primary stolon' remained 'semi-liquid' and 'sticky'. However, behind the tip the chitinous perisarc of the rest of the stolon became hardened, securing the young *Tubularia* polyp to the substrate. The ability of hydroid perisarc to attach to hard substrata was earlier shown by the experiments of Goldin and Barth (1941) and Hauschka (1944) and newly secreted chitin has previously been

described as "semi-liquid" and "sticky" (Berrill, 1949, 1952; Hawes, 1958).

Early reports supported conflicting views of the mechanism of 'settlement attachment' in *Tubularia*. Pyefinch and Downing (1949) thought the actinula larva was secured to the substratum by a muco-protein secretion similar to the attachment cement of barnacles and other fouling groups. They postulated that this "extra-chitinous cement" was then hardened or tanned by the action of a quinone securing the actinula to the substrate. Other studies, however, found no 'extra-chitinous cement', but attributed the 'settlement attachment' of *Tubularia* actinulae to the secretion of new perisarcal chitin (Ciamician, 1879, in Pyefinch and Downing, 1949; Berrill, 1952; Hawes, 1958) and in *Obelia*, (Berrill, 1949). The results of the present study confirm the view that the secretion of perisarcal chitin from the aboral pole is the means by which 'settlement attachment' is achieved, and are in agreement with the conclusions of Hawes (1958) that 'no extra-chitinous cement is present or necessary".

Additional circumstantial evidence of the role of newly secreted chitin in effecting permanent attachment to the substrate was seen when newly settled *Tubularia* actinulae were exposed to a 10% sodium hypochlorite solution. These young polyps soon became detached and appeared to 'dissolve'. According to Brown (1950a) a sodium hypochlorite solution was able to disperse chitin-tanned protein complexes, and Chapman (1968) reported that the cuticular podocyst in *Aurelia* "dissolved" within 33 hours of being exposed to a 10% solution. Further morphological evidence that 'settlement attachment' was achieved without the need for the "attachment cement" proposed by Pyefinch and Downing (1949) was shown when some larvae (having effected 'temporary attachment') failed to make contact between the aboral pole

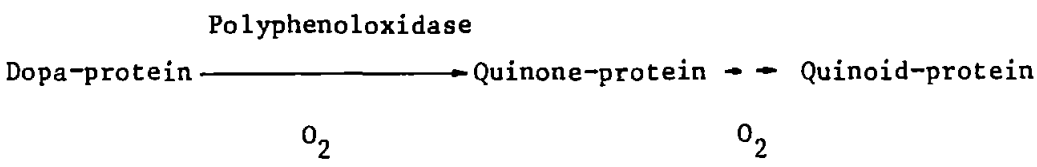
and the substratum (see Plate 4.1.26). In these individuals, the formation of stolon buds and elongation of the hydrocaulus were still observed, although Pyefinch and Downing (1949) had previously reported it was necessary for the aboral pole to contact the substratum to stimulate development. Longitudinal sections of these actinulae revealed that the coelenteron was continuous with the stolon bud, which itself was seen as an 'outpushing' or 'growth' of the aboral pole (see Plate 4.1.27). The characteristic ectodermal tissue of the aboral pole and 'stolon bud' also formed the zone of growth in the tip of the primary stolon, which was typically made up of simple columnar ectodermal cells containing large numbers of intensely staining granules. The aboral pole of the *Tubularia* actinula larva, therefore, can be considered as little more than a stolon tip, confirming the findings of Berrill, (1952).

The results of histochemical observations of the ectodermal cells in the 'primary stolon tip' and its chitinous secretions were also similar to those recorded in the aboral pole and 'stolon bud' tissue. The presence of a non-acidic mucopolysaccharide together with a protein complex containing a number of tyrosine residues was again demonstrated. The presence of structural proteins may also have been shown by the positive reaction to the alkaline tetrazolium test with the mixture of Barrnett and Seligman which is positive to SS and SH groups (Pearse, 1968). According to Dennell and Malek's (1955) classification of chitin reactions with Mallory's trichrome stain, the newly secreted perisarc at the stolon tip was untanned. Whole mount histochemical methods indicated that the chitinous secretions of the aboral pole and the primary stolon tip may subsequently be tanned by the action of a quinone.

The presence of a phenolic compound, probably an orthodihydroxy-phenol, together with phenol oxidase activity, was demonstrated in the perisarc and ectodermal cells in both the aboral pole and the 'primary' stolon. The evidence presented here suggests that the phenolic substrate and phenol oxidase originate in the ectodermal cells and are released to the inner surface of the perisarc, where oxidation of the phenol by the enzyme would produce a quinone to induce cross-linking of structural proteins, hardening the chitin.

Quinone tanned proteins are widespread in occurrence, although the sequence of events leading to tanning may differ. Ever since Pryor's (1940) pioneering work in the tanning of cockroach oothecae, tyrosine derivatives have been commonly implicated in the process of sclerotization, particularly in the hardening of insect cuticles, via the formation of N-acetyldopamine (Figure 4.6.1). N-acetyldopamine seems to lead to either of two types of sclerotization. The first of these, termed β sclerotiaation by Anderson (see Richards, 1978) because it involves the β carbon of the side chain (Figure 4.6.2.) results in hardening with little or no coloration. Secondly, N-acetyldopamine may lead to quinone sclerotization (Figure 4.6.3.) first proposed by Pryor (1940), and gives rise to coloured products. The tanning of aromatic amino acids occurs also in the hardening of the byssus in *Mytilus* (Brown, 1950b). Recently, Waite (1983) has investigated the secretion of the foot in *Mytilus* and proposed the metabolic pathway responsible for hardening of the byssus threads. He reported that the enzyme polyphenoloxidase induced intermolecular cross-linking of proteins in the byssus and suggests that the natural substrate of the enzyme may be the dopa-containing polyphenolic

protein:



Amongst the coelenterates, Chapman (1968) reported a protein with a large number of tyrosine residues was involved in the stabilization of the podocyst cuticle in *Aurelia*, by a mechanism of 'autotanning' (Figure 4.6.4). Subsequently, Knight (1968, 1970, 1971) reported a comprehensive study of the sclerotization of the perisarc in the calyptoblastic hydroid, *Laomedea (Campanularia) flexousa*. He demonstrated . the presence of the orthodihydroxyphenol, dopamine, and the enzyme orthodihydroxyphenol oxidase in specialised "tanning" cells of the ectoderm, and suggested oxidation of the dopamine to a quinone led to the tanning of structural proteins which formed a hard exoskeleton. Knight (1970) considered that, since tyrosine was a para substituted phenol, the positive reaction to Gibb's reagent in the protein-containing inclusions of the ectodermal cells of the podocyst in *Aurelia* (Chapman, 1968) was unlikely to be due to tyrosine (see Smith, 1960) but probably resulted from the presence of an orthodihydroxyphenol. Earlier, Pyefinch and Downing (1949) had reported a personal communication that dihydroxyphenols were present in the 'attachment cement in *Tubularia* and were involved in the tanning of that material.

Although proteins with a number of tyrosine residues were identified in the present study in the ectodermal cells, perisarc of the aboral pole and 'primary' stolon of recently settled *Tubularia*, positive reactions to all methods (including Gibb's test) to demonstrate an orthodihydroxyphenol, were also recorded. These findings suggest that

the mechanism of hardening of the chitin during the process of 'settlement attachment' of *Tubularia* actinulae may be the result of the phenol oxidase oxidising an orthodihydroxyphenol, and not a process of 'autotanning' involving the oxidation of the hydroxyl groups of tyrosine residues (Brown, 1950b, Chapman, 1968). The work of Knight (1970) included fluorescence techniques and gel electrophoresis, not repeated in this study, which further identified the orthodihydroxyphenol substrate as a catecholamine, probably dopamine. Clearly, whatever the precise sequence of events leading to hardening of the perisarc in recently settled *Tubularia* actinulae, a phenolic tanning process involving the formation of a quinone was responsible for the stabilisation of the newly secreted chitin.

The components of the phenolic tanning mechanism appeared to be secreted from the characteristic columnar ectodermal cells of the aboral pole and 'primary' stolon in *Tubularia*, and were added to the inner layers of the perisarc. Electron microscopical investigations revealed the presence of two distinct, membrane bound secretory packages (Type A and Type B vesicles) occupying the apical half of the cell. The contents of Type A vesicles were distinctly heterogeneous suggesting the structures were divided into compartments, and may be the sites of storage of both the phenolic substrate and phenol oxidase. Similar spherical inclusions in the young "tanning" cells in *Laomedea (Campanularia) flexuosa* consisted of a core "which may represent a store of phenol oxidase", and a matrix "which may contain the phenol" (Knight, 1969). Earlier, Smyth and Clegg (1959) had reported that the vitelline cells of Trematodes and Cestodes also appeared to store both the phenolic substrate and phenol oxidase in the same spherical inclusions; whilst proteins, phenols and polyphenol oxidase may be structurally separated in the heterogeneous granules of

the ∝ cells in the cypris cement glands of *Balanus balanoides*. (Walker, 1971). According to Knight (1971) separation of the enzyme from the substrate in the same vesicle in *Laomedea* may represent a control of quinone-formation. The contents of Type B vesicles in *Tubularia* were more moderately electron dense and homogeneous in nature. These structures may be the sites of storage of a mucopolysaccharide-protein complex contributing to the chitinous perisarcal sheath.

Secretions from the apical surfaces of the ectodermal cells in the aboral pole and the 'primary' stolon tip were laid down against the inner layers of the perisarcal chitin. No specialised "tanning" cells (Knight, 1968) were observed in *Tubularia*. Knight (1968) found "tanning" cells occupied an interstitial position throughout the ectoderm in colonies of *Laomedea*, and reported that they were "particularly numerous in the tips of actively growing stolons where they out-numbered the ectodermal epithelial cells". Cells similar in appearance to the "tanning" cells reported by Knight (1968, 1970) were first described by Allman (1872) in *Obelia dichotoma*. According to Billard (1904, in Knight, 1970) these distinctive cells were excretory in function and present in 19 species of calyptoblastic hydroids, 1 species of gymnoblastic hydroids and 2 species of siphonophores. Berrill (1949) described similar distinctive, ovoid, cells lying parallel to the surface in the epidermis, particularly in the growing stolon tip in *Obelia*. He suggested that they secreted the chitin of the perisarc. 'Tanning' cells have also been reported in the hardening of the periostracum in the bivalve, *Mercenaria mercenaria* (Hillman, 1961) and the test of the tunicate *Dendrodoa grossularia* (Barrington and Thorpe, 1968).

The evidence of this part of the study showed that specialised 'tanning' cells were not present in the ectoderm of actively growing tissues in the gymnoblastic hydroid, *Tubularia larynx*. Rather, the columnar ectodermal cells of the aboral pole and 'primary' stolon tips would appear to be the sites of synthesis (Plates 4.1.32 and 4.1.33) storage (Plate 4.1.31) and secretion (Plates 4.1.35 and 4.1.36) of the orthodihydroxyphenol and phenol oxidase. Figure 4.1.37 illustrates the ultrastructure of these highly active cells and summarizes the possible secretory pathway of the components of the phenolic tanning mechanism. There may, however, be a short delay in the formation of a highly reactive quinone, allowing the newly secreted chitin to remain labile, and to enable 'growth' of the stolon bud and 'primary' stolon. But it appears that the contents of Type A vesicles are released to the inner surface of newly secreted chitin, and the apparent absence from recently secreted chitin of the phenol and phenol oxidase may therefore be a result of fixation; whereas these materials are more readily retained in the more stable, hardened and semi-hardened chitin proximal to the tip of the 'primary' stolon and in the aboral pole. Consequently, there may be a 'control' mechanism preventing immediate quinone-formation. Clearly, more work is needed to precisely detail the events at the stolon tip and in stolon bud formation at the aboral pole. However, within the scope of the present study it appears that the phenolic tanning of recently secreted, unpolymerized chitin at the aboral pole is responsible for securing the actinula larvae of *Tubularia larynx* to the substrate, and is the mechanism by which 'settlement attachment' in the species is effected.

Figure 4.1.1.

Laboratory settlement in Tubularia larynx.

The sequence of events following release of the actinula larva to permanent or 'settlement attachment'.

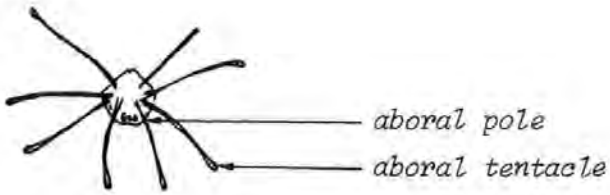
(a) Free actinula with stiffly radiating aboral tentacles.

(b) Temporary attachment effected by discharge of nematocyst threads from swollen tips of aboral tentacles.

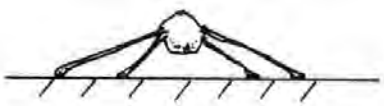
(c) Elongation of the hydrocaulus brings aboral pole into contact with substratum.

(d) Permanent attachment. The aboral pole is securely attached to the substratum. Subsequent flexing movements of the hydrocaulus (together with polyp elongation) and the aboral tentacles occur, breaking contact of the tentacles with the substratum.

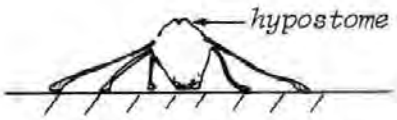
(e) The young *Tubularia* polyp adopts the feeding posture and the detached aboral tentacles are raised.



a *Free actinula*

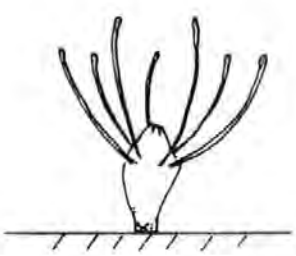


b *temporary attachment*



c *Elongation of hydrocaulus*

d *Settlement attachment*



e *Young polyp*

Plate 4.1.2

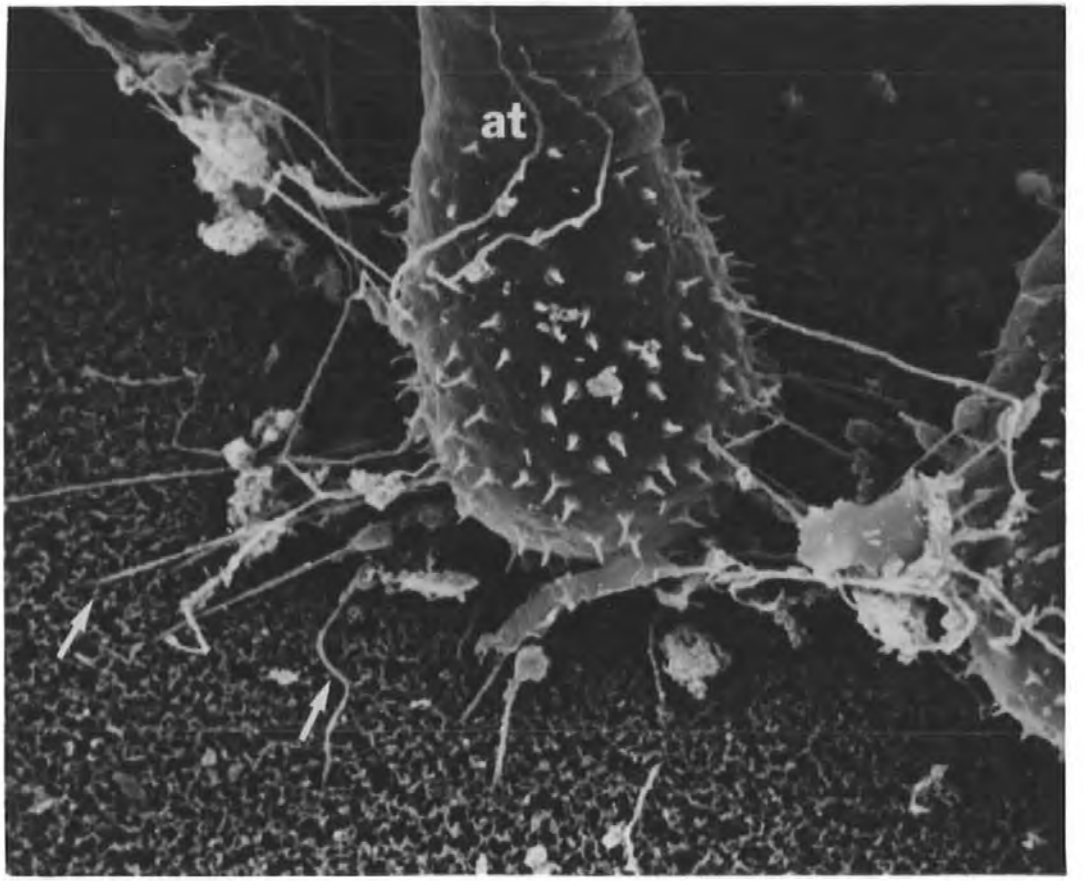
(x420)

Temporary attachment in *Tubularia larynx;* effected by discharged nematocyst threads (arrows) from the swollen tips of the aboral tentacles (at)

Plate 4.1.3

(x420)

Temporary attachment in *Tubularia larynx.* Arrows indicate discharged nematocyst threads.


at

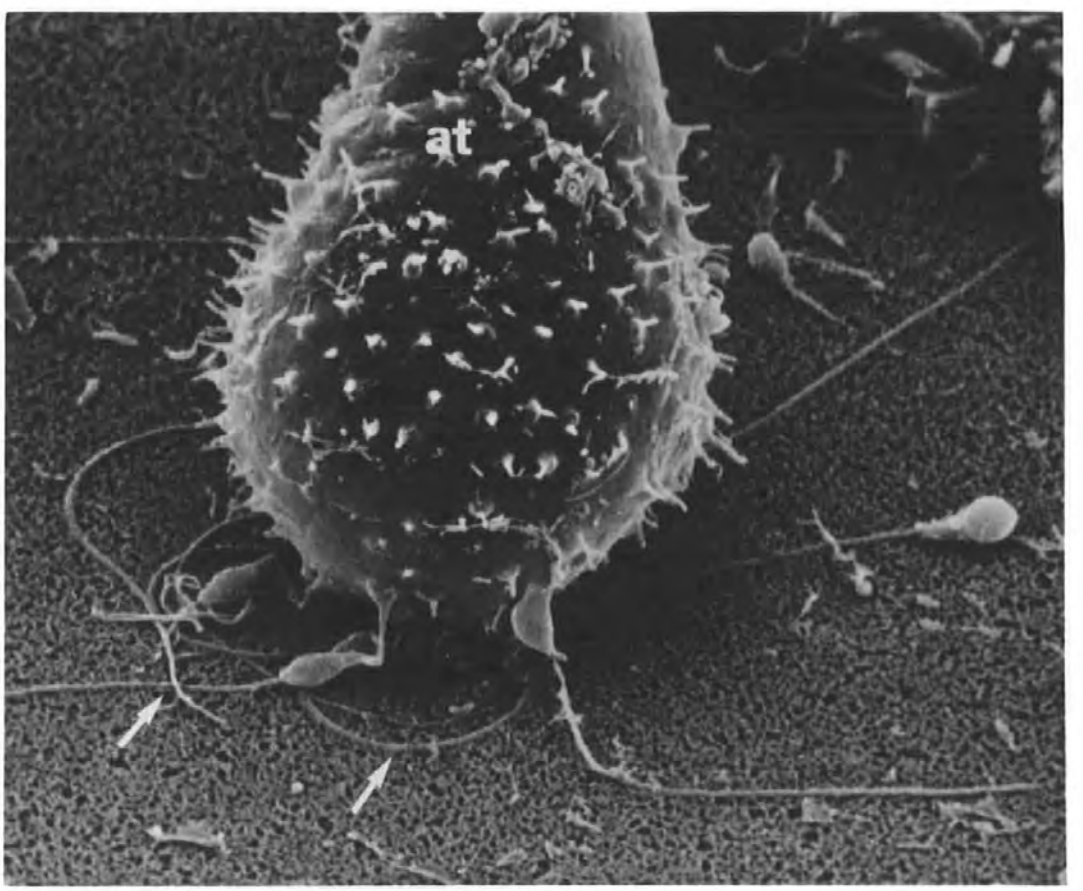

at

Plate 4.1.4

(x600)

Temporary attachment in *Tubularia larynx*. Arrows indicate discharged nematocyst threads from the tip of the aboral tentacle (at), covering the substratum.

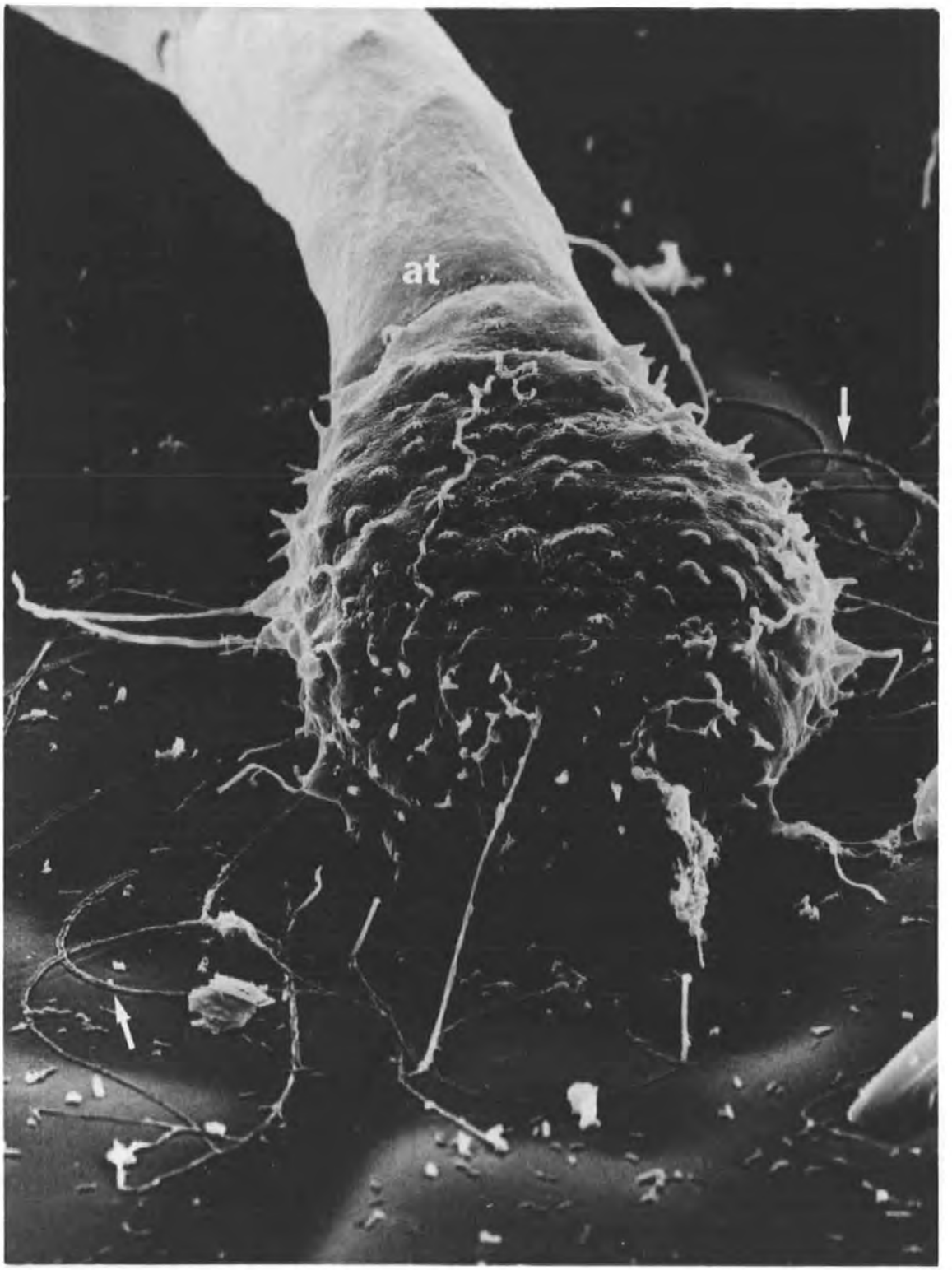

at

Plate 4.1.5

(x60)

Temporary attachment in *Tubularia larynx*. Almost all of the aboral tentacles (at) may be involved in securing 'temporary attachment' to the substrate.

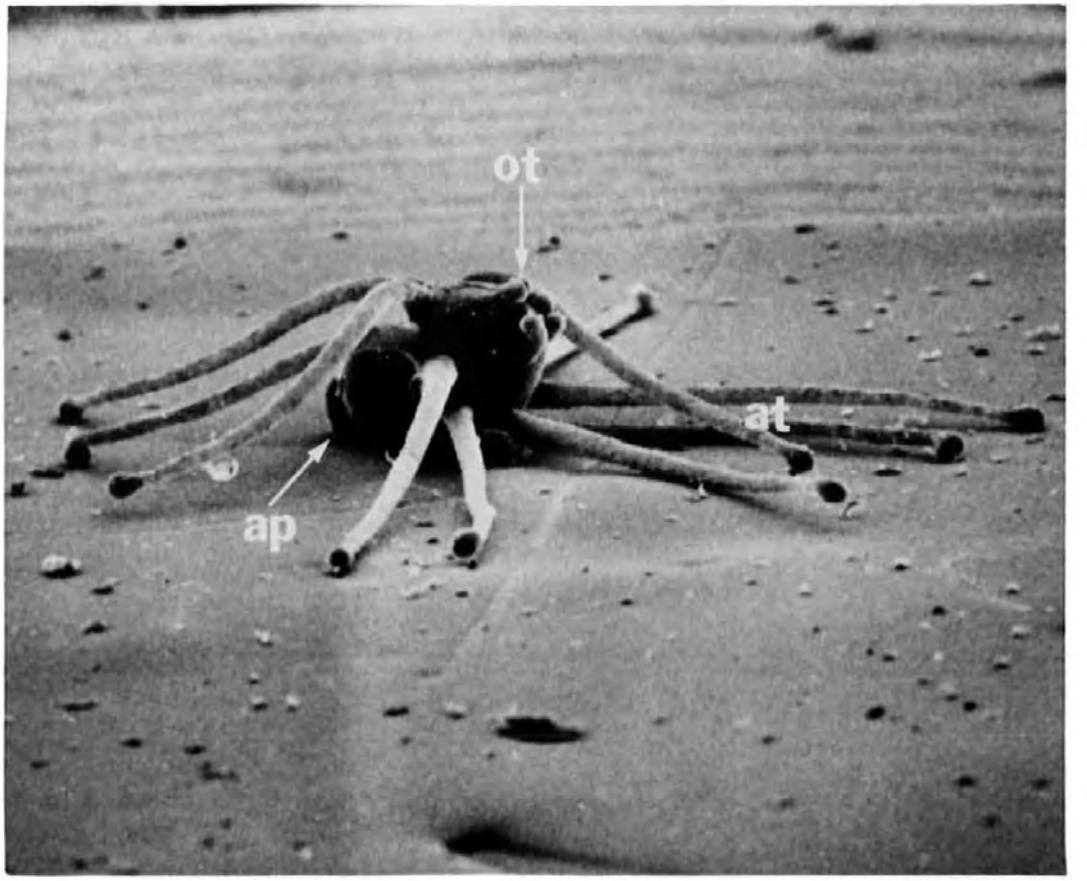
ot = oral tentacles

ap = aboral pole

Plate 4.1.6

(x44)

Temporary attachment in *Tubularia larynx*. Initially, only one or two aboral tentacles (arrows) may be involved, indicating the tenacity of the 'temporary attachment' brought about by the nematocyst threads.


ot
at
ap

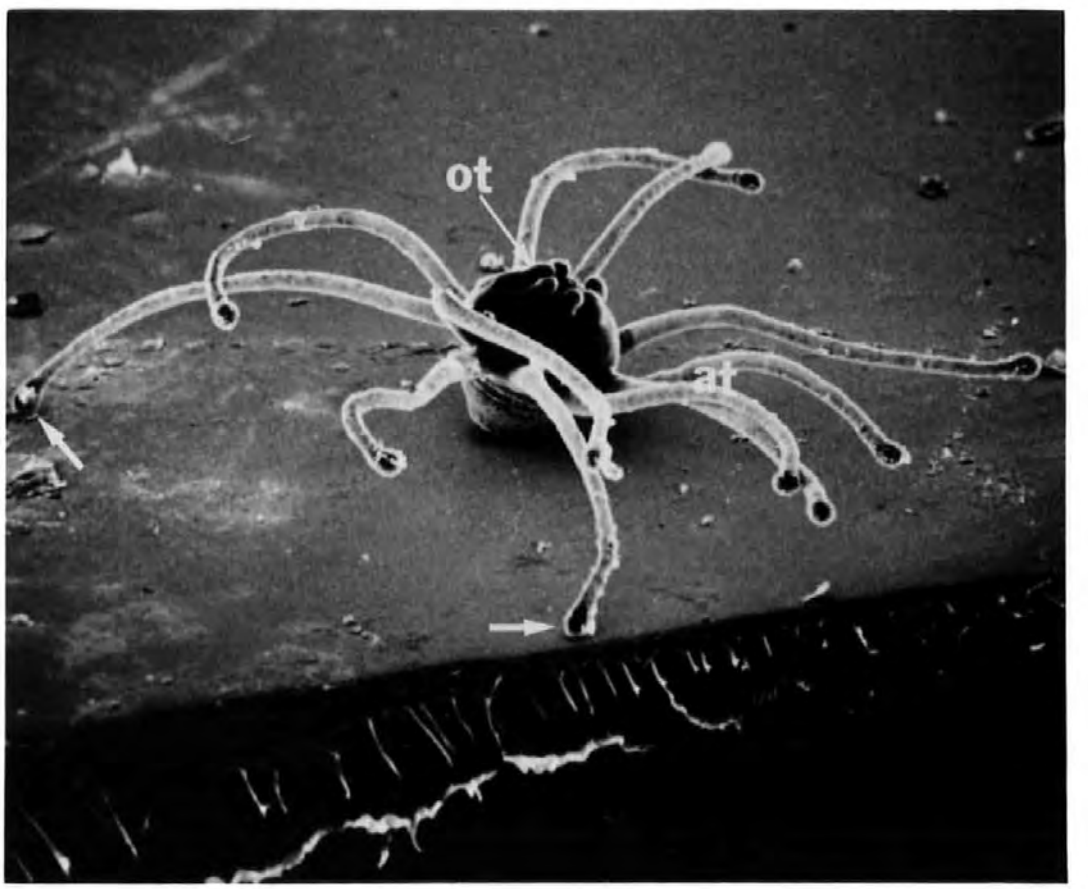

ot
at

Plate 4.1.7

(x380)

Scanning electron micrograph of the raised 'button' or 'pad' of tissue forming the aboral pole (ap) in the recently released, free, actinula larva in *Tubularia*.

at = aboral tentacle

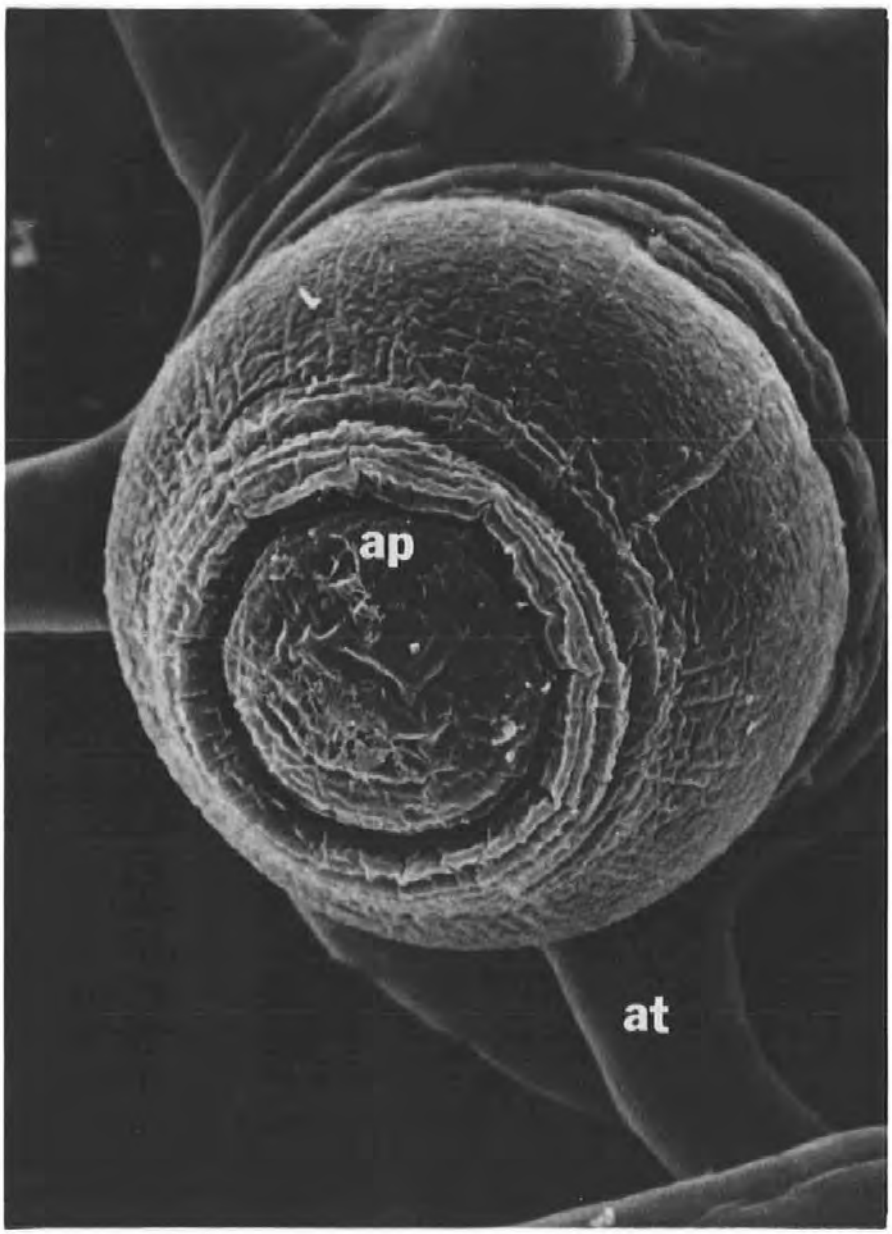

ap
at

Plate 4.1.8

(x240)

L.S. Newly released actinula larva (1 um, toluidine blue). The 'button' of tissue forming the aboral pole (ap) consists of deeply columnar ectodermal cells with a large proximal nucleus. Apically, the cells are characteristically densely packed with large numbers of intensely-staining granules (g). Externally, the thin chitinous sheath (up to 10-15 um) investing the larva is seen.

at = aboral tentacle

op = oral pole

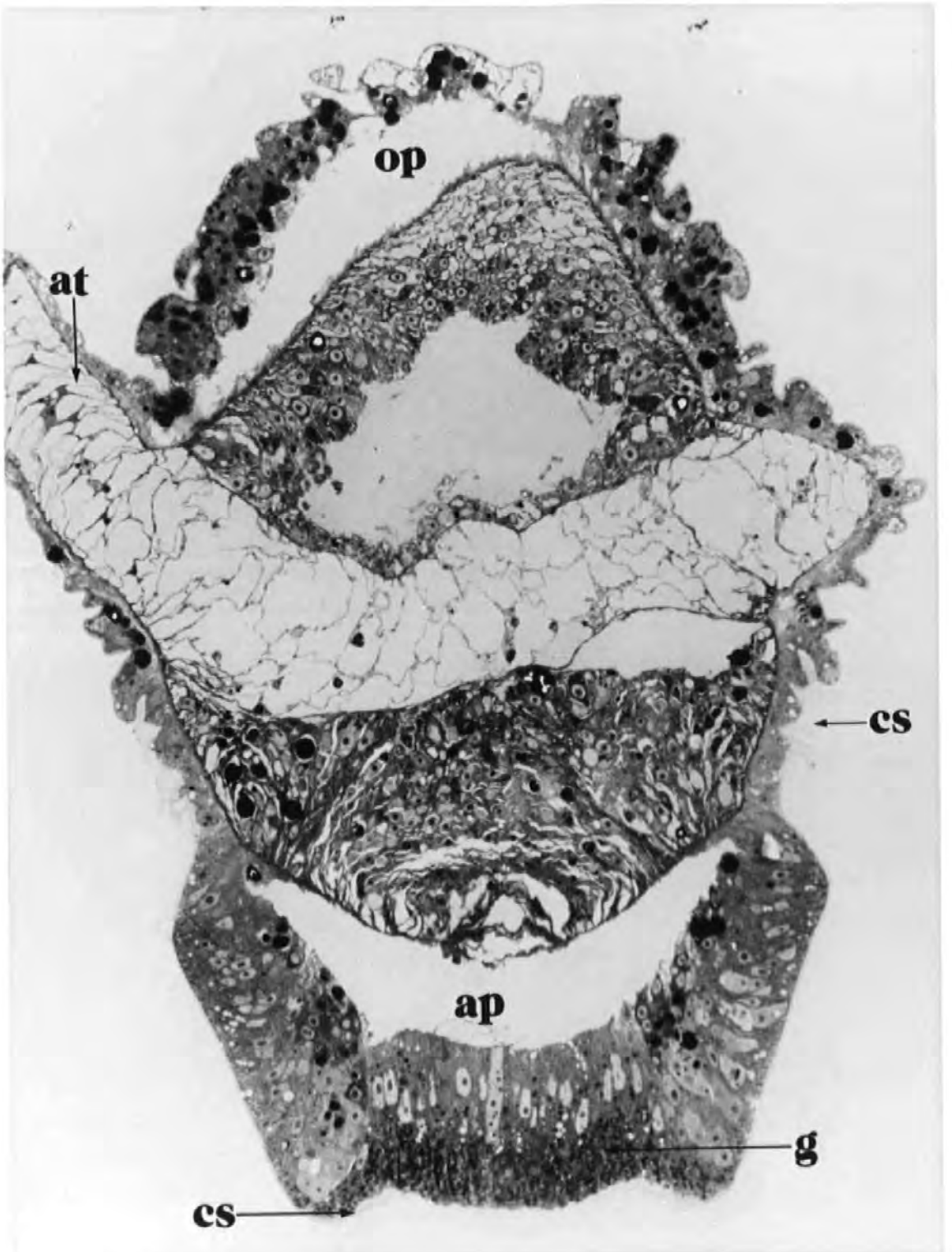

op
at
cs
ap
g
cs

Plate 4.1.9

(x240)

Temporary attachment in *Tubularia larynx*. The aboral pole (ap) is brought into contact with the substratum.

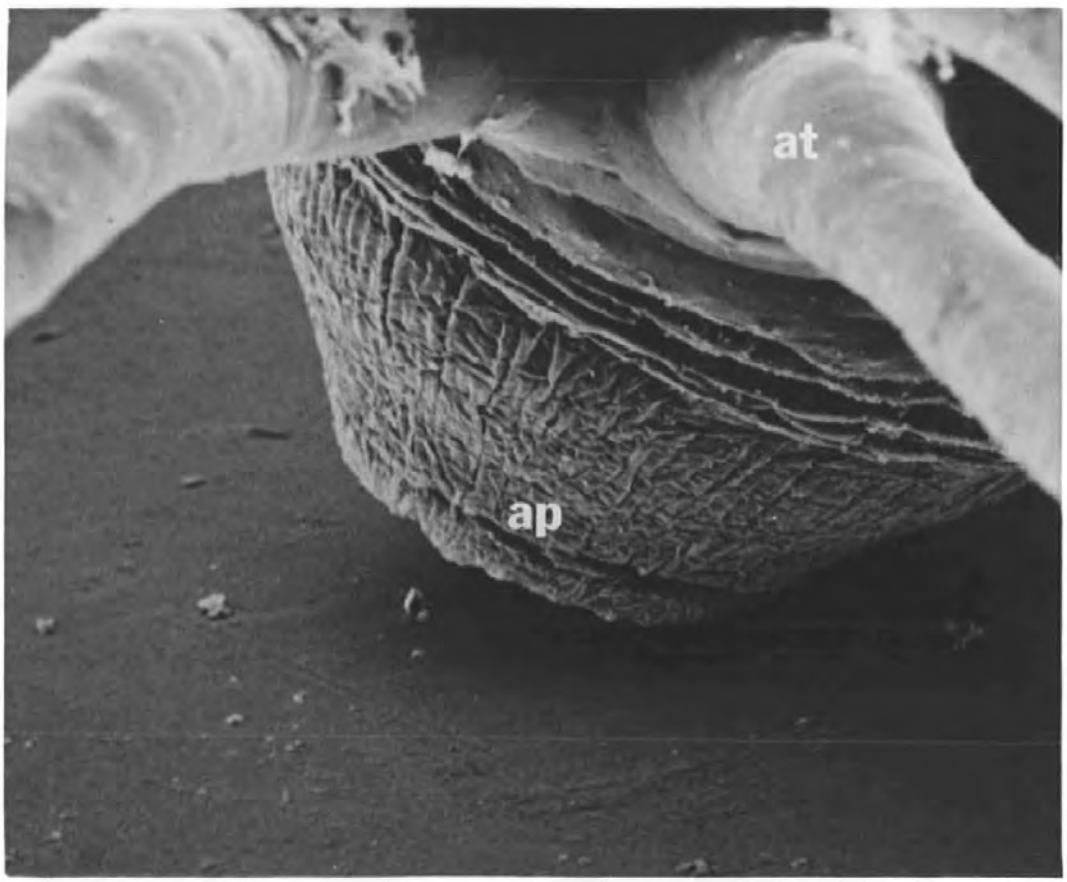
at = aboral tentacle.

Plate 4.1.10

(x240)

Scanning electron micrograph showing the first stage in the events leading to settlement attachment. The aboral pole (ap) is seen to 'grow' outwards in the direction of the arrows.

at = aboral tentacle


at
ap

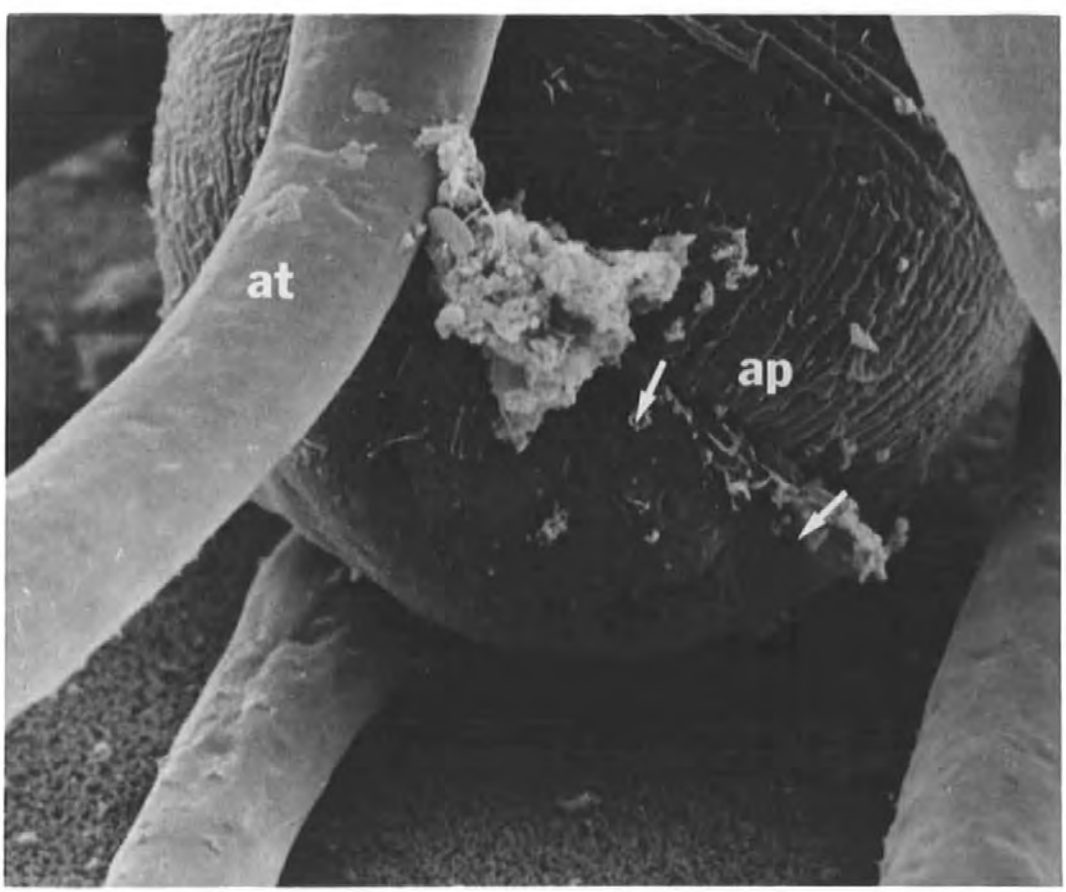

at
ap

Plate 4.1.11

(x260)

The protrusion from the aboral pole (ap) forms a bulbous mass or stolon bud (sb) which appears to make a 'sticky' or 'adhesive' contact with the substratum; n.b. sticky threads (st) of material are seen extending from the aboral pole (ap) in the region of the secretion of the stolon bud (sb), making contact with the substratum.

at = aboral tentacle

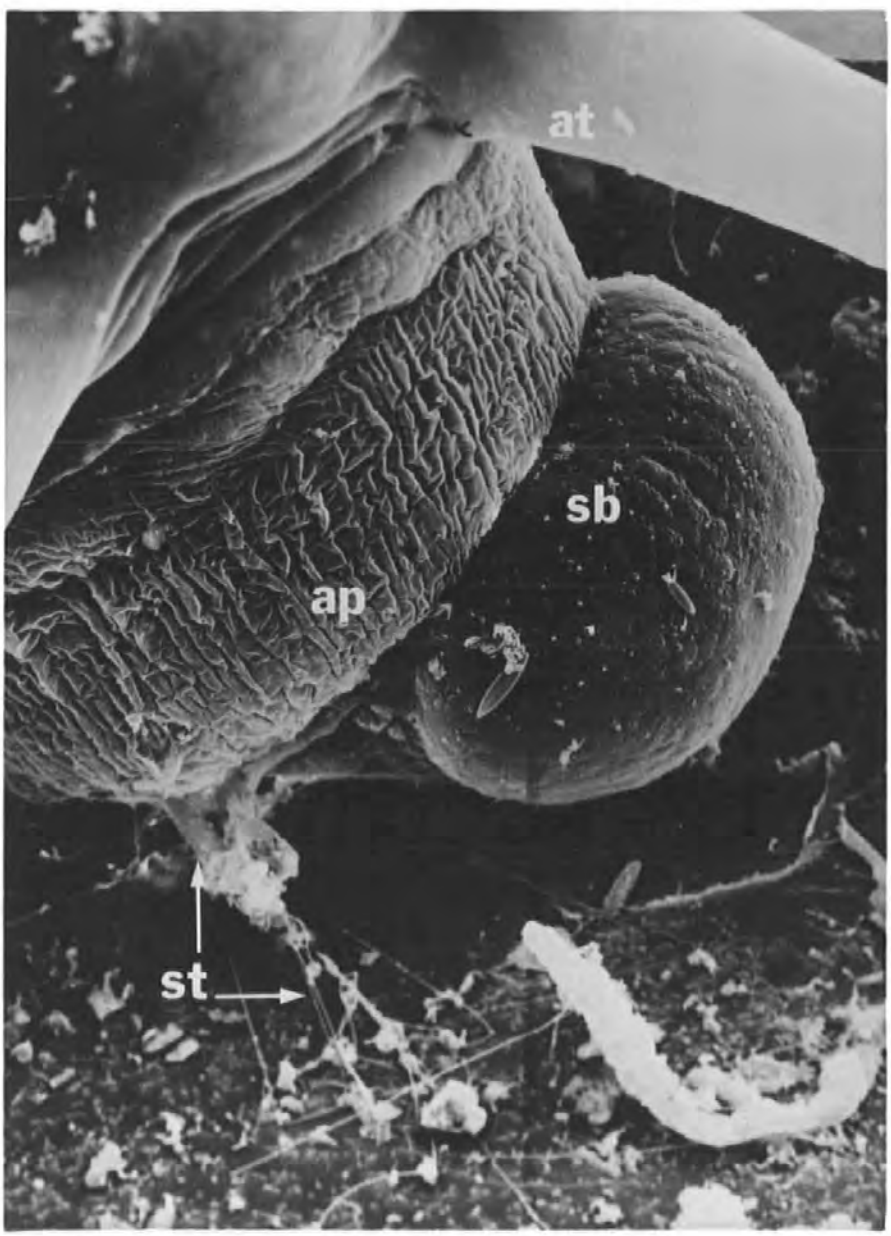

at
sb
ap
st

Plate 4.1.12

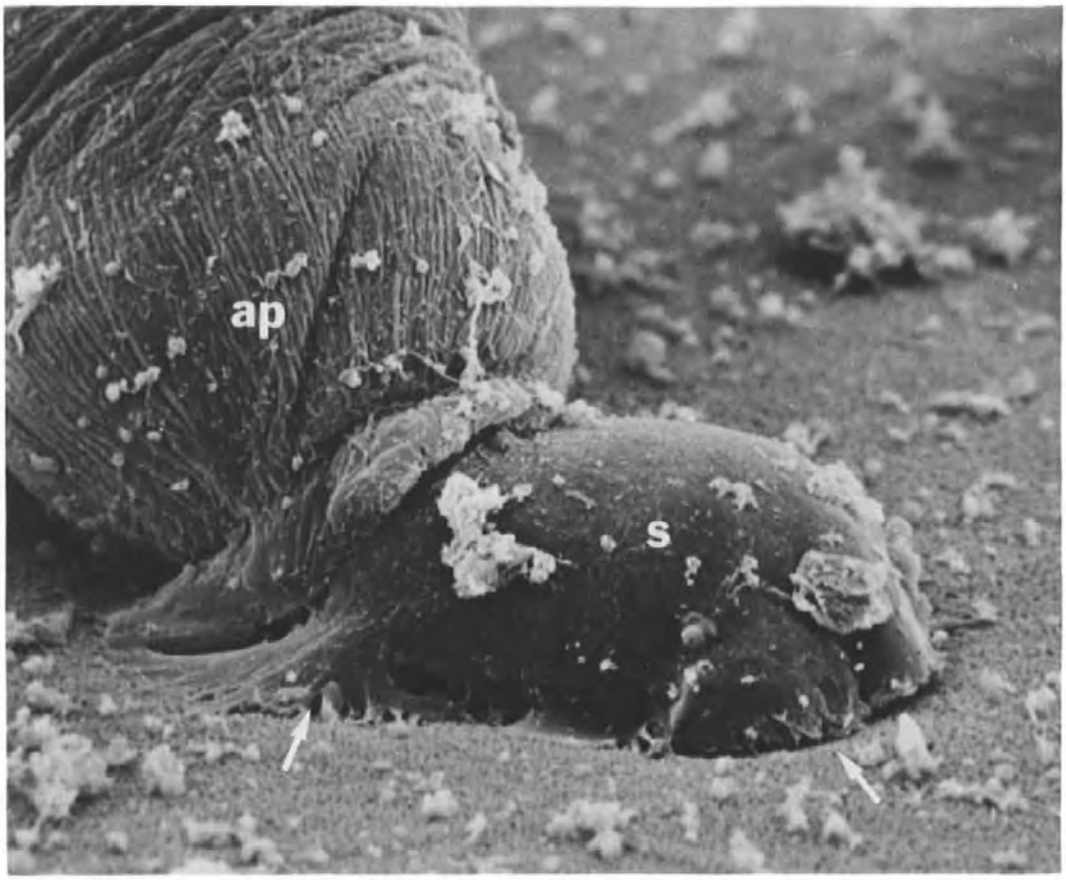
(x280)

Plate 4.1.13

(x320)

The secretion of the initial 'stolon bud', gives rise to the 'primary' stolon (s) which grows across the substratum. At this stage the chitinous secretion of 'primary' stolon remains 'semi-liquid' and is unpolymerized; n.b. the smooth appearance of the 'primary' stolon and the sculptured surface of the hardened perisarc of the hydrocaulus. The leading edges (arrows) of the 'primary' stolon appear to maintain a 'sticky' contact with the substratum.

ap = aboral pole


ap
s

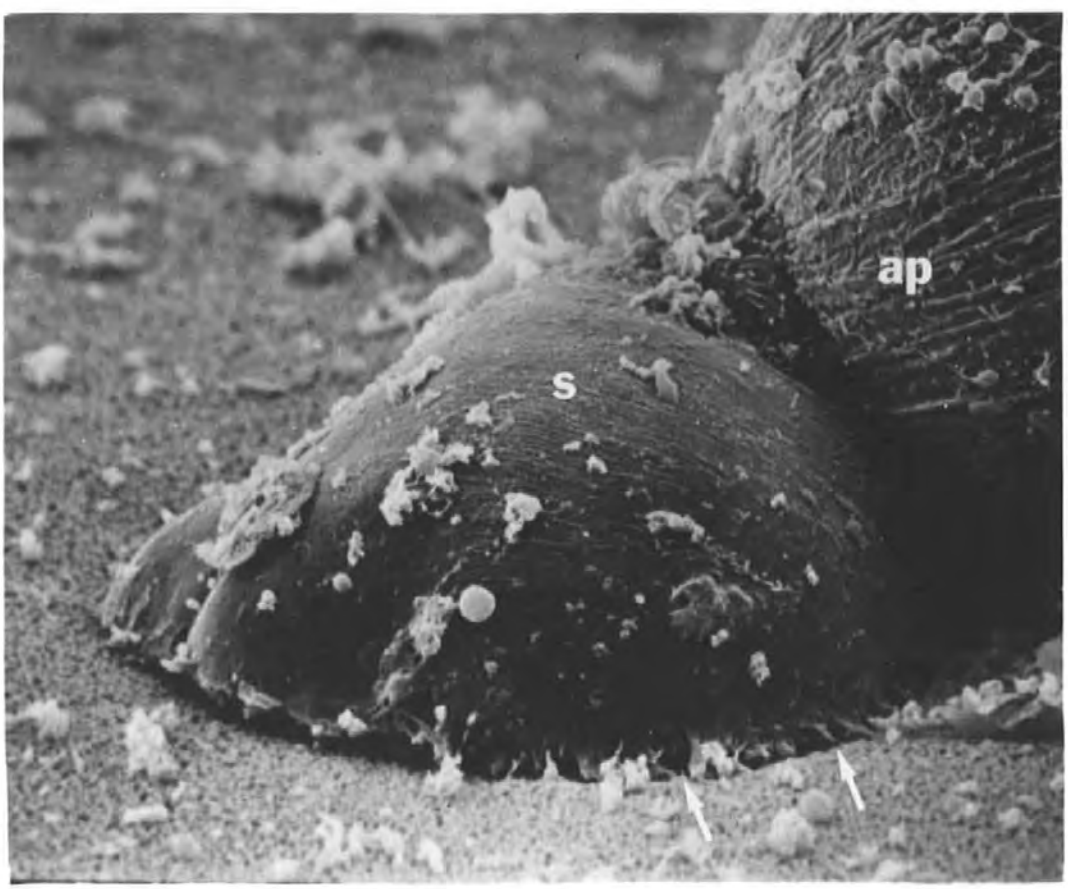

ap
s

Plate 4.1.14

(x280)

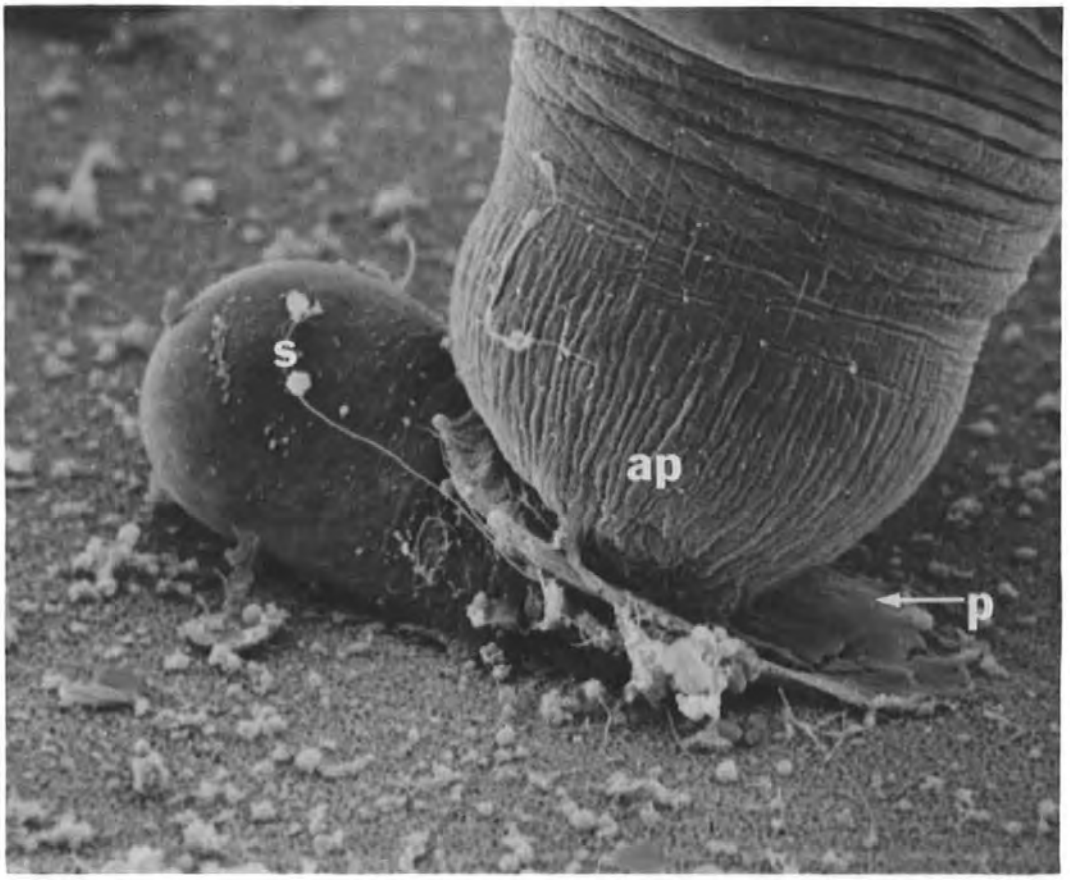
The emergence and 'growth' of both stolon buds and the 'primary' stolon (s) appears to rupture the chitinous perisarc (p) bounding the newly settled individual, in the region of the aboral pole (ap).

Plate 4.1.15

(x220)

The 'adhesive' nature of the 'primary' stolon (s) secretion, appears often to make an initially large area of contact with the substratum (arrows) at the aboral pole (ap).

at = aboral tentacle


s
ap
p

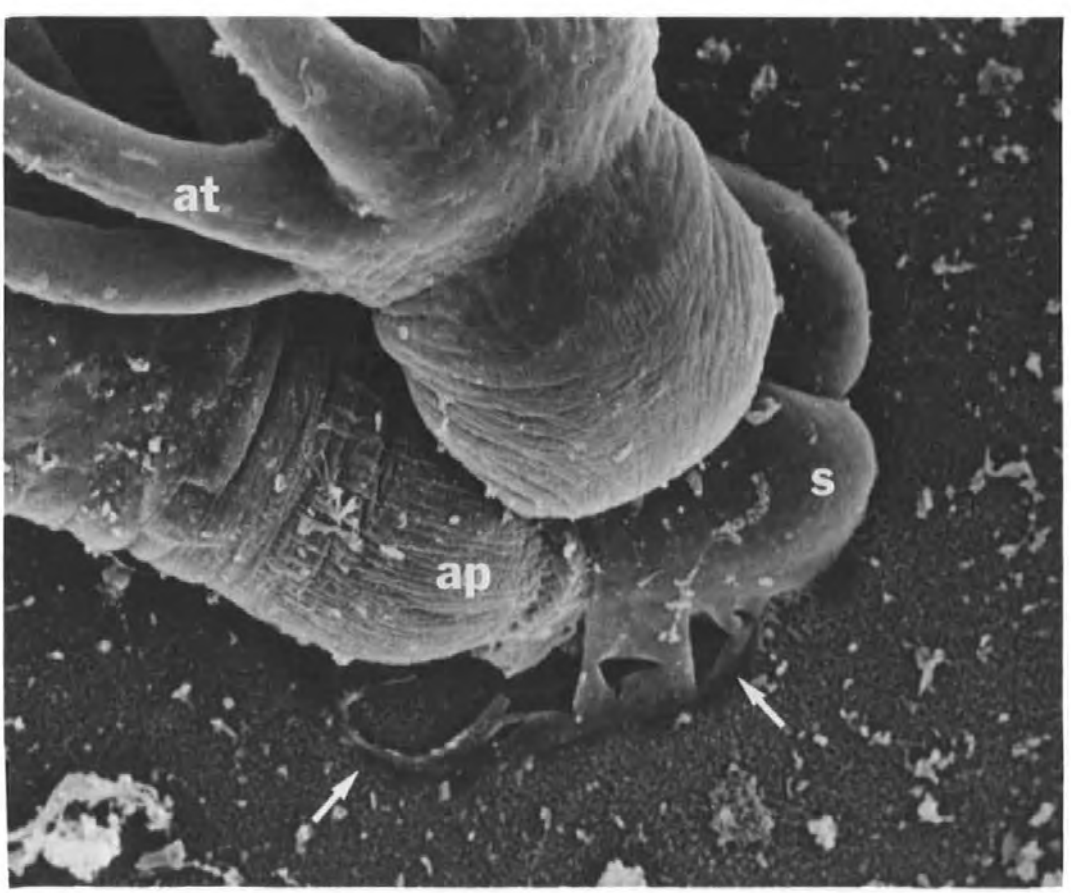

at
s
ap

Plate 4.1.16

(x320)

A number of stolon bud (sb) and subsequently 'primary' stolon 'outgrowths' may be found radiating from the base of the young polyp, at the aboral pole (ap). At the stage of active stolon bud and 'primary' stolon proliferation, the aboral tentacles are detached and, with the hardening of the chitinous secretions, settlement attachment is effected.

ap = aboral pole

ps = perisarcal sheath

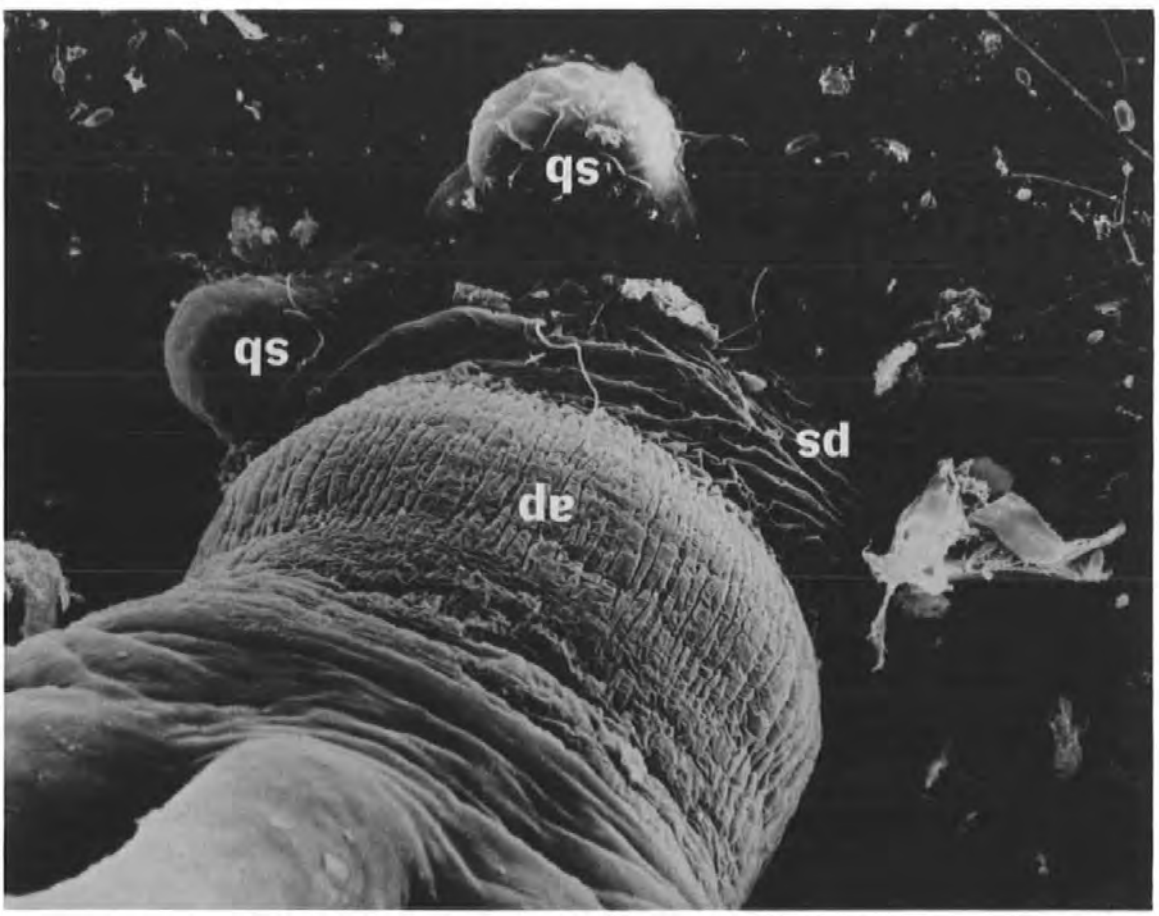

qs
qs
sd
de

Plate 4.1.17

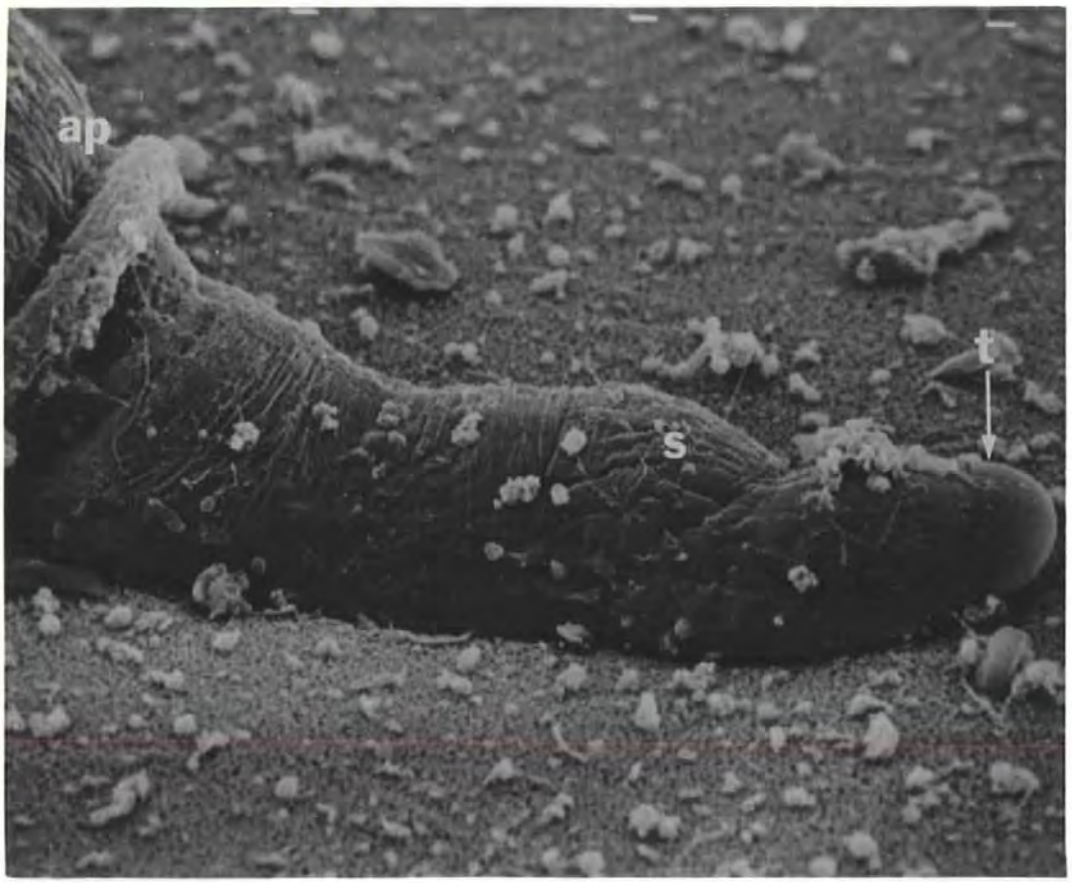
(x240)

and

Plate 4.1.18

(x240)

Subsequent growth of the 'primary' stolon (s) occurs across the substratum. The tip (t) of the stolon remains 'semi-liquid' and unpolymerized, whilst older portions of the stolon become hardened, 'cementing' the stolon to the substrate.


ap
s
t

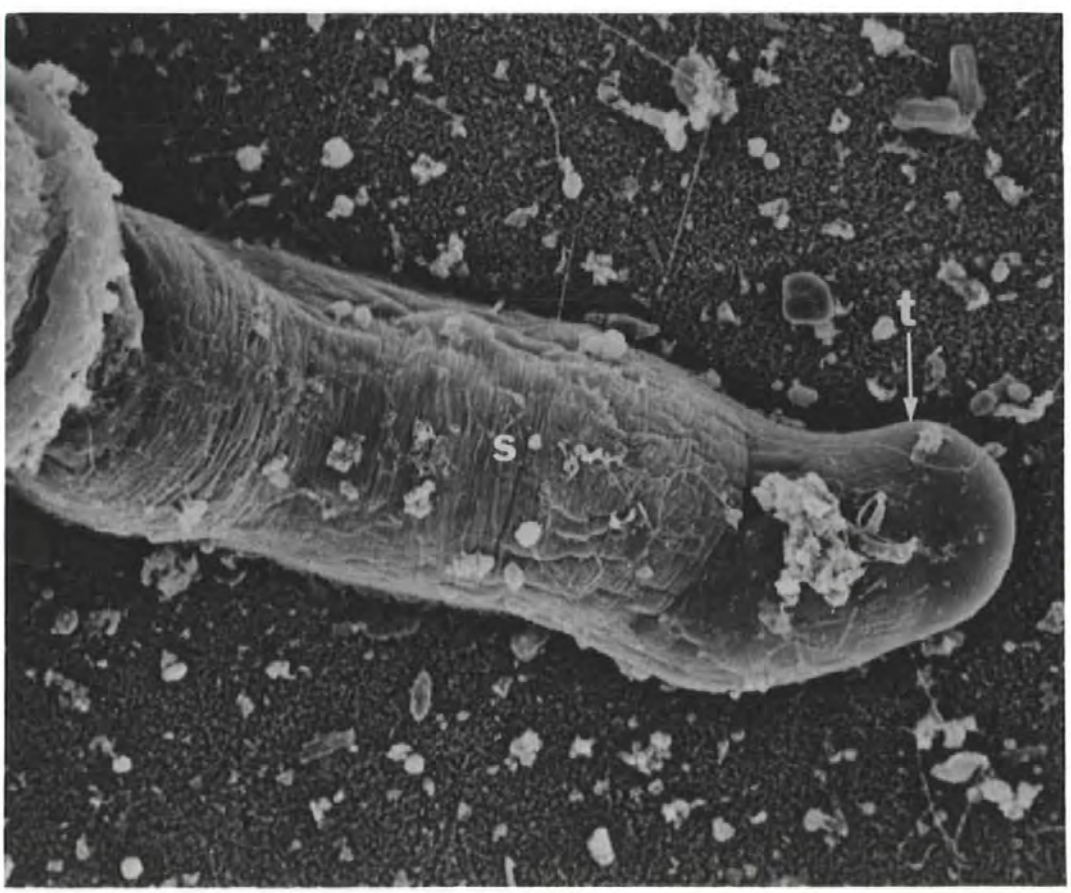

s
t

Plate 4.1.19.

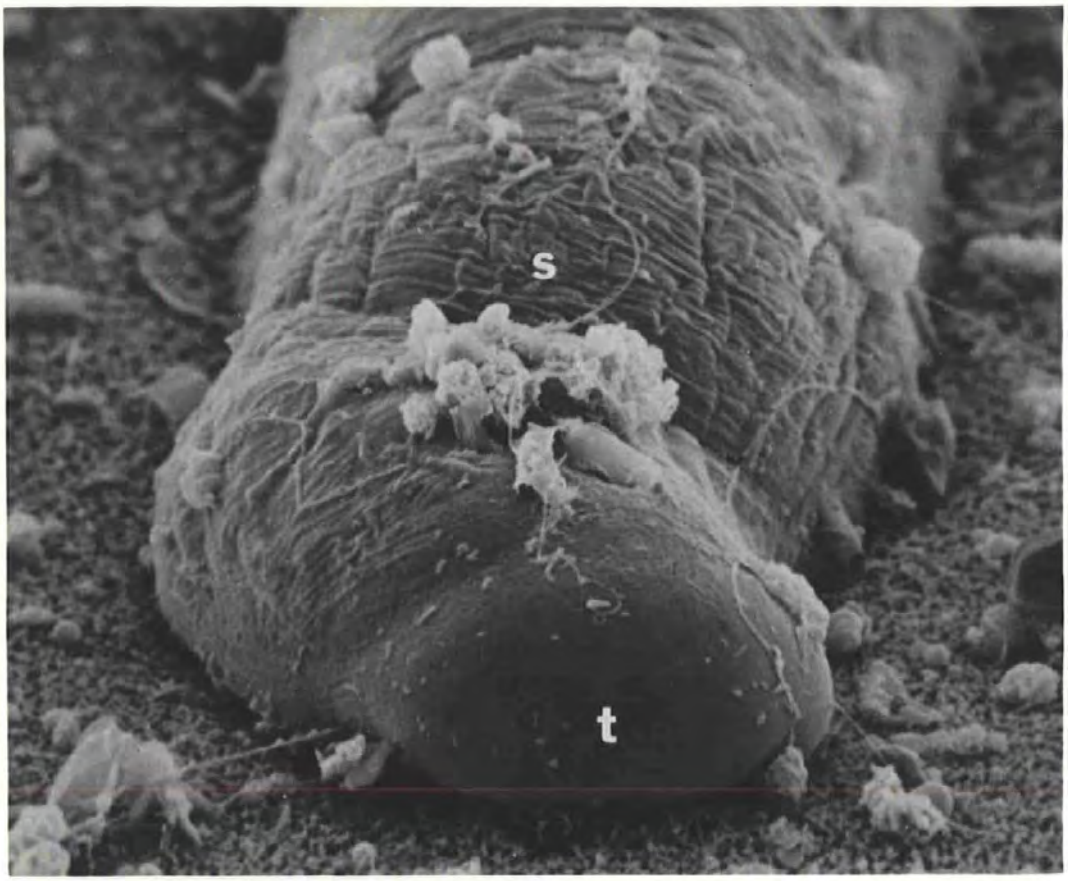
(x380)

Scanning electron micrograph of a 'primary' stolon (s). The tip (t) remains unpolymerized and is smooth in appearance, whilst the surfaces of older, hardened, portions of the stolon (s) are sculptured.

Plate 4.1.20.

(x180)

L.S. 'primary' stolon (1 um, toluidine blue). The actively growing tissue at the tip (t) of the 'primary' stolon (s) consists of columnar ectodermal cells, and is similar in appearance to the tissue of the aboral pole of the actinula larva. At the tip (t) a massive secretion of material adding to the chitinous sheath (cs) is seen.


s
t

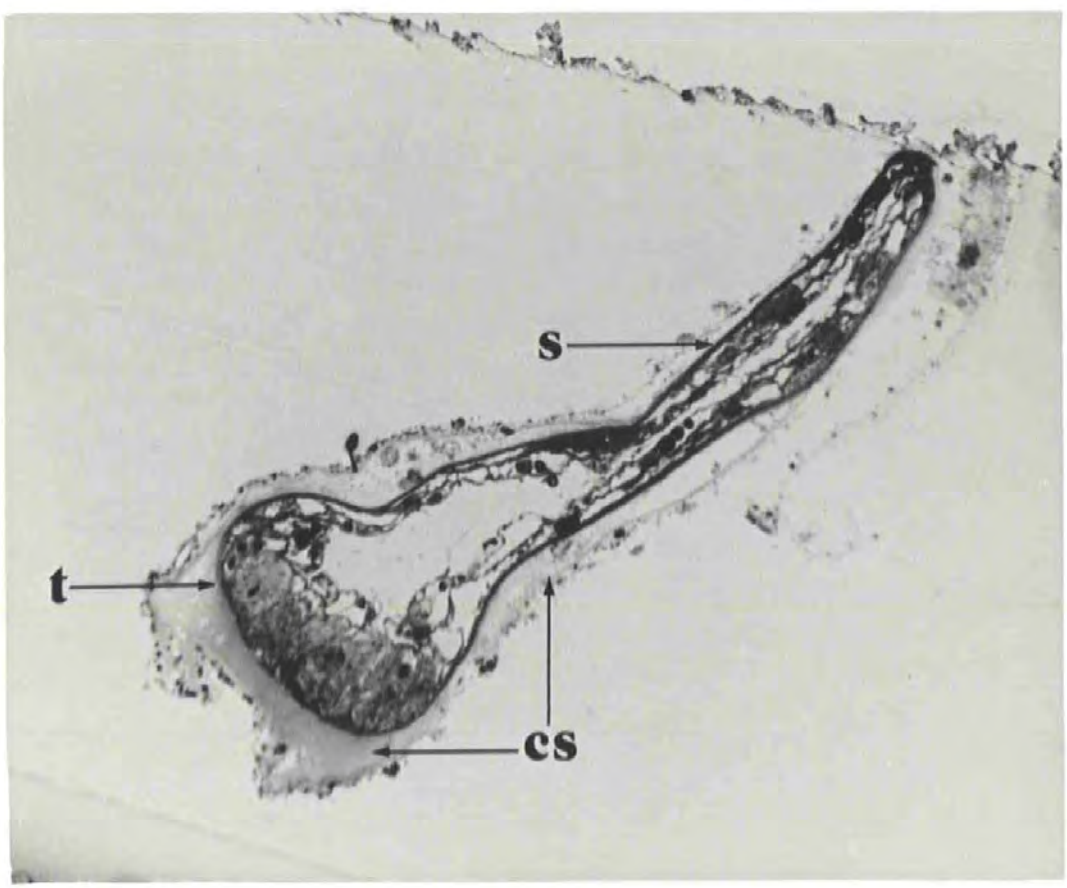

s
t
cs

Plate 4.1.21

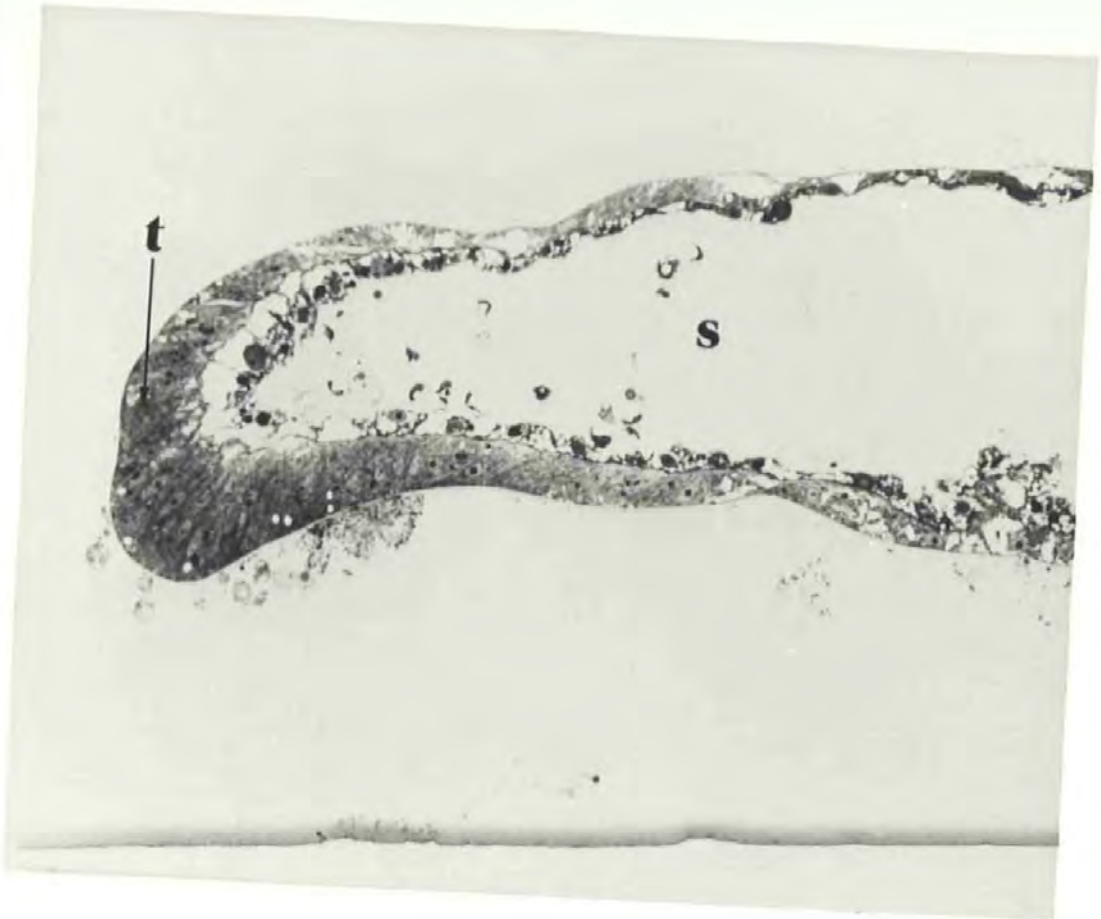
(x180)

L.S. 'primary' stolon (1 um, toluidine blue). The actively growing tissue at the tip (t) of the 'primary' stolon (s), consists of deeply columnar ectodermal cells.

Plate 4.1.22

(x180)

L.S. 'primary' stolon (1 um, toluidine blue). The columnar ectodermal cells at the tip (t) of the 'primary' stolon (s) characteristically contain large numbers of intensely staining granules (g), apically.


t
s

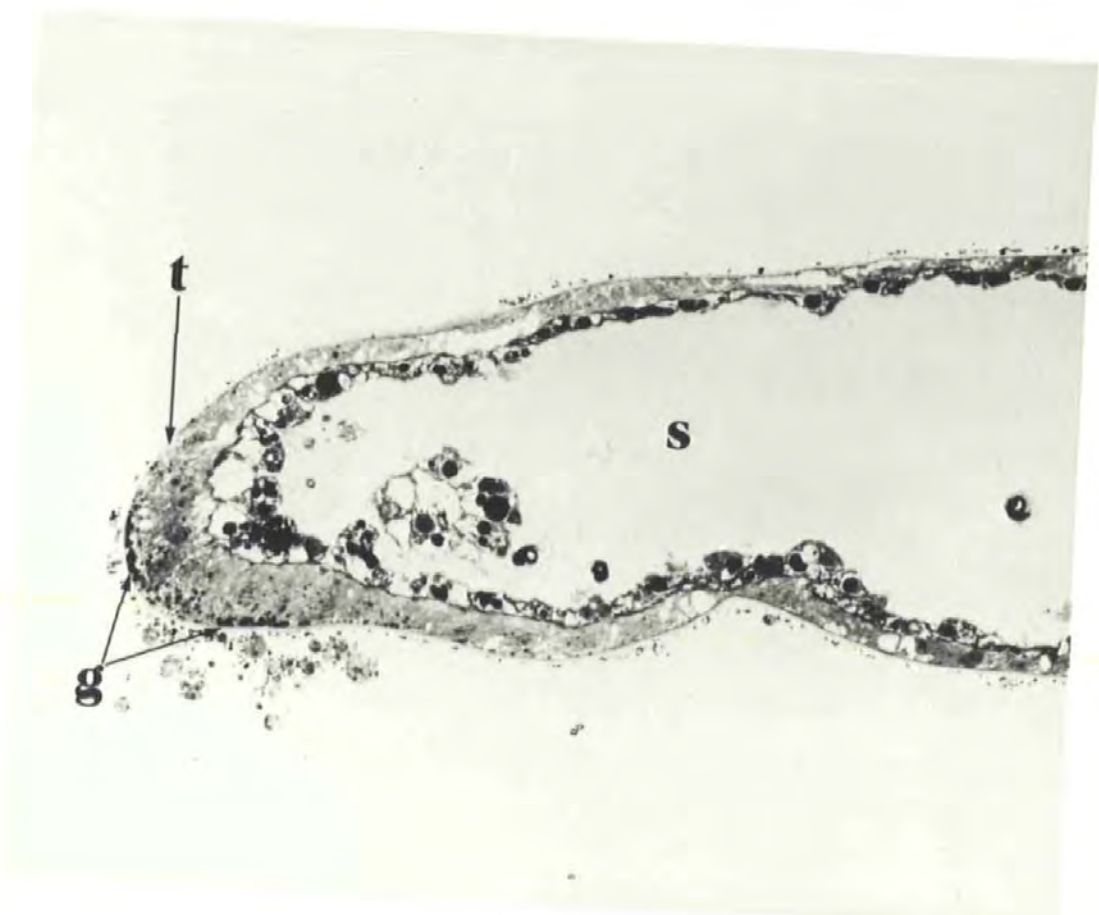

t
s
g

Plate 4.1.23

(x220)

Scanning electron micrograph showing the formation of new zones of growth (arrows) at the tip (t) of a 'primary' stolon (s). Typically, the older, 'primary' stolon (s) is hardened and its surfaces appear sculptured; whereas the newly secreted chitin of the two new outgrowths of stolonic tissue (arrows) remains unpolymerized.

Successive divisions in the zone of growth at stolon tips may eventually give rise to the ramifying network of basal stolons characteristic of the adult hydroid colony.

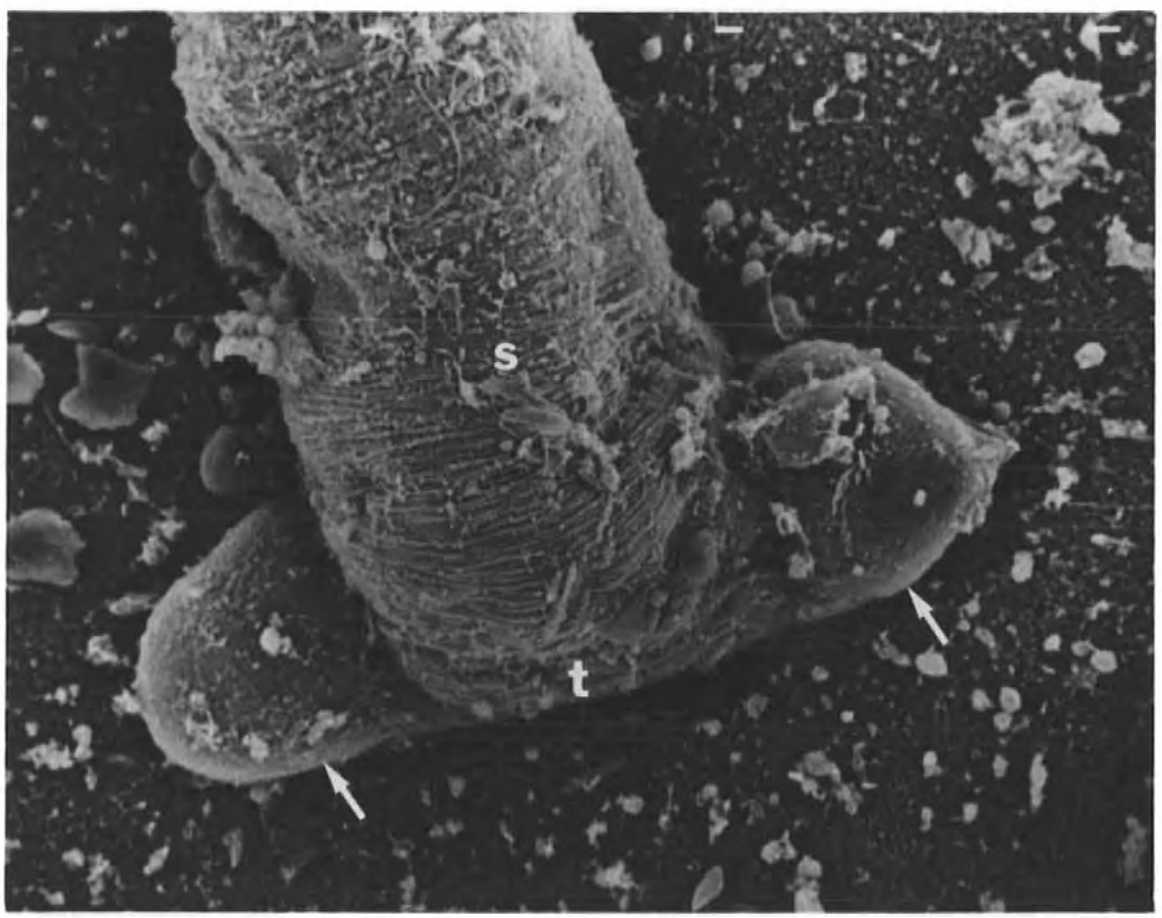

s
t

Plate 4.1.24.

(x80)

Young *Tubularia* polyp approximately 16 hours after the initiation of settlement attachment. Secure attachment to the substratum is effected by the radiating growth of a number of 'primary' stolons (arrows). The temporary attachment provided by the aboral tentacles (at) is broken and the young polyp assumes the feeding posture.

ot = oral tentacles

h = hydrocaulus

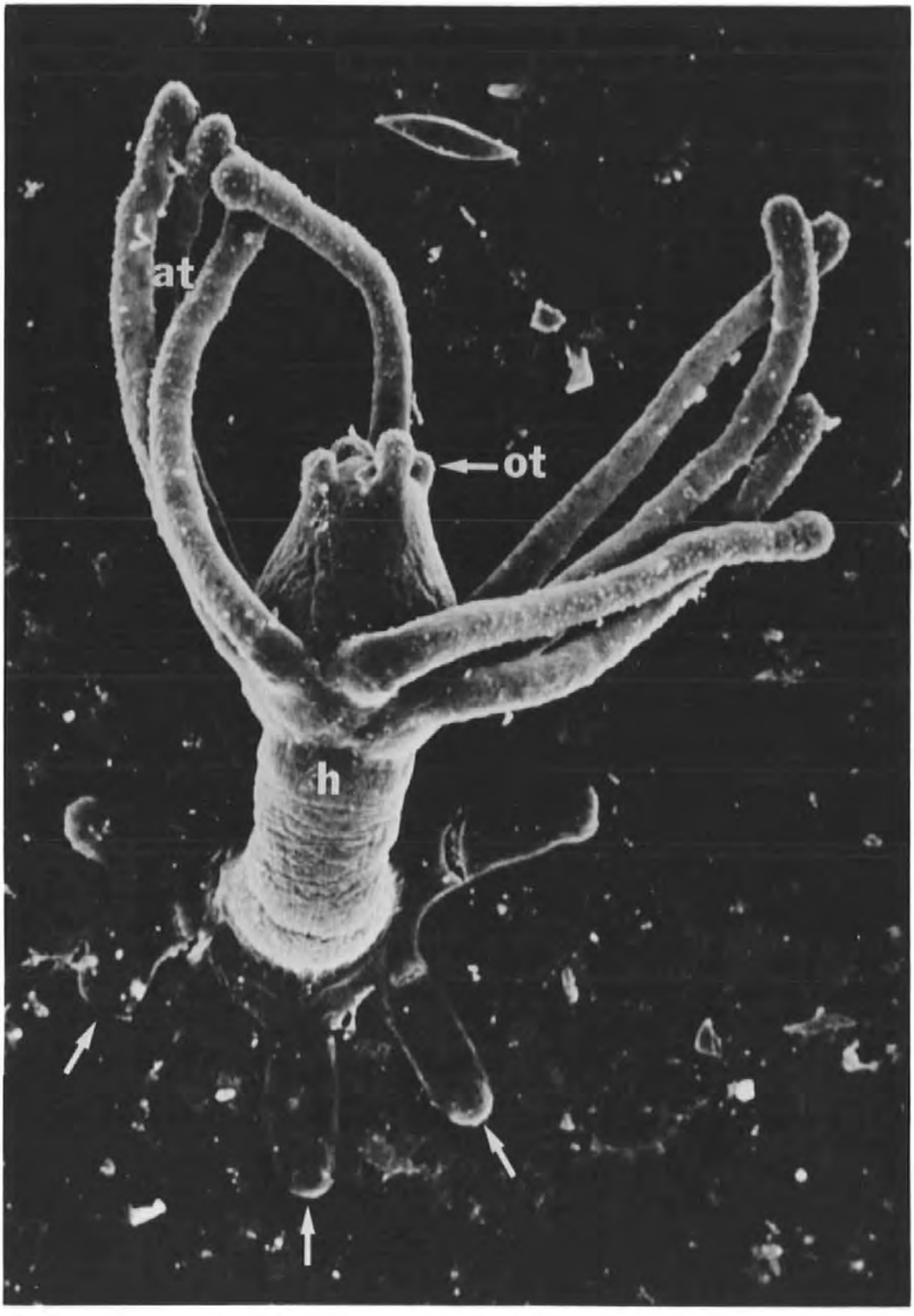

at
ot
h

Plate 4.1.25

(x100)

Another view of the recently settled polyp seen in Plate 4.1.25, clearly showing the beginnings of a network of basal stolonic growth (s).

at = aboral tentacles

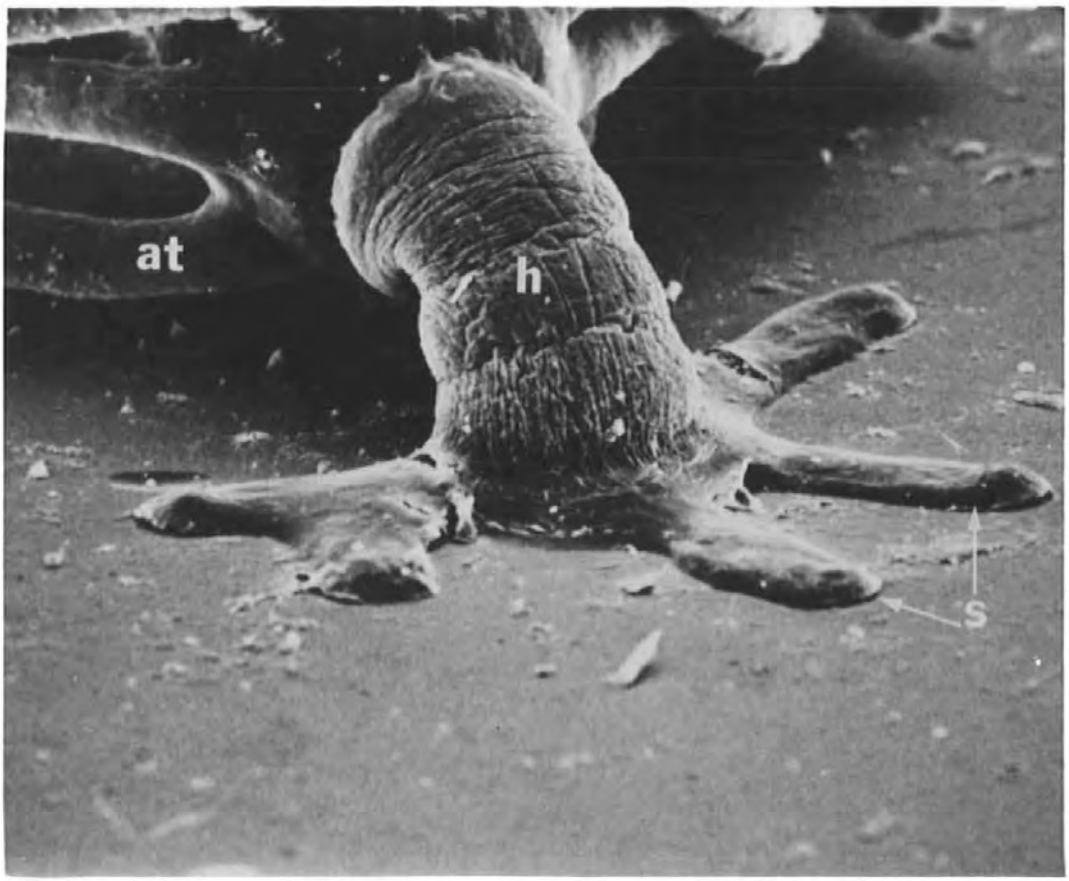
h = hydrocaulus

Plate 4.1.26.

(x48)

Scanning electronmicrograph of a 'temporarily attached' individual, showing that the aboral pole (ap) need not be brought into contact with the substratum to stimulate 'growth' and stolon bud or 'primary' stolon production.

at = aboral tentacles


at
h
s


at
ap

Plate 4.1.27.

(x170)

L.S. 'temporarily attached' individual, as seen in Plate 4.1.26 (1 um, toluidine blue). Although the aboral pole (ap) is not in contact with the substratum and attachment is provided by the aboral tentacles (at), stolon bud production, seen as an 'outpushing' of the aboral pole (ap) continuous with the larval coelenteron (co), still occurs. The elongation of the larval body may be due to a reorganization of tissues, whilst the zone of true growth appears to be the characteristic columnar ectoderm of the aboral pole (ap), the cells of which were typically densely packed with intensely staining granules (g) at the apical surface.

ot = oral tentacles.

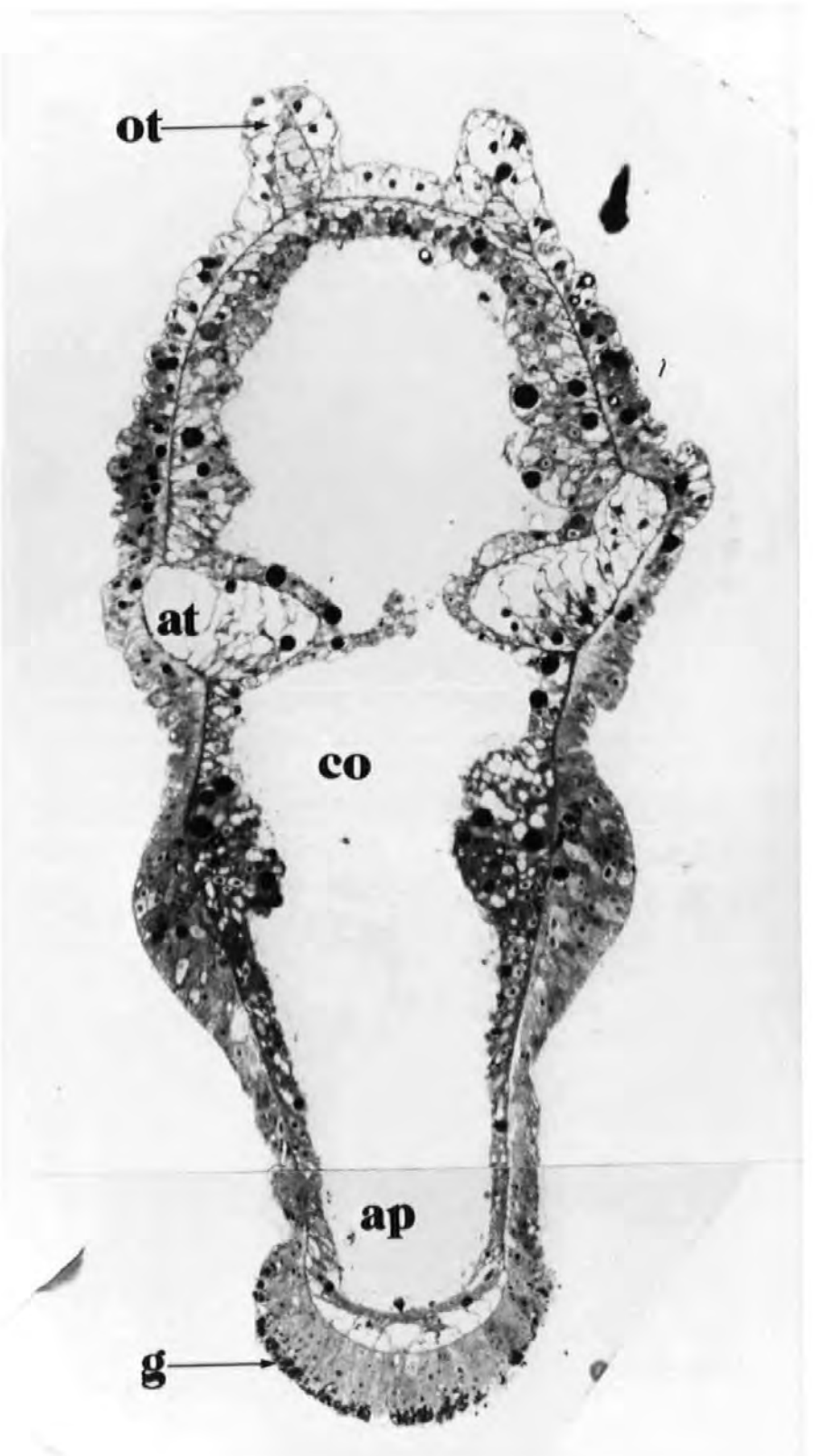

ot
at
co
ap
g

Plate 4.1.28

(x2750)

Electronmicrograph of the aboral pole in the recently released actinula larva. The columnar ectodermal cells possess a large proximal nucleus and, distally, are packed with secretory granules (g). The chitinous sheath (cs) of variable thickness is clearly visible external to these cells.

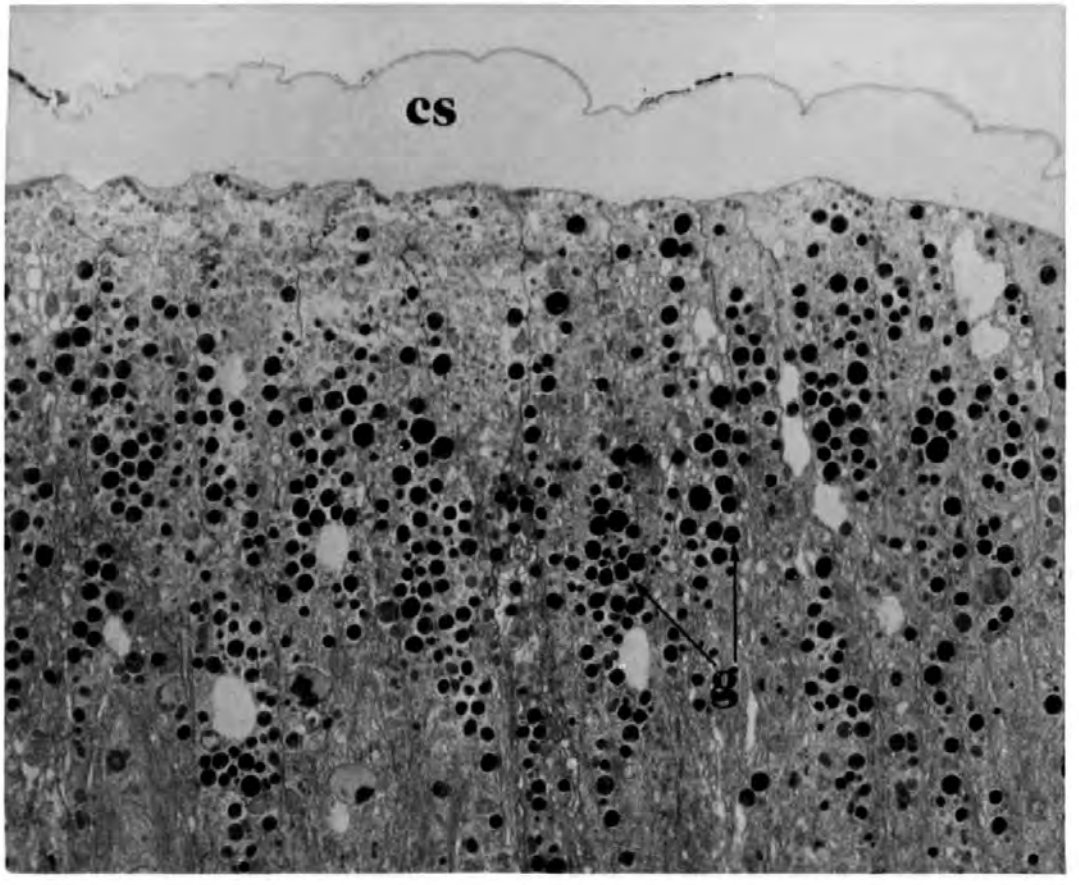
Plate 4.1.29

(x 17,400)

Higher magnification of the apical surface of the columnar ectoderm in the recently released actinula larva. Two types of secretory inclusion are seen; Type A vesicles (A) which varied in diameter up to 0.6 um, and Type B vesicles (B) also variable in diameter up to 0.6 um, but more usually between 0.1 um + 0.2 um.

m = mitochondrion

cs = chitinous sheath


cs
g

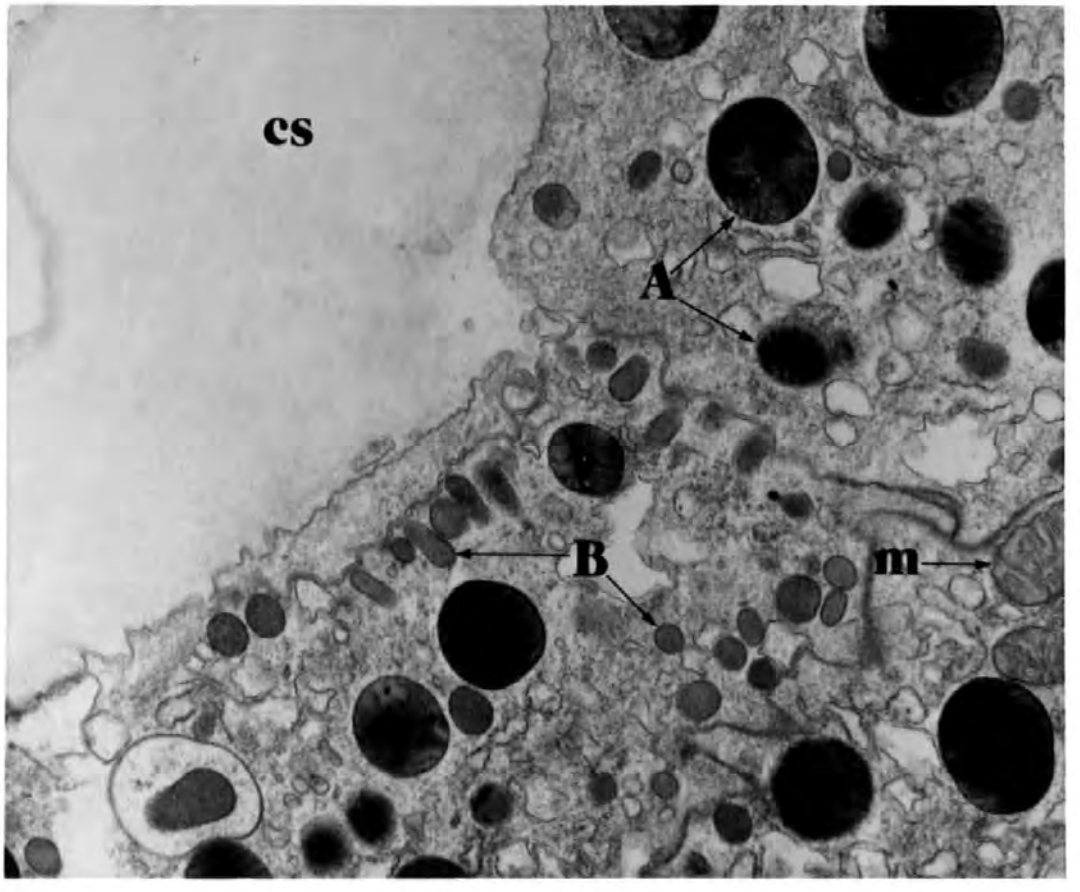

cs
A
B
m

Plate 4.1.30

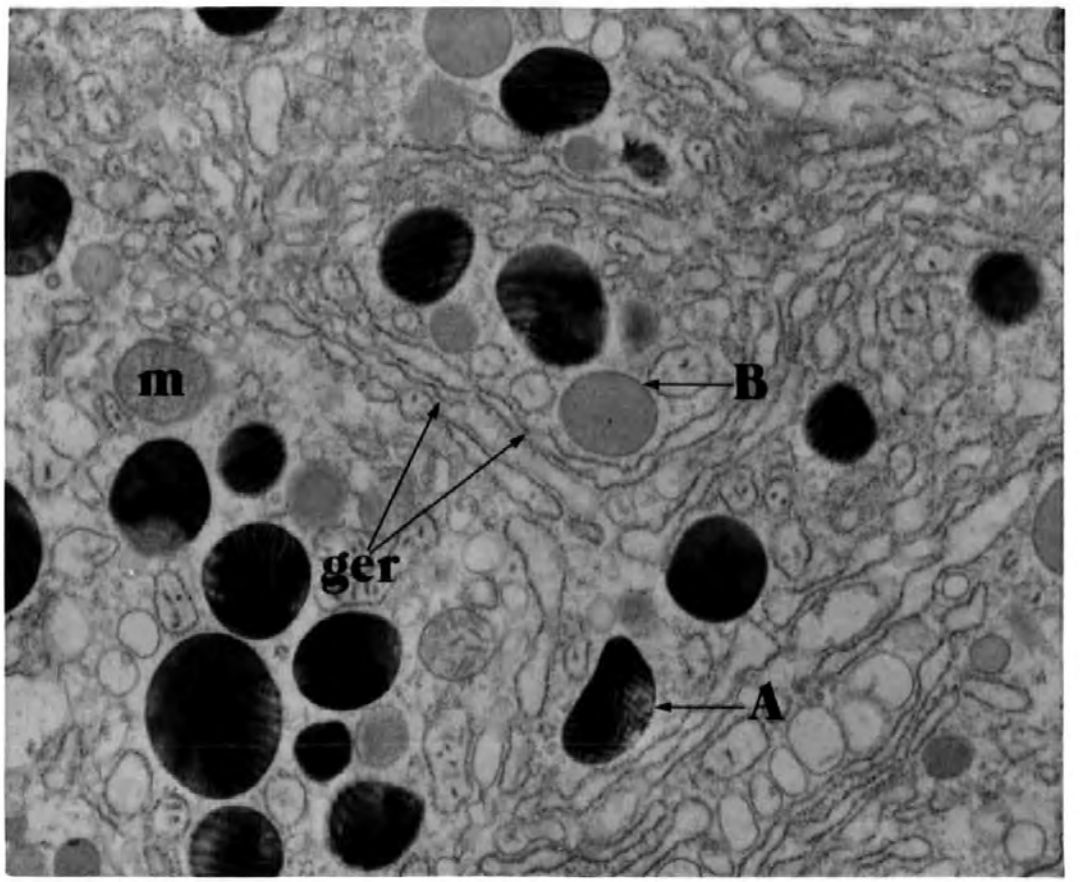
(x16,200)

Electronmicrograph, mid-cell region of a columnar ectodermal cell in the aboral pole of the actinula larva. Both the characteristically striated Type A vesicles (A) and the homogeneous, less intensely staining Type B vesicles (B) are seen in close association with profiles of granular endoplasmic reticulum (ger). The cytoplasm is distinctly vesicular and often contains a number of rounded mitochondria (m).

Plate 4.1.31

(x25,500)

Type A vesicles. These membrane-bound, spherical or rod shaped bodies characteristically contain stacks of electron-dense lamellae (la) organized into regular patterns, together with a densely granular matrix.


m
B
ger
A

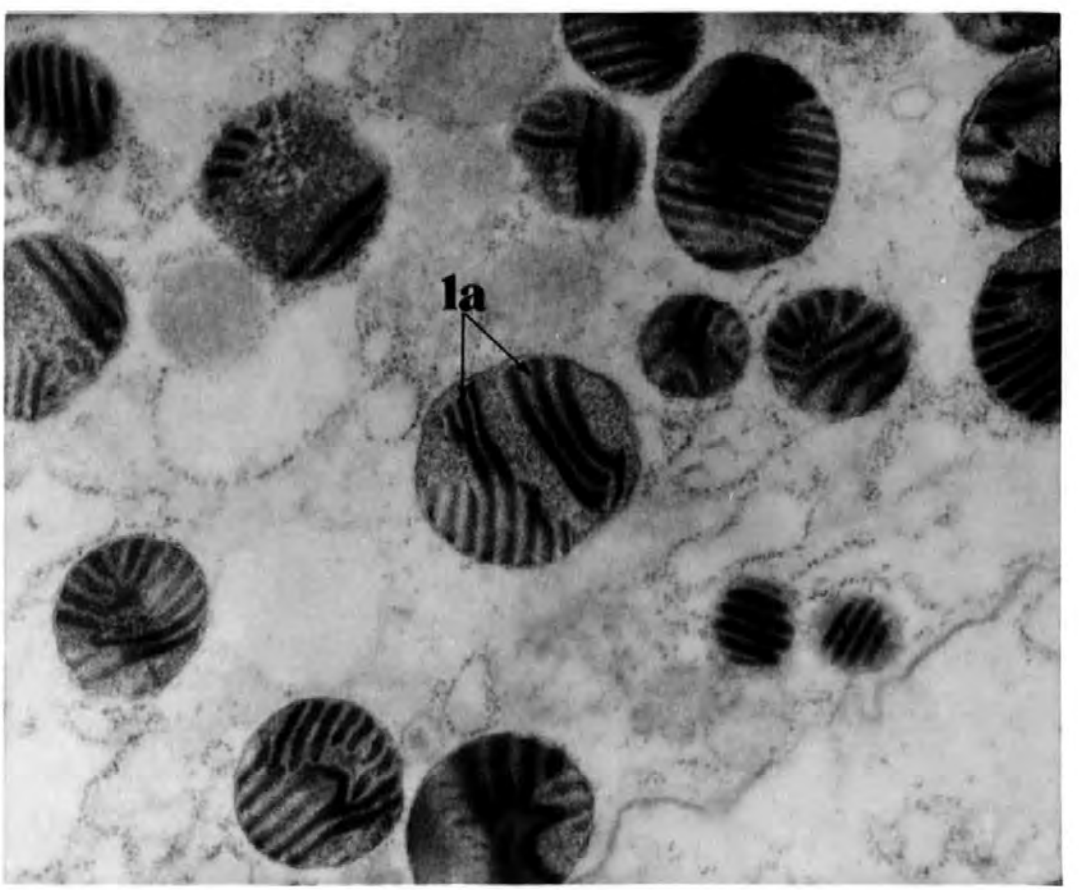

la

Plate 4.1.32

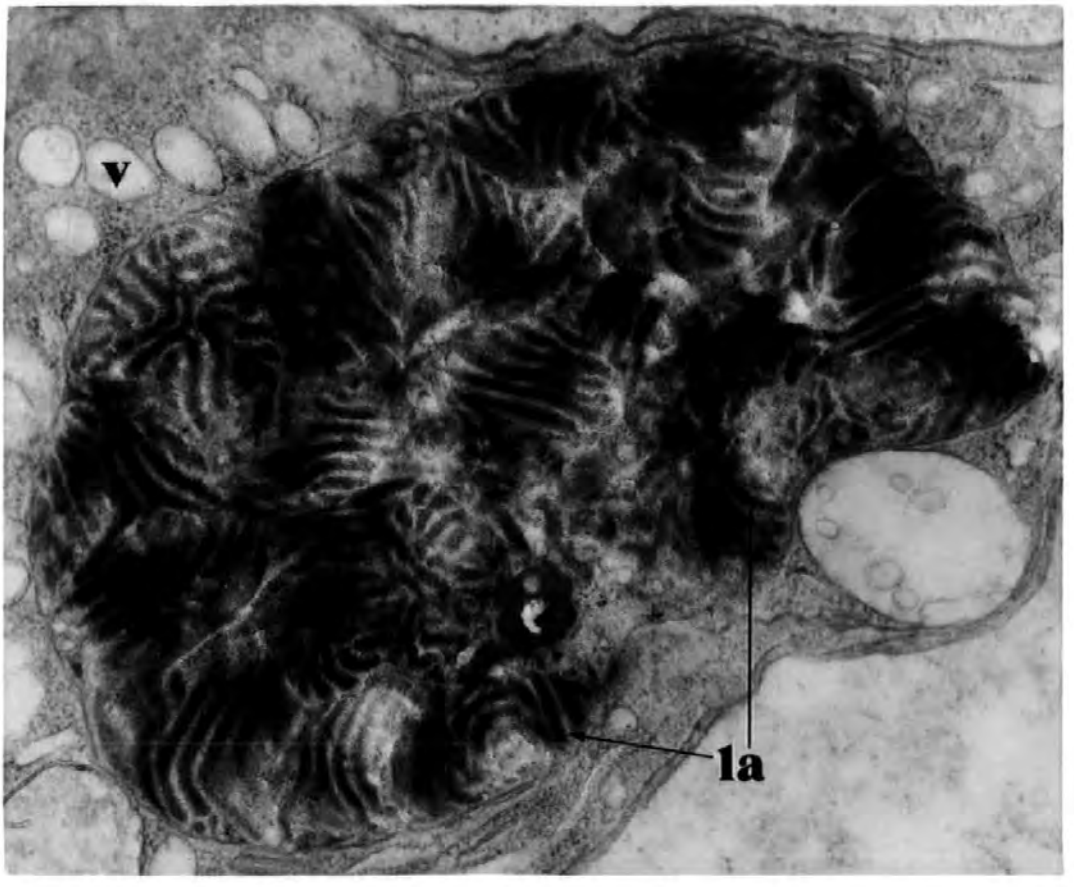
(x 20,500)

Large aggregations of Type A vesicles were found in the mid cell-region, closely associated with profiles of granular endoplasmic reticulum, and vesicular structures (v). The highly electron dense lamellae (la), however, appeared to be undergoing organization and the membrane-bound, Type A vesicle was not yet formed.

Plate 4.1.33

(x 16,800)

Large multivesicular bodies in the mid-cell region may represent sites of synthesis of Type A vesicles (A) and may precede the stage seen in Plate 4.1.33. The highly electron dense lamellae typical of Type A vesicles are seen in close association with numerous vesicles (v) of the multivesicular body and appear to be undergoing organization to form Type A vesicles (A). The multivesicular body is itself closely associated with profiles of granular endoplasmic reticulum (ger) and many rod-shaped mitochondria (m).


v
la

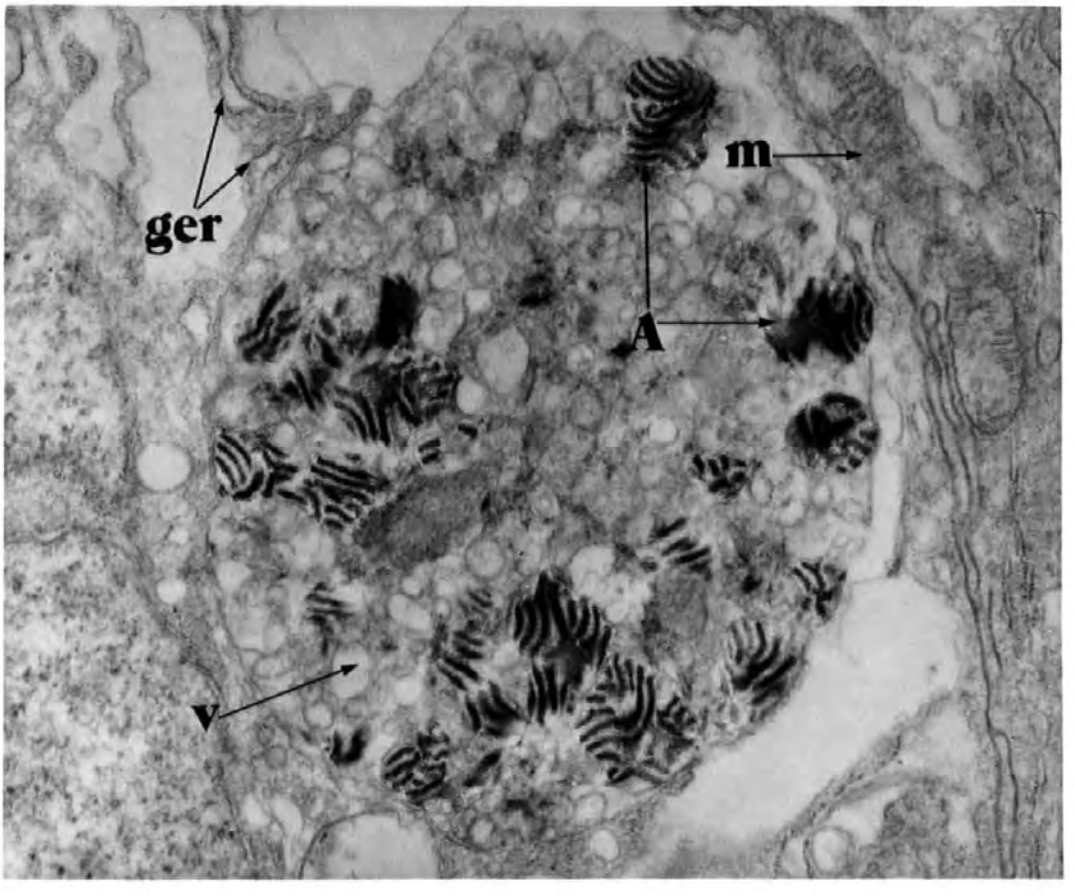

ger
m
A
v

Plate 4.1.34

(x 11,500)

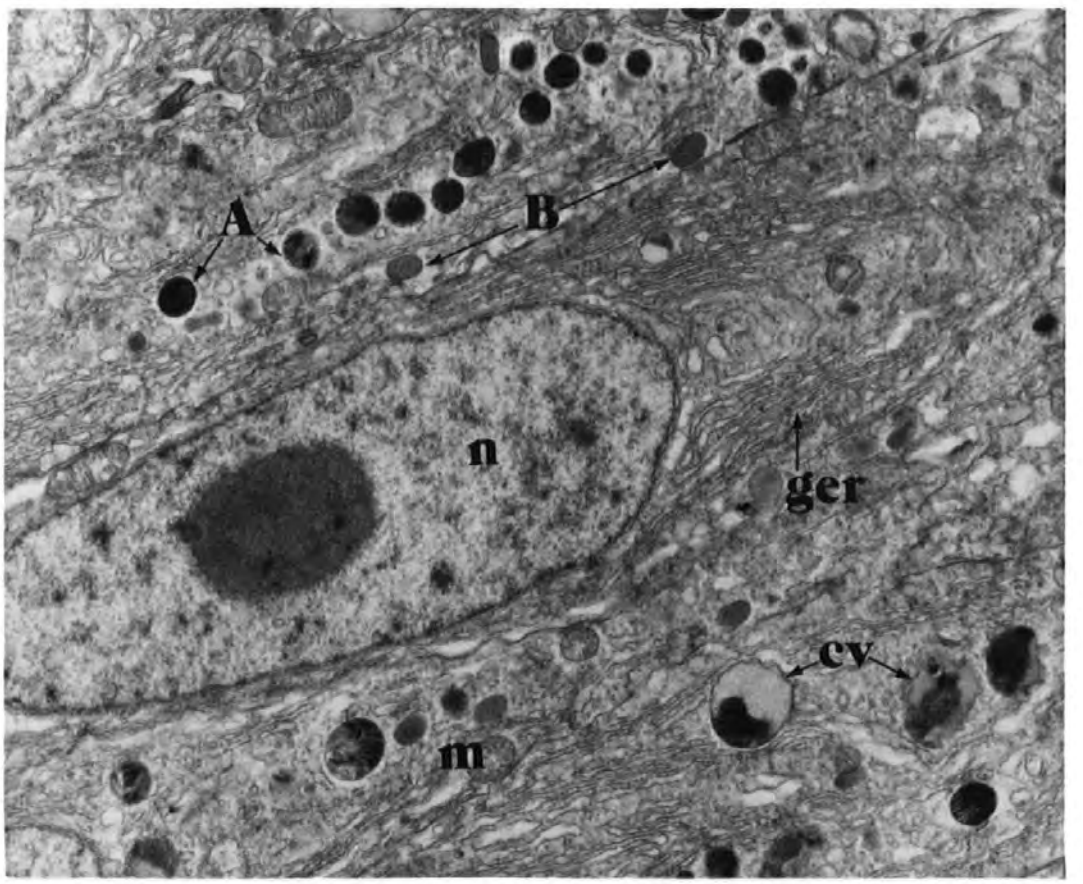
Electronmicrograph of the mid-cell region in the columnar ectoderm of the aboral pole in the recently released actinula larva. A large mid-cell to proximal nucleus (n) is seen, with a prominent nucleolus, closely associated with numerous profiles of granular endoplasmic reticulum (ger). Both Type A (A) and Type B (B) vesicles are seen in the cytoplasm, together with spherical and rod-shaped mitochondria (m). Often, cored vesicles (cv) were found associated with the granular endoplasmic reticulum (ger) in this area of the cell.

Plate 4.1.35.

(x 7,400)

Electronmicrograph of the 'primary' stolon tip. The columnar ectodermal cells are packed distally with Type A vesicles (A). Secretory droplets (sd) appear to be released from the apical surfaces of the cells, to the inner layers of the chitinous sheath (cs).

n = nucleus


A
B
n
ger
cv
m

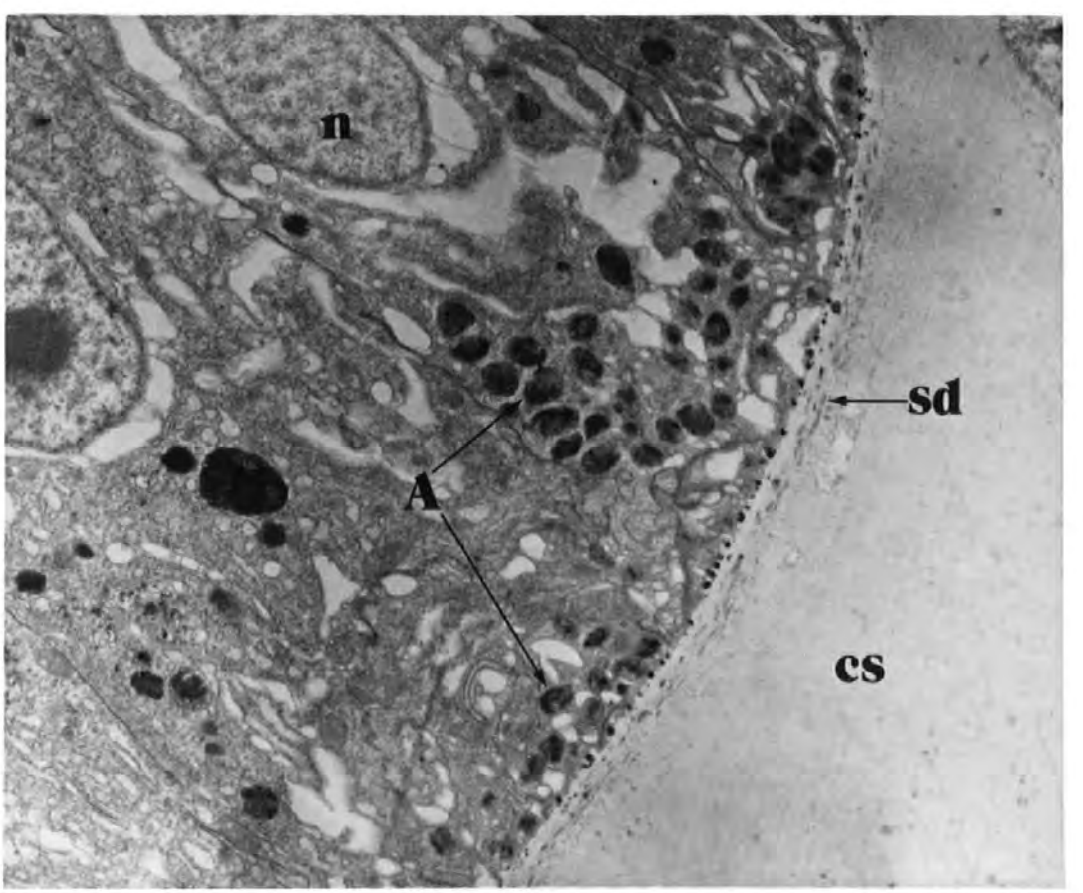

n
sd
A
cs

Plate 4.1.36

(x 9,200)

Electronmicrograph of the 'primary' stolon tip. Both Type A (A) and Type B (B) vesicles are found close to the apical surface. Secretory droplets (sd) appear to be added to the inner layers of the chitinous sheath (cs). The large nucleus (n) was found in close association with profiles of granular endoplasmic reticulum (ger).

m = mitochondrion.

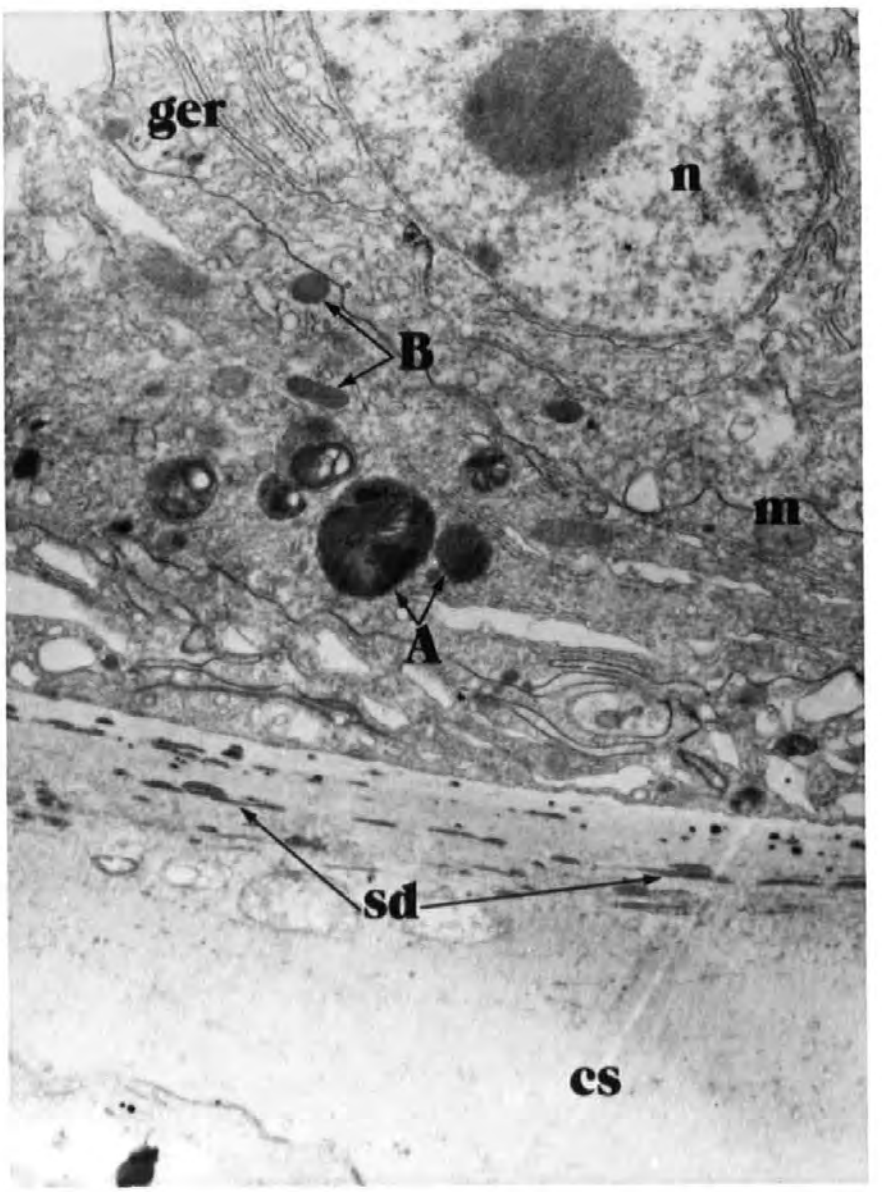

ger
n
B
m
A
sd
cs

Figure 4.1.37.

Laboratory settlement in Tubularia larynx

Schematic columnar ectodermal cell (x8000 approx.) from the 'primary' stolon tip; broad arrows indicate possible route of synthesis, storage and secretion of products involved in the phenolic tanning of the external chitinous perisarc.

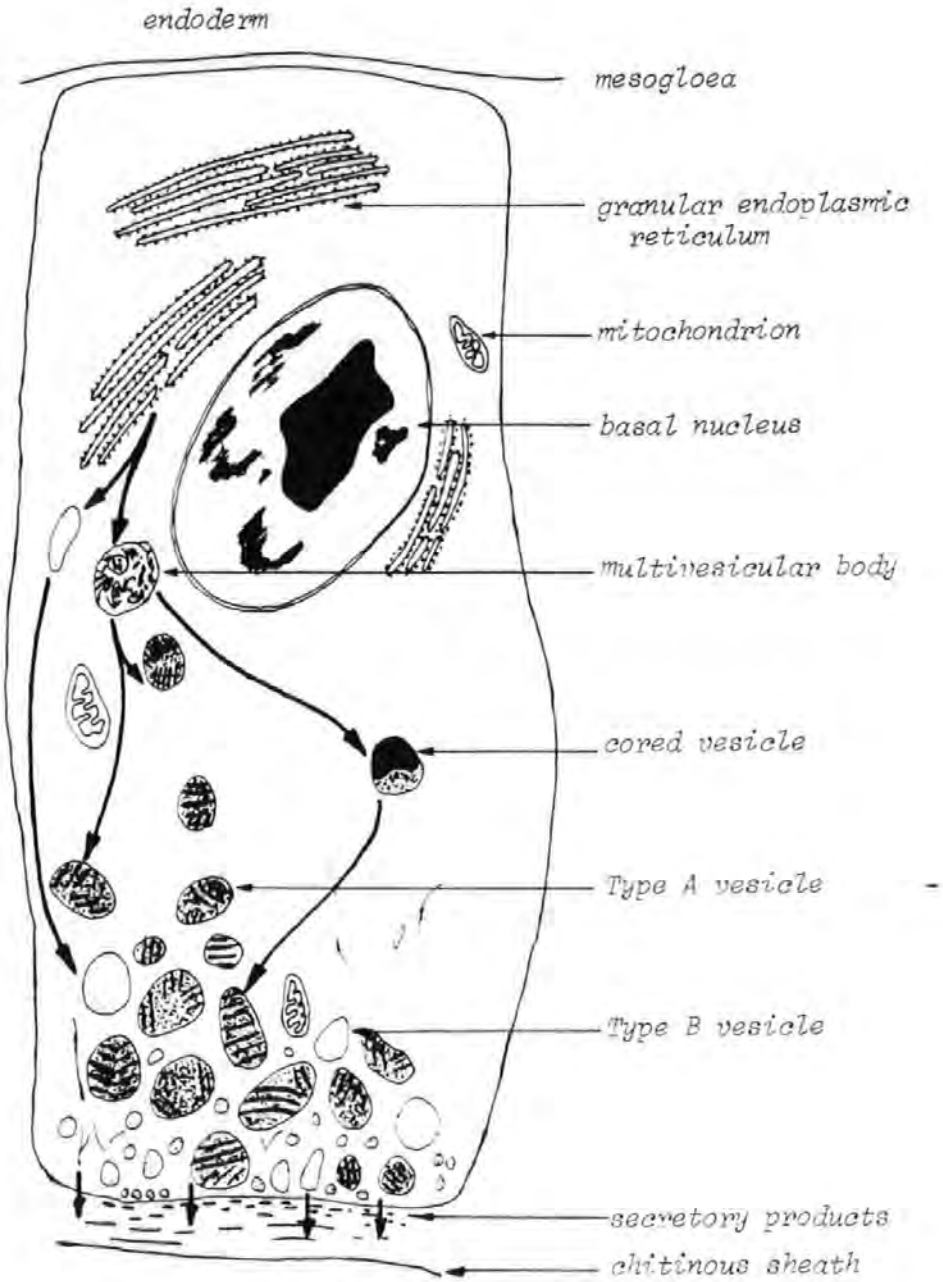

endoderm
mesogloea
granular endoplasmic reticulum
mitochondrion
basal nucleus
multivesicular body
cored vesicle
Type A vesicle
Type B vesicle
secretory products
chitinous sheath

Table 4.2.1

Laboratory settlement in Tubularia larynx.

Histochemistry.

+++ = strong reaction

++ = moderate reaction

+ = weak reaction

- = negative

1. *Carbohydrate*

Test	Aboral pole (actinula)	Aboral pole (settled)	1° stolon tip	hydrocaulus
PAS	+++	+++	++	++
Lead tetra-acetate Schiff	+++	++	++	++
AB 1.0	+	+	+	+
AB 2.5	+	+	+	+
AB (5.7) CEC				
0.1	+	++	+	-
0.2	-	+	+	-
0.5	-	-	-	-
0.6	-	-	-	-
0.8	-	-	-	-
1.0	-	-	-	-

2. *Protein*

Bromophenol Blue	++	++	+++	++
Millons	++	++	+++	++
Diazotised Coupling	++	++	+	++
Sakaguchi	-	-	-	-

3. *Chitin*

Alkaline tetrazolium	+	+/++	+	+
Mallory's trichorome	+	+/++	+/++	+

Table 4.2.2.

Laboratory settlement in Tubularia larynx

Whole mount histochemical results for the demonstration of a phenolic tanning mechanism.

+++ = strong reaction

++ = moderate reaction

\+ = weak reaction

\- = negative

1. *Phenolic substrate* Test	Aboral pole (actinula)	Aboral pole (settled)	1^{o} stolon tip	hydrocaulus
Chromaffin	++	++	++	+
Iodaffin	+	+	+	+
Gibb's method	+	++	++	+
Alkaline diazonium coupling	+	+	+	+
Vulpians ferric chloride	+	+	+	+
Molybdate method	+	+	+	+
Argentaffin	+	+	+	+
2. *Phenol oxidase*				
Catechol oxidase				
(a) dopamine	+	++	-	+++
(b) tyrosine	-	-	-	-
(c) catechol	+	++	-	+++
Rubeanic acid	-	-	-	-

Plate 4.2.3 (x 80)

Whole mount staining for phenol oxidase activity; catechol oxidase test with catechol. Enzyme activity was mainly confined to the ectodermal cells and the reaction product (rp) was most intense in the region of the hydrocaulus (h) and stolons (s). The newly secreted chitin of the chitinous sheath (cs) at the tip of the 1^{o} stolon was unstained.

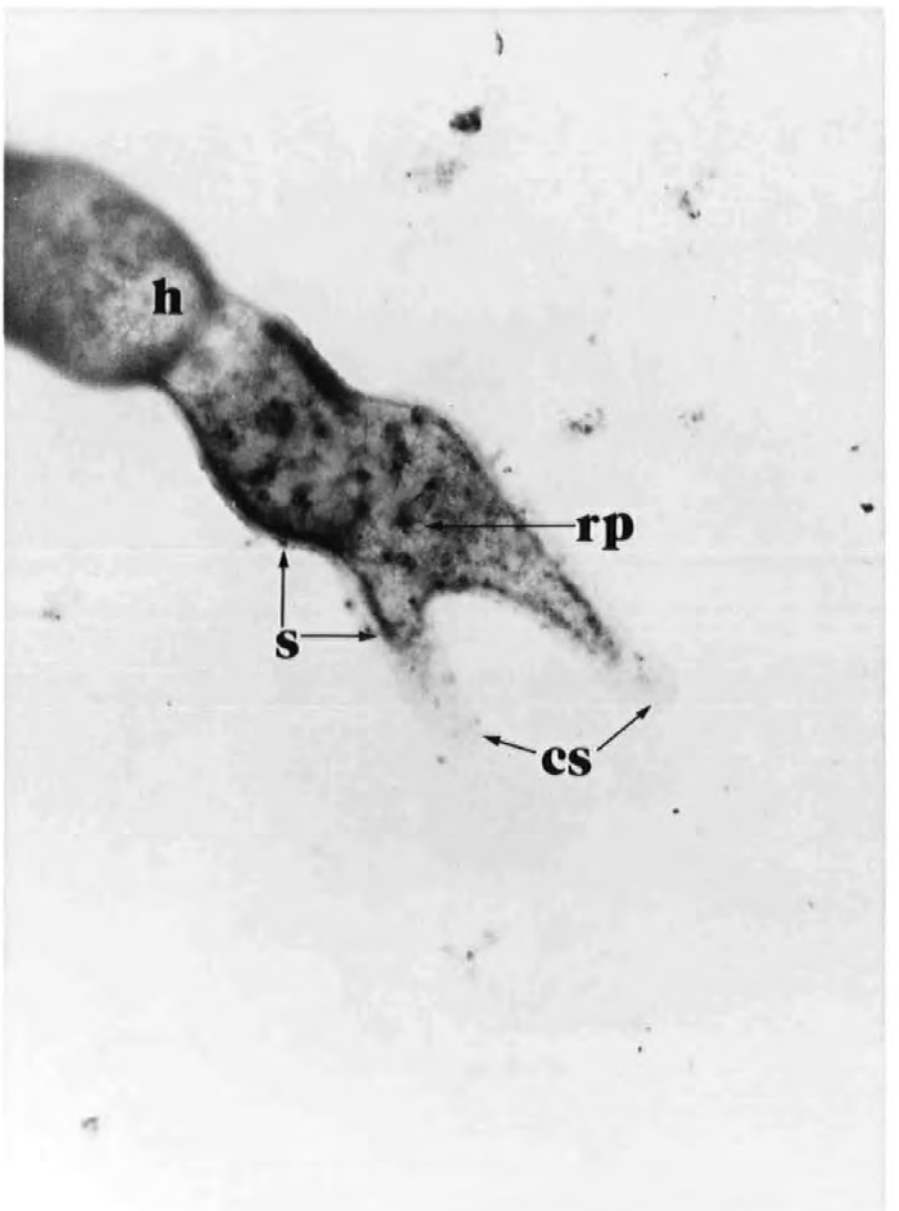

h
rp
s
cs

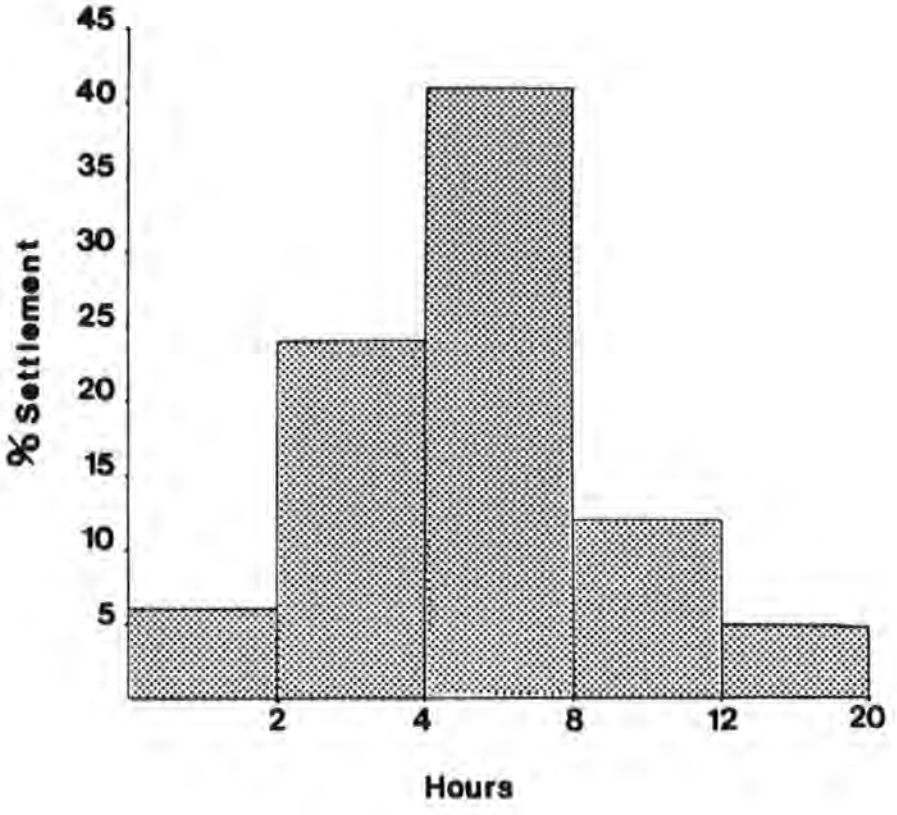
Figure 4.3.1.

Laboratory settlement in Tubularia larynx

Duration of larval phase, and mean % settlement over 20 hours after release of the actinula larvae.

Table 4.3.2.

Duration of larval phase.



Expt.	2	4	8	12	20
1	2	27	49	3	2
2	3	39	33	15	5
3	9	19	26	24	12
4	2	26	37	10	8
5	16	12	60	10	1
Total	32	123	205	62	28
Mean	6	24	41	12	5

Figure 4.4.1

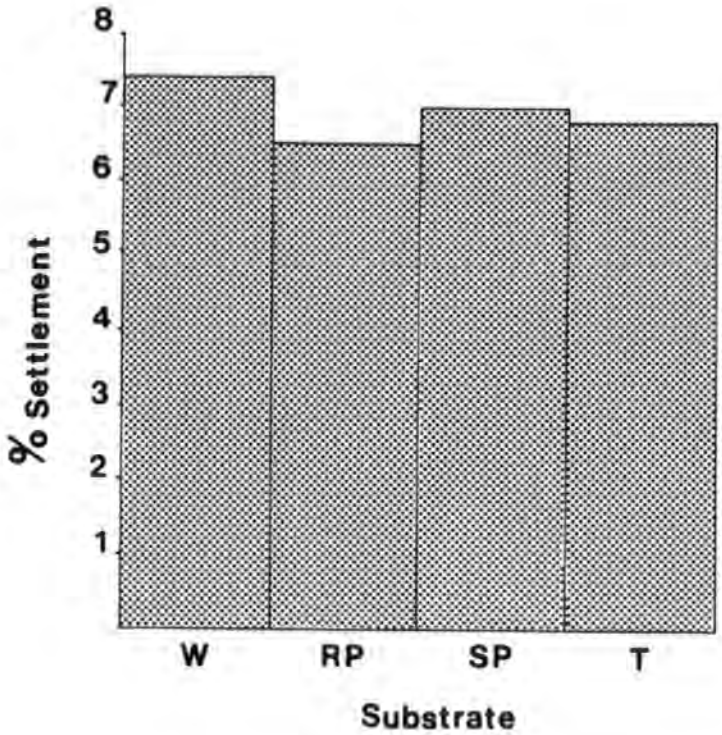
Laboratory settlement in Tubularia larynx

Mean % settlement and substrate preference, 20 hours after release of actinula larvae.

W = wood
RP = rough perspex
SP = smooth perspex
T = tufnol

Table 4.4.2

Substrate preference.



Expt.	W.	R.	S.	T.	Total
1	5	9	8	6	28
2	3	5	12	7	27
3	10	6	5	11	32
4	8	5	6	2	21
5	11	7	4	8	30
Mean	7.4%	6.5%	7.0%	6.8%	

Table 4.5.1.

Tubularia Field Settlement and Growth.

Turtle raft study, physical data.

	Salinity °/oo		Temperature °C	
	High tide	Low tide	High tide	Low tide
April	33.4	31.7	9.1	8.9
May	33.8	30.7	9.8	9.2
June	33.4	31.8	10.6	9.9
July	32.6	31.3	13.4	12.9
August	32.9	31.8	15.6	15.2
September	31.4	30.2	15.2	14.6

Table 4.5.2.

Tubularia Field Settlement and Growth

The seasonal pattern of settlement and growth in *Tubularia larynx*, in light zones A, B and C.

Panels	A	B	C
April	12% cover Many isolated young colonies up to 10 m.m. high. Most with gonophores	Few young tufts. Colonies 15 m.m. high. Few gonophores. 16% cover	Some large colonies up to 40m.m. high Ripe gonophores and large hydranths. 22% cover
May	Colonies isolated but up to 20% cover. Max. height to 35mm. No actinulae being shed.	Many large colonies 22% cover with some small tufts. Subject to predation and mullet grazing (many hydranths lost).	Luxurious growth 42% cover. Mostly large, old colonies up to 90 m.m. high. Some younger colonies. Hydrants in good condition with gonophores
June	6% cover Many hydranths lost. Poor condition.	Many older colonies broken away. Still few hydranths on younger colonies	44% cover. Many hydranths lost and older stolons gone. Some younger tufts still in good condition.
Mid-July	No hydranths. few tufts of old stolons.	A few stunted tufts left with few hydranths in poor condition.	12% cover, some hydranths remaining but in poor condition.
August	Odd tufts of old stolon	A few tufts, no hydranths.	A few new hydranths growing from old tufts. Only 4% cover.
September	Isolated new young colonies 15 m.m. high. Few hydranths.	Only new colonies up to 15 m.m. high No re-growth from old stolon tufts. 18% cover. Hydranths in good condition.	Numerous small new colonies 15 m.m max. with many hydranths. Old original stolons, rapid re-growth with large hydranths to 40 m.m. high.

Figure 4.5.3.

Field settlement in Tubularia larynx

The mean % cover in light zones A, B and C at the turtle raft field study.

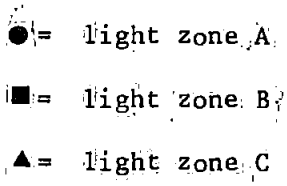
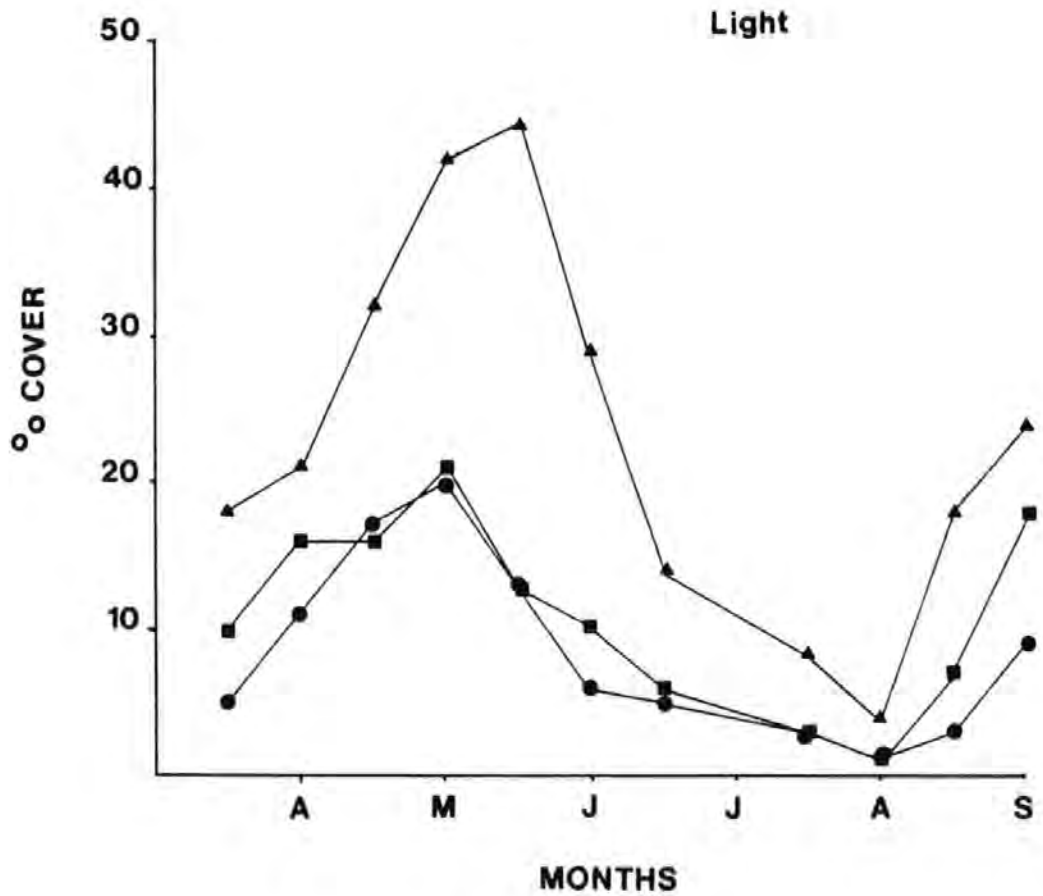


Table 4.5.4.

Analysis of variance table and the effect of light.



Analysis of variance.

Source	Df	SS	MS	f	P
Light	2	1409.5	704.7	8.79	0.001
Error	30	2405.9	80.2		
Total	32	3815.4			

Figure 4.5.5

Field settlement in Tubularia larynx.

The mean % cover of each test substrate, at the turtle raft field study.

□= wood

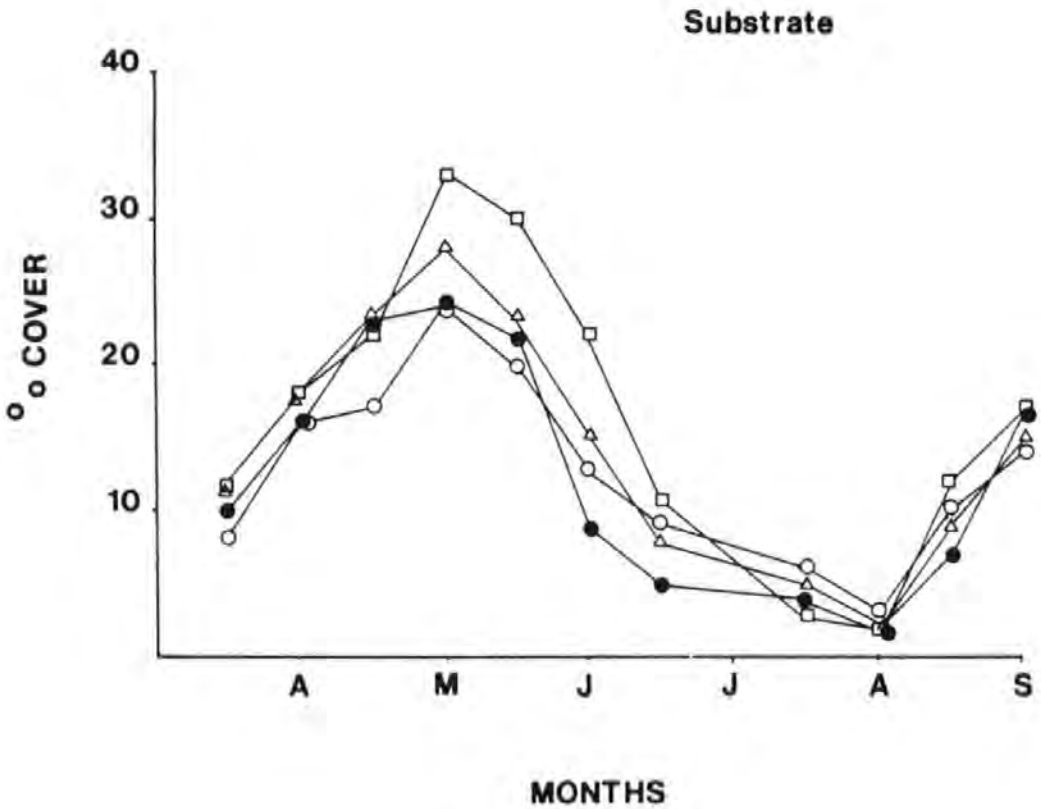
○= rough

●= smooth perspex

△= tufnel

Table 4.5.6.

Analysis of variance table and the effect of substrate.



Analysis of variance.

Source	Df	SS	MS	f	P
Substrate	3	137.0	45.7	0.68	NS
Error	40	2668.6	66.7		
Total	43	2805.6			

Figures 4.6.1 to 4.6.4.

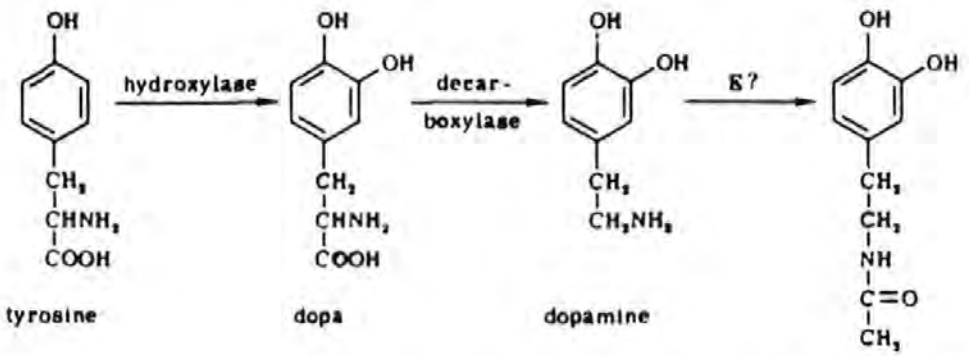
'Settlement attachment' in Tubularia larynx schemes for the sclerotization of recently secreted chitin.

Figure 4.6.1 (from Richards, 1978).

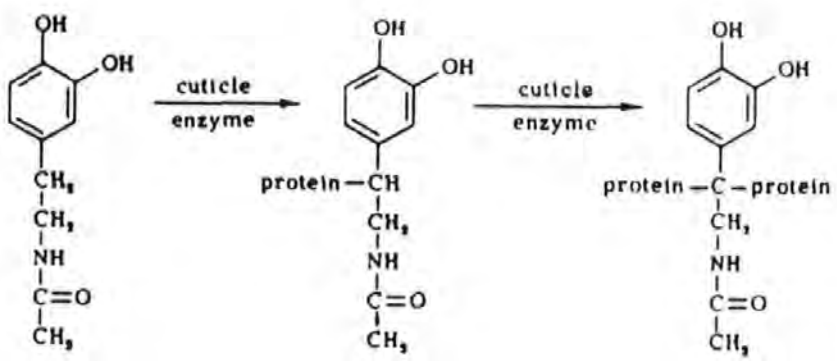
Steps in the conversion of tyrosine to N-acetyldopamine.

Figure 4.6.2 (from Richards, 1978).

Scheme for β-sclerotization.



Steps in the conversion of tyrosine to *N*-acetyldopamine.



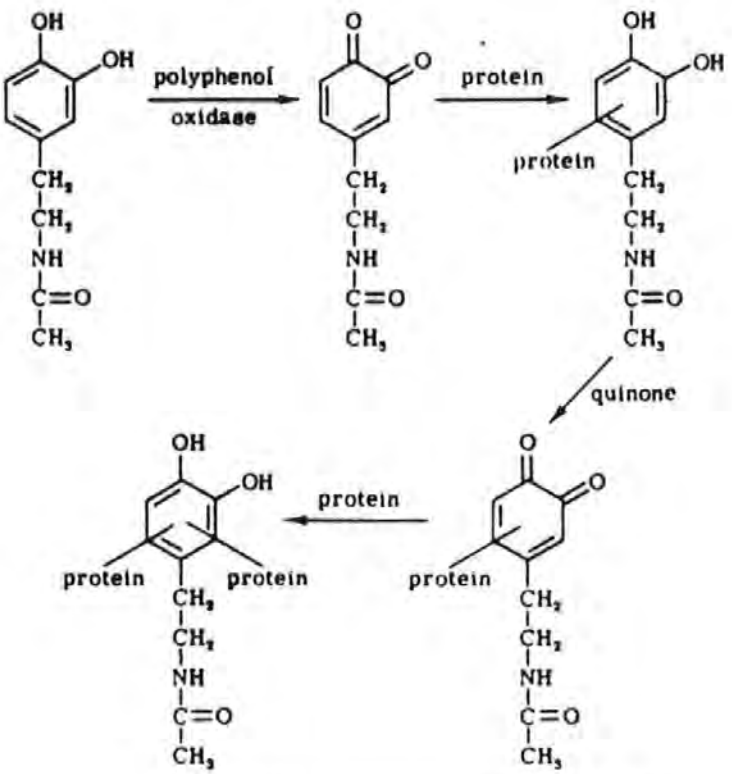
Scheme for β-sclerotization.

Figure 4.6.3. (from Richards, 1978).

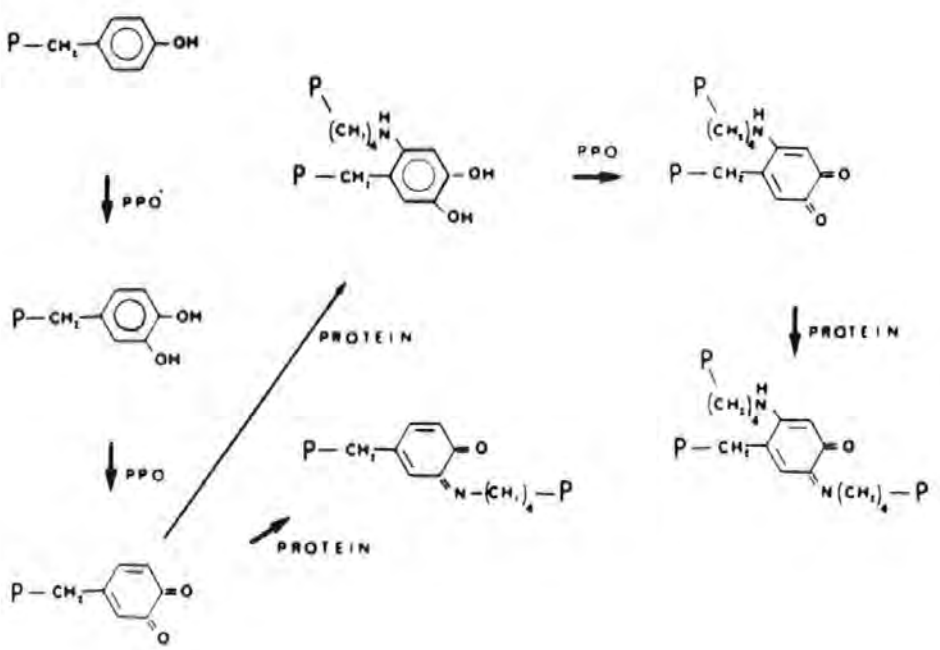
Scheme for quinone sclerotization.

Figure 4.6.4

The mechanism of autotanning (from Lissitzky, Rolland and Lasry, 1962).



Scheme for quinone sclerotization (tanning).



CHAPTER 5

Growth in Tubularia larynx (Ellis and Solander)

Limited tolerance tests carried out in order to design the experimental range of temperatures and salinities for growth studies in *Tubularia*, showed that hydranths were shed at temperatures greater than 20°C., and also when temperatures were reduced to less than 5°C. The salinity range was much narrower, with hydranths being lost when the experimental salinity was reduced to 75% seawater (26°/oo salinity).

5.1 *'Actinula' growth*

Following 'settlement attachment' and when the young *Tubularia* individual was securely attached to the substratum, a period of rapid basal stolonic growth was observed. This activity was usually preceded by elongation of the hydrocaulus to approximately 2 mm. During this time the basal 'stolon buds' became apparent as one or two, but sometimes up to five or more outgrowths from the aboral pole. The 'stolon buds' gave rise to 'primary stolons'. Basal stolonic growth occurred irregularly across the settlement plates, and after between 4 mm and 6 mm of growth some degree of branching was often observed and a new 'upright' plus hydranth bud was formed. At each of the temperature and salinity combinations, some young polyps failed to elongate and grow after settlement, but overall development rates were high throughout the experiment. On average, up to 80% of the young *Tubularia* polyps in each experimental tank grew into small 'colonies'.

Throughout the range of experimental conditions, the mean rate of stolonic growth was exponential (Figures 5.1.1 to 5.1.3, and tabulated in Appendix 4 , illustrate the logarithmic nature of the early

stolonic growth). Although in this part of the study the index of growth was the increase in stolon length, the number of hydranths and hydranth buds were also noted. The mean rate of stolonic growth recorded in young *Tubularia* polyps held in $34^{o}/oo$ and $30^{o}/oo$ salinity seawater at both $18^{o}C$ and $12^{o}C$ was very similar. However, a greater number of hydranths was supported by those *Tubularia* developing at $12^{o}C$, in each salinity. By day 14, young colonies held in $34^{o}/oo$ and $30^{o}/oo$ at $18^{o}C$., averaged 26.1 mm and 21.5 mm of stolonic growth respectively; whilst the tufts of colony supported an average of 3 hydranths plus the original polyp. *Tubularia* 'colonies' developing in the same salinities at $12^{o}C$., averaged 22.2 mm and 21.2 mm respectively (stolonic growth), but also supported an average of 4 hydranths and hydranth buds in addition to the original individual. The mean rate of stolonic growth amongst the animals growing in the lowest salinity of $28^{o}/oo$, at both $18^{o}C$., and $12^{o}C$., was much reduced, and by day 14, mean stolon lengths of 10.4 mm and 8.5 mm respectively were recorded. At these temperature and salinity combinations 2 to 3 new hydranths were developed in each of the growing 'colonies' over the 14 day experimental period.

At $6^{o}C$., the maximum mean rate of stolonic growth was observed in the colonies developing in $34^{o}/oo$ salinity seawater. Here, by day 14 an average stolon length of 10.2 mm was recorded. In these 'colonies' an average of 2 to 3 new hydranths were developed during the period of observations. Young *Tubularia* polyps growing in $30^{o}/oo$ and $28^{o}/oo$ salinity sea water at $6^{o}C$., however, developed slowly, and most failed to produce any significant basal stolonic growth, the bulk of stolonic growth being observed as an increase in length of the hydrocaulus. By day 14, the mean stolonic growth of polyps held at $30^{o}/oo$ and $28^{o}/oo$ salinity was 5.1 mm and 3.8 mm respectively. No hydranths or hydranth

buds were formed.

Both temperature and salinity main effects were important determinants of the growth response of newly settled *Tubularia* polyps. Both factors were significant at P = <0.001 (Table 5.1.4). The interaction effect of temperature and salinity was also shown to be highly significant at P = <0.01. The effects of temperature and salinity were linear. The highest mean rates of stolonic growth throughout the salinity range were recorded from young polyps growing at 18°C., whilst the lowest growth rates in all salinities were observed at 6°C. Similarly, *Tubularia* polyps held in 34°/oo salinity seawater at each temperature, grew faster than those colonies developing at the reduced salinities of 30°/oo and 28°/oo. Notably, the rate of stolonic growth of young *Tubularia* 'colonies' developing in 30°/oo salinity seawater at both 18°C., and 12°C., was very similar to the rate of growth achieved at 34°/oo salinity at both temperatures. However, at the lowest temperature of 6°C., there was a marked difference in the rate of stolonic growth of polyps developing at 34°/oo and 30°/oo salinity. At this temperature, *Tubularia* polyps at 30°/oo salinity, showed a 50% reduction in the rate of stolonic growth to that attained at 34°/oo salinity.

5.2 *'Colony' growth*

5.2.1 *The Effect of Temperature and Salinity*

The pattern of 'colony' growth in *Tubularia larynx* was similar under all the experimental conditions of temperature and salinity (see figures 5.2.1 to 5.2.3). The explanted hydranth rapidly developed a stolon from the cut end, which grew and attached to the surface of the perspex plate within 30 hours. Mean weekly increases in hydranth production appear in Appendix 5. Eventually, after a variable amount of

stolonic growth had occurred, the stolon gave rise to branches and to uprights which subsequently developed hydranths and hydranth buds. However, this sequence of events occurred within varying time periods and the mean rate of growth or hydranth production of *Tubularia* was markedly different in most of the temperature and salinity combinations. This early growth phase was usually very rapid in all the full salinity ($34^{o}/oo$) tanks throughout the temperature range, often taking only 3 days to the production of a second hydranth. The fastest rate of growth was seen amongst young colonies growing at $12^{o}C.$, in $34^{o}/oo$ salinity sea water. These colonies reached a mean hydranth number of over 15 at the end of the 36 day experimental period. At $12^{o}C.$, the rate of hydranth production amongst colonies maintained in $34^{o}/oo$ and $30^{o}/oo$ salinity sea water accelerated after day 18. This increase in the rate of growth was, however, more rapid amongst those colonies developing at $34^{o}/oo$ salinity. In the lowest test salinity ($28^{o}/oo$) at $12^{o}C.$, the rate of hydranth production, although much reduced, was relatively constant throughout the experimental period (Figure 5.2.2). The *Tubularia* colonies growing at $18^{o}C/34^{o}/oo$ and $18^{o}/28^{o}/oo$ also showed an acceleration in the rate of hydranth production, although under these test conditions the increase was less rapid (than at $12^{o}C/34^{o}/oo$ and $12^{o}C/30^{o}/oo$) and did not occur until day 24. Animals maintained at $18^{o}C/30^{o}/oo$ did not exhibit such a response and the rate of growth was constant to age 36 days. Notably, between day 33 and day 36, some of the *Tubularia* colonies maintained at $18^{o}C/28^{o}/oo$ salinity had lost hydranths, and the mean number of hydranths per colony fell from 6.75 on day 33 to 6.5 by day 36 (Figure 5.2.1).

Throughout the temperature range, $6^{o}C.$, to $18^{o}C.$, the fastest rates of growth (at each temperature) were observed in *Tubularia* held

at full salinity ($34^{o}/oo$). Colonies growing at $6^{o}C.$, in $34^{o}/oo$ salinity sea water showed no acceleration in the rate of hydranth production, as observed in *Tubularia* after day 18 at $12^{o}C/34^{o}/oo$, and after day 24 at $18^{o}C/34^{o}/oo$. However, during the early phase of growth (before the observed marked accelerations in the rate of hydranth production) young *Tubularia* colonies held at each of the temperatures, $6^{o}C.$, $12^{o}C.$, and $18^{o}C.$, in $34^{o}/oo$ salinity sea water, grew at very similar rates. Young colonies developing in the 'intermediate' salinity of $30^{o}/oo$, grew rapidly at both $18^{o}C.$, and $12^{o}C$. These colonies did not differ markedly from those growing in the higher salinity of $34^{o}/oo$ at both temperatures, and the rate of hydranth production was similar. The poorest rates of growth were recorded from colonies growing in $28^{o}/oo$ salinity sea water, throughout the temperature range (and also at $6^{o}C/30^{o}/oo$. New hydranths or hydranth buds were not added until day 9, or after, and the pattern of hydranth production was often irregular. Observations showed that *Tubularia* of these groups underwent loss and regeneration of some hydranths during the experimental period, although such activity was limited to a few explants and only occasionally observed. Throughout the range of temperature and salinity combinations, almost all the explants survived and grew into young *Tubularia* colonies. With the exception of animals growing at $18^{o}C/28^{o}/oo$, the *Tubularia* colonies were seen to be actively growing, albeit at different rates, on day 36 and none had yet reached a 'steady-state' growth level.

In summary, it was seen that the growth response of young *Tubularia* colonies was primarily determined by the main effects of temperature and salinity (see Table 5.2.4), although the interaction effect of temperature, and salinity was also highly significant at $P = <0.001$. These effects were most pronounced at the lowest experimental temperature of

6^{o}C., across the salinity range, and at 28^{o}/oo salinity in each temperature group (Figures 5.2.5 and 5.2.6).

In addition to the effects on the rate of hydranth production, differences in hydranth size and 'condition' were also observed in young *Tubularia* colonies grown under each set of experimental conditions. At 12^{o}C., in both 34^{o}/oo and 30^{o}/oo salinity sea water, the hydranths were often larger, and on average possessed more proximal and distal tentacles than observed in colonies of the other test groups. Many of the hydranths of *Tubularia* growing at 18^{o}C and 12^{o}C., in both 34^{o}/oo and 30^{o}/oo salinity sea water, possessed gonophores at various stages of maturation, by day 36. In contrast, young colonies growing at 28^{o}/oo salinity throughout the temperature range, possessed mostly small, but 'active' hydranths, few of which bore gonophores.

5.2.2 *Effect of Ration*

A parallel series of experiments, at the two higher temperatures of 18^{o}C and 12^{o}C (in 34^{o}/oo, 30^{o}/oo and 28^{o}/oo salinity sea water) was run to investigate the effect of increased ration on the rate of growth in *Tubularia*. An increase in the rate of feeding from one first stage *Artemia* nauplius per hydranth per day to one nauplius/hydranth/three times daily, resulted overall in only marginally increased hydranth production under each set of experimental conditions. However, in the full salinity (34^{o}/oo) tanks the rate of hydranth production was noticeably greater over the initial period of growth. For instance, at 12^{o}C/34^{o}/oo 'control' explants fed at the rate of one *Artemia* nauplius/hydranths/day supported an average of 2.8 hydranths by day 9 and 4.8 hydranths by day 18; whereas *Tubularia* under the increased feeding regime bore a mean of 4.9 and 8.5 hydranths per colony at the

same respective time intervals. An increase in the rate of hydranth production with increased ration was similarly observed amongst *Tubularia* growing in the lower salinities at each temperature. Under each set of conditions, the hydranth buds rapidly developed into large hydranths, although gonophores were present mainly on *Tubularia* at the two higher salinities in each temperature, and few were borne by animals growing at $28^{o}/oo$ salinity. Many of the gonophores developed rapidly and matured within the experimental period. Some actinulae were shed from day 33 onwards and readily settled on the surfaces of the culture tanks. A few actinulae also settled on the hydrocauli of the parent *Tubularia* and care was taken to discount any F_2 hydranths from the observations of colony growth.

The effect of increased ration was shown to be not significant at $18^{o}C/34^{o}/oo$; $30^{o}/oo$ and $28^{o}/oo$, and at $12^{o}C/34^{o}/oo$ and $30^{o}/oo$, but significant at $P = <0.05$ at $12^{o}C/28^{o}/oo$ salinity (see Figure 5.2.7 and Table 5.2.8). Mean weekly increases in hydranth production appear in Appendix 6.

5.2.3 ***Effect of Light***

The pattern of colony growth was similar to that observed amongst *Tubularia* developing in the same salinity-temperature ($12^{o}C/34^{o}/oo$) combination in Experiment One (see section 5.2.1). The fastest rate of hydranth production was recorded from animals maintained under 'shaded' conditions ($4x10^{-2}$ lux), of reduced light intensity. This rate of growth, however, was only marginally greater than that attained by *Tubularia* growing under a 12 hour daylength period ($4x10^{-3}$ lux). In each set of light conditions, the young colonies appeared in good 'condition' with mostly large 'active' hydranths. The slowest rates of growth were observed in *Tubularia* developing under conditions of continuous light ($4x10^{-3}$ lux) and continuous darkness. However, on

average, these colonies supported only one less hydranth than observed in *Tubularia* growing in 'shaded' conditions and under a 12 hour daylength period. The effect of light was shown to be not significant (see Figure 5.2.9 and Table 5.2.10). Mean weekly increases in hydranth production appear in Appendix 7.

5.3 *Discussion*

Following permanent or 'settlement-attachment' the early development and growth of *Tubularia* polyps into young colonies was very rapid. Logarithmic growth rates (of increase in stolon length) of up to 0.26k (at $18^{o}C/34^{o}/oo$, see figure 5.2.1) were recorded during this early growth phase, and were comparable to the exponential growth reported in other colonial hydroids (see Tusov and Davis, 1971). The pattern of early growth with, initially, stolonic growth being predominant until about day 6 (at $18^{o}C/34^{o}/oo$) when the first hydranth was produced, largely confirmed the laboratory observations in *T.larynx* of Pyefinch and Downing (1949), although they found that hydranth production occurred earlier (after 4 days) and proceeded at a faster rate. Basal stolonic growth, however, did not usually occur until after the hydrocaulus had elongated to 2-3 mm and the pattern of early growth was similar to that reported in *T.crocea* (Mackie, 1966). In the present study, most of the fastest developing 'colonies' possessed 5 hydranths supported by over 20 mm of total stolon length after 14 days, whereas Pyefinch and Downing (1949) reported only a third of this stolonic growth, but up to 8 hydranths per 'colony' 11 days after settlement. Similarly, gonophore buds did not appear until day 10 at the earliest (at $18^{o}C/34^{o}/oo$ and $12^{o}C/30^{o}/oo$, whilst Pyefinch and Downing (1949) found gonophore buds had developed within 7 days in their cultures. Although there may be a degree of variation in colony form and

maturation in *Tubularia*, clearly the early rate of growth may be very rapid. The laboratory results of this study confirm the observations of Berrill (1952) who described the basal stolons in young colonies of *T. crocea* being in "vigorous growth at virtually all points".

The rate of stolonic growth (and hydranth production) immediately following settlement of the actinulae larva was, however, variable with both temperature and salinity conditions. The fastest rates of stolonic growth were observed in *Tubularia* developing in full salinity (34^{o}/oo) seawater throughout the temperature range, and when the environmental salinity was reduced to 28^{o}/oo, the rate of stolonic growth (and hydranth production) was depressed. Reduced growth at reduced salinities was most marked when the temperature was lowered to 6^{o}C., where colonies held at 30^{o}/oo salinity also grew slowly. These results showed a general, linear trend in the effects of salinity and temperature on the early growth rate in *Tubularia larynx*. Similarly, Berrill (1949) reported that stolonic growth in the colonial hydroid *Obelia* was also temperature dependent and increased with increasing temperature over the range, 10^{o}C., to 20^{o}C. By comparison, Wyttenbach (1968) reported stolonic growth in *Campanularia* was "virtually identical" over a wide range of temperature (from 14^{o}C., to 20^{o}C), although the rate of growth slowed considerably at 12^{o}C. The possible influence of the combined effects of temperature and salinity have also been shown; where the adverse growth conditions of low salinity (28^{o}/oo) were partly 'offset' at higher temperatures (18^{o}C), and similarly, the adverse effect of low temperatures (6^{o}C) on the rate of stolon growth was partly 'overcome' in the higher (34^{o}/oo) salinity tanks, (see figures 5.1.1 to 5.1.3). However, to date, the effects of salinity variations on the early growth

of *Tubularia* have not been investigated, whilst previous reports of the effects of temperature were mostly concerned with hydranth autotony and regeneration (e.g. Moore, 1939; Berrill, 1952). A limited series of tolerance tests carried out in the present study showed that *T. larynx* colonies acclimatised to temperatures above 20°C and below 5°C soon lost hydranths, although regeneration occurred within 2 to 3 days at the higher temperatures. Similarly, when the salinity was reduced to around 75% sea water (26°/oo) hydranths were also shed and the experimental tanks soon became fouled. These results confirmed the earlier findings of temperature tolerance in *Tubularia* reported by Morse (1909), Elmhirst (1922), Moore (1939), Berrill, (1948, 1952), who found the critical temperature, above which hydranths were lost in *Tubularia*, was between 20°C. and 21°C.

The effects of temperature and salinity may be more clearly seen in the experiments of 'colony' growth (i.e. from a single explanted nutritive hydranth) in *Tubularia*, where observations were carried out over a 36 day experimental period. Explanted *T. larynx* hydranths rapidly produced stolonic growth from the cut end and became attached to the perspex plates within 36 hours. Subsequent basal stolonic growth, branching, and production of uprights, produced the first hydranth within 3 days under the best conditions. Similarly, Kinne and Paffenhofer (1966) reported that reattachment of polyps of the colonial hydroid *Clava multicornis*, occurred "within 24 hours" and that all test individuals showed "rapid growth". Observations of the rate of hydranth production in the 'colony' growth of *T. larynx*, showed that the maximum rates of growth were attained at the intermediate temperature of 12°C, (in both 34°/oo and 30°/oo salinity seawater). Growth in these colonies was faster than the rate of growth of *Tubularia* held at 18°C.,

(the 'optimum' temperature for stolonic growth in the newly settled *Tubularia* individual, see section 5.1). At 12°C., (in both 34°/oo and 30°/oo salinity seawater) there was a marked acceleration in the rate of hydranth production from age 18 days, whereafter the rate of growth more than doubled. This acceleration in the rate of growth corresponded with a mean number of 4.6 to 4.8 hydranths per colony, by day 18, at 30°/oo and 34°/oo salinity respectively. These results suggest it is possible that, at 12°C., developing *Tubularia* colonies may reach a 'threshold' size or number of hydranths, after which the process of growth or assimilation of food may reach its maximum performance. A similar acceleration in the rate of hydranth production was not, however, observed in the animals growing at 18°C/34°/oo until after day 24 (when the colonies possessed an average of 6.4 hydranths); whilst no increase in hydranth production was seen at all amongst *Tubularia* developing at 30°/oo salinity. The lowest rates of growth across the temperature range were observed at 6°C., although the reduction in the rate of hydranth production was 'minimized' amongst *Tubularia* maintained at 34°/oo salinity; whereas at the intermediate salinity level of 30°/oo, a 50% reduction in the rate of growth was observed. Colonies growing in the lowest salinity seawater (28°/oo), throughout the temperature range showed the least growth, and hydranth production often did not occur until day 9 or 12 in each case. There was, therefore, a linear trend in the effect of salinity, with hydranth production increasing with increased salinity, and maximum growth rates were observed in *Tubularia* growing in full salinity sea water (34°/oo); whilst the relationship between temperature and the rate of growth was less obvious.

These results do not agree with previous studies of the effects of salinity on the growth of colonial hydroids. According to Kinne and Paffenhofer (1966) the rate of hydranth production may be stimulated by reduced salinities and maximum rates of hydranth production in *Clava multicornis* were attained in 24°/oo salinity sea water. Similarly, the maximum growth of *Bougainvillia* (Tusov and Davis, 1971) and *Campanularia* (Stebbing, 1981a) colonies occurred in less than fully saline conditions, at around 80% sea water. Stebbing (1981b) comprehensively described the process of growth in the colonial hydroid *Campanularia* and showed that when the specific growth rate (R) of experimental colonies was considered against time, the simple growth curve revealed detailed information about the growth response following exposure to environmental stress, including reduced salinities (Stebbing, 1981a. He concluded that maximum rates of growth may occur in response to low level inhibition (including reduced salinities) causing an over-reaction from the organism. In the present study, where growth data was presented in a cumulative form (as increases in number of hydranths, or stolon length), no such growth-response was observed, in *Tubularia*, to salinity stress, and maximum growth rates occurred in full salinity seawater (34°/oo). Laboratory observations showed that exposure to salinities of 26°/oo and below, resulted in cessation of growth and loss of hydranths, and the geographical range of *Tubularia* may be limited primarily by salinity tolerance. These considerations may partly explain the absence of *Tubularia* growths from most estuarine situations, although Calder (1976) found colonies of *T. crocea* flourished in salinities as low as 24°/oo.

In addition to inhibiting overall colony growth in *Tubularia*, exposure to reduced salinities also resulted in a slower rate of sexual maturation. The hydranths of colonies developing at 28°/oo salinity, bore little gonophore growth and the rate of maturation appeared to parallel both the rate of hydranth production and development across the salinity range. These findings contrast with the observations of Stebbing, (1981a) in the calyptoblastic hydroid *Campanularia flexuosa*, who found that the frequency of gonozooids increased up to 10 fold upon exposure to 70% salinity seawater.

The maximum rates of growth in *Tubularia larynx* occurred at the cooler water temperature of 12°C., and agree with similar findings by Crowell (1957), who reported greater growth in *Campanularia* at cooler temperatures, and found that temperatures of 16°C., promoted the most rapid hydranth development. Rapid growth of hydroids in cooler water may be widespread, and although hydranths in *Obelia* continued to grow in temperatures up to 26°C., new hydranths could not develop until the water temperature was below 20°C., (Berrill, 1949). However, the higher rates of hydranth production at 12°C., and the lower rate of growth observed in *Tubularia* at 18°C., may result from a feeding effect (i.e. a comparatively higher cost of maintenance and growth at 18°C., may require a more continuous supply of food for maximum performance). This view was earlier put forward by Kinne and Paffenhofer (1966) in a study of growth in *Clava*, but when the ration for *Tubularia*, this study, was increased threefold at the higher temperature of 18°C., there was still little extra scope for growth and only marginally increased rates of hydranth

production were observed. The effect of increased ration was not significant (see Table 5.2.6) although there was a more marked 'within groups' variation in the rate of growth of *Tubularia* fed three times per day. These results, therefore, appear to indicate that 12°C., may be close to the 'physiological optimum' in *T. larynx*. Increased food ration did, however, generally result in slightly larger hydranths in most colonies supporting the findings of Crowell and Wyttenbach (1957) who observed that terminal growth in *Campanularia* was very sensitive to nutritional level.

Hydranth dimensions were also affected by temperature and salinity, with larger hydranths (bearing most tentacles) being developed in *Tubularia* growing in full salinity seawater (34°/oo) at both 12°C., and 18°C. Reductions in salinity and temperature resulted in smaller hydranths being formed. These observations are opposite to the findings of Kinne and Paffenhofer (1958, 1965) who reported that the hydranths in both the brackish water hydroid *Cordylophora* and the marine hydroid, *Clava* showed maximum dimensions, and number and length of tentacles in combinations of low temperature and low salinity, and high temperature and high salinity. No 'abnormal' hydranths were found in the *Tubularia* colonies throughout the range of experimental conditions, and there was minimal loss and regeneration activity over the 36 day period of observations. In comparison, Crowell (1957) reported that 'abnormal' hydranths were commonly developed in low temperatures between 2°C., and 6°C., in the calyptoblastic hydroid *Campanularia*, and also observed a "cyclical pattern of hydranth loss

and replacement in the same animal, and *Obelia*.

The influence of light did not significantly affect the rate of growth in *Tubularia larynx* (Table 5.2.7.). At an observational level, the fastest rates of hydranth production were found in *Tubularia* colonies exposed to 'shaded' light conditions (4×10^{-2} lux), and colonies under a 12 hour daylength period (4×10^{-3} lux); whilst the poorest growth rates occurred under conditions of continuous light or continuous darkness. Although the relationship between light conditions and the rate of growth in *Tubularia* is not clear, it appears that denser growths of the hydroid are most often found under conditions of reduced light intensity. For instance, raft studies of settlement and growth in *Tubularia* (see section 4.5) showed that the most luxuriant growths of *T. larynx* were found on the underside of the 'turtle' in the zone of least incident light: e.g. during the second settlement and growth peak in September the % cover of *Tubularia* on the illuminated panels, A, was 9%, but was increased to 25% cover in the shaded zone, C. Similarly, the hydroid was most abundant on the "shaded" surfaces of piles (McDougall, 1943) below depths of 10m on North Sea oil and gas platforms (Oldfield, 1980: Kingsbury, 1981), and below the surface zones of the offshore buoys in the present study (see section 3.1).

However, McDougall(1943) also put forward the view that settlement in *Tubularia* was "very largely a matter of chance", and could occur equally on both illuminated and shaded substrates. He suggested that direct sunlight had an inhibitory effect upon the growth of newly

settled *Tubularia* individuals and that (whilst the rise in temperature associated with summer was the "principal factor") the accompanying increase in light intensity may contribute directly to the decline of the species during the summer. Earlier, Morse (1909) had shown that direct sunlight had no effect upon the growth of *Tubularia*, and that no hydranths were lost as long as the temperature remained below 20°C. Laboratory findings in the present study agreed with the view of McDougall (1943) on *Tubularia* settlement, and revealed the lack of any recognized behavioural pattern in the actinula, which, together with the apparent ability of the larva to readily settle on any hard substrate (section 4.4 and 4.5) indicates that *Tubularia* settlement may occur throughout the water column. In addition, experiments to investigate the laboratory settlement of *Tubularia* (section 4.1; 4.3; 4.4) showed that large numbers of actinula larvae were able to attach and settle under fully illuminated conditions. Many of the findings of the present study appear, therefore, to substantiate the observations of De Bastida, et. al., (1980) that settlement and growth in *Tubularia* may also occur throughout the illuminated zone in surface waters. Consequently, it seems probable that the comparative sparcity of *Tubularia* growth in the zone of most incident light (panels, A) of the turtle raft (see section 4.5) may be a result of the apparent reduced larval settlement at the surface (probably mainly due to the effects of algal growths) and was not due to the direct influence of light on the development of young colonies.

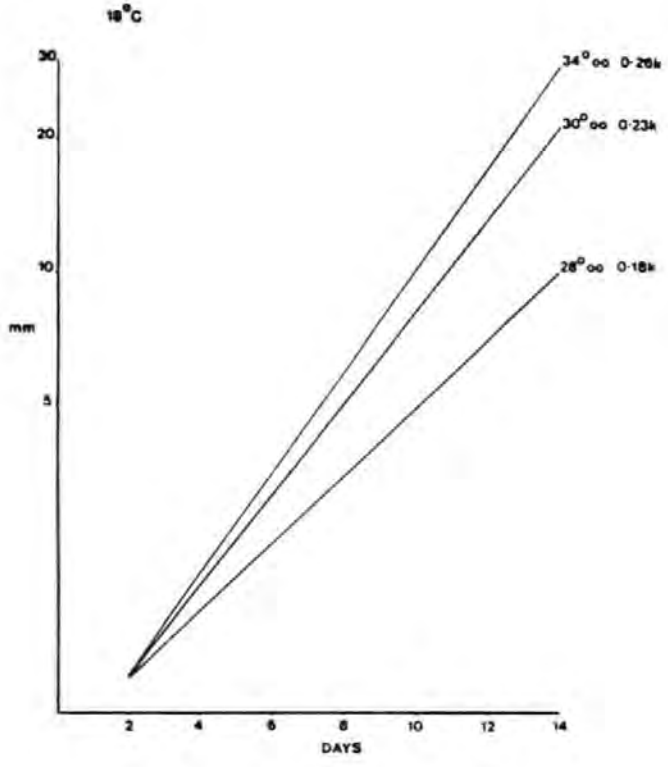
The rate of development of *Tubularia larynx* colonies was very rapid, from both newly settled actinulae and explanted hydranths. Results from the latter experiments (see section 5.2) showed that many colonies developing in the higher salinity and temperature combinations,

matured within 33 days and released second generation actinula larvae. This rate of growth was a little slower than the estimate of Pyefinch and Downing (1949) who found laboratory cultures of *Tubularia larynx* matured within 24 days after settlement. However, it is possible that faster rates of development may occur under more 'natural' conditions in the field. Notably, colonies developing on the 'turtle' raft in the field study in the river Yealm (section 4.5) often grew to maturity within 3 weeks during April and May, and usually possessed large hydranths with prominent gonophore clusters. These observations largely confirm the earlier report of Orton (1914) who recorded that colonies of *Tubularia larynx* matured within 26 days on experimental panels at Plymouth, and agree also with the field studies of McDougall (1943) and Fry (1975).

The rapid rate of development over the temperature range 12°C., to 18°C., and the fast rate of growth observed even in temperatures as low as 6°C., in full salinity seawater (34°/oo) may partly explain the abundance of *Tubularia larynx* in British waters and the successful colonization of hard substrata over much of the year.

Figures 5.1.1 to 5.1.3.

'Actinula growth' in Tubularia larynx. The effect of temperature and salinity on the early stolonic growth of the recently settled polyp.


18°C
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20
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28°oo 0·18k
2
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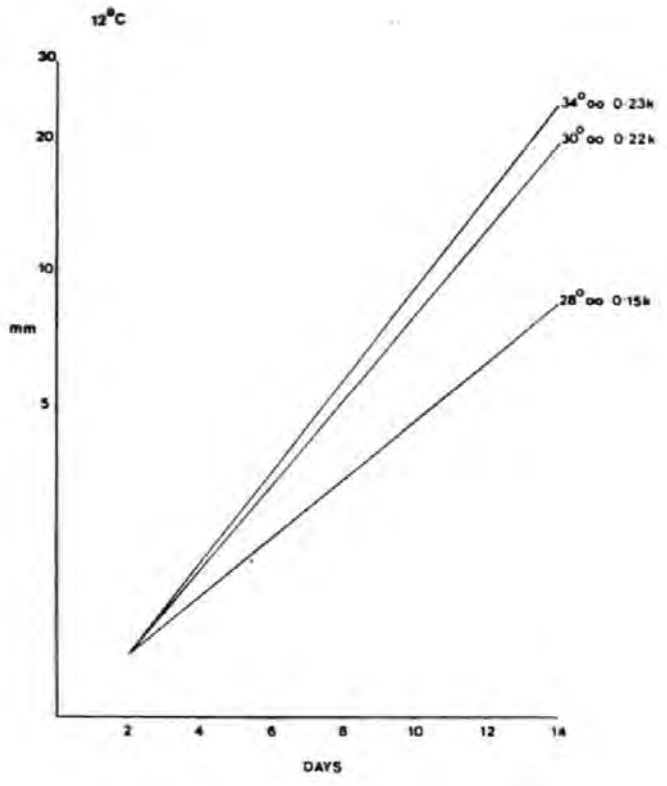
DAYS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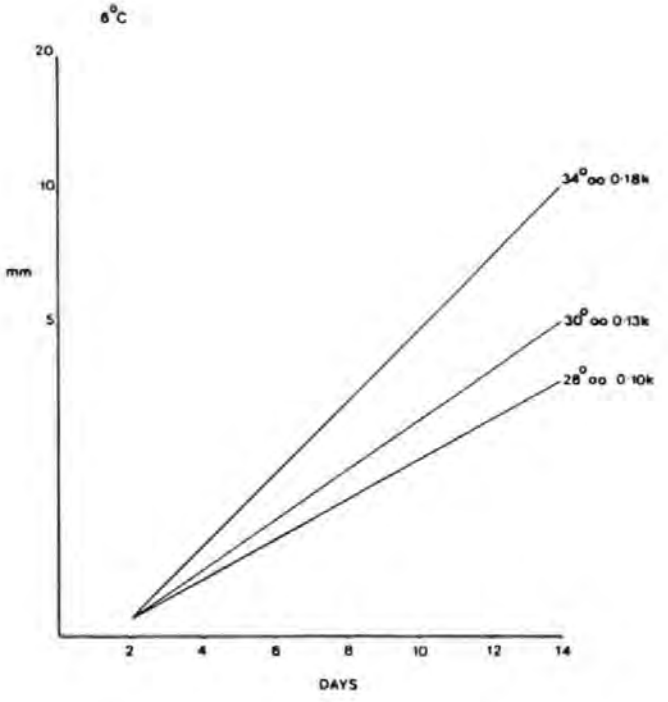
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DAYS

Table 5.1.4

'Actinula growth' in Tubularia larynx. Table of variances; the effect of salinity, temperature and temperature x salinity.

Source of variation	Df	SS	MS	f	P
Salinity	2	861.296	430.648	97.9	<0.001
Temperature	2	667.974	333.987	75.9	<0.001
Temp. x salinity	4	78.098	19.525	4.4	<0.01
Error	81	356.160	4.397		
Total	89	1963.487	788.587		

Figures 5.2.1 to 5.2.3.

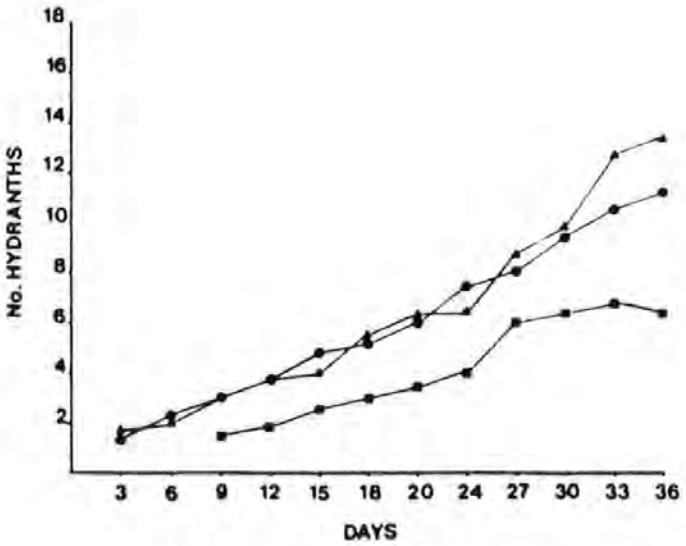
'Colony growth' in Tubularia larynx. The effect of temperature and salinity on the rate of hydranth production.

▲= 34°/oo salinity

●= 30°/oo salinity

■= 28°/oo salinity

18°C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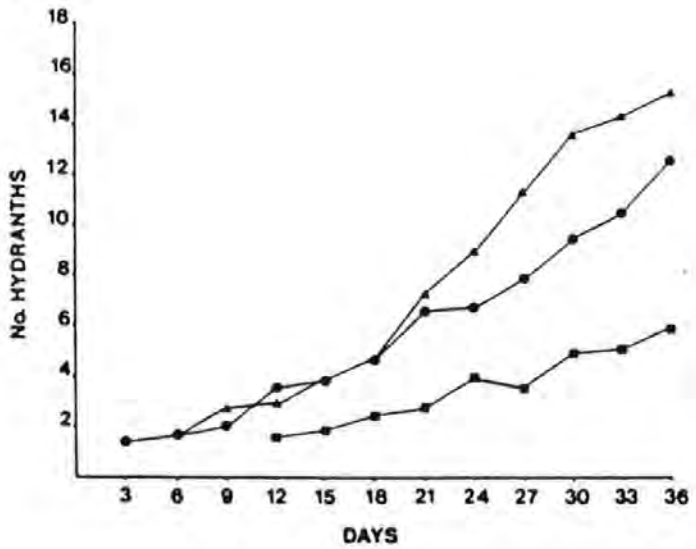
3 6 9 12 15 18 20 24 27 30 33 36
DAYS

12°C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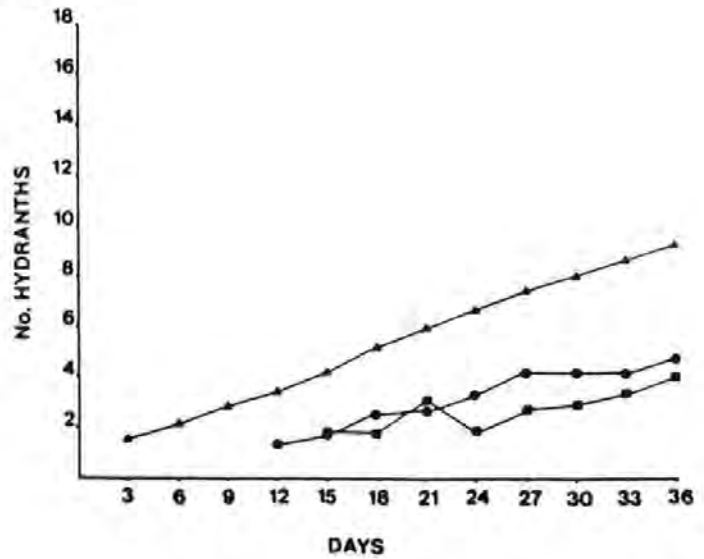
3 6 9 12 15 18 21 24 27 30 33 36
DAYS

6°C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10
8
6
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2
3 6 9 12 15 18 21 24 27 30 33 36
DAYS

Table 5.2.4.

'Colony growth' in Tubularia larynx. The effect of temperature and salinity on the rate of hydranth production. Analysis of variance table.

Figure 5.2.5.

'Colony growth' in Tubularia larynx. The mean number of hydranths per colony, at age 36 days x temperature (^{o}C).

▲ = 34^{o}/oo salinity

● = 30^{o}/oo salinity

■ = 28^{o}/oo salinity

Figure 5.2.6.

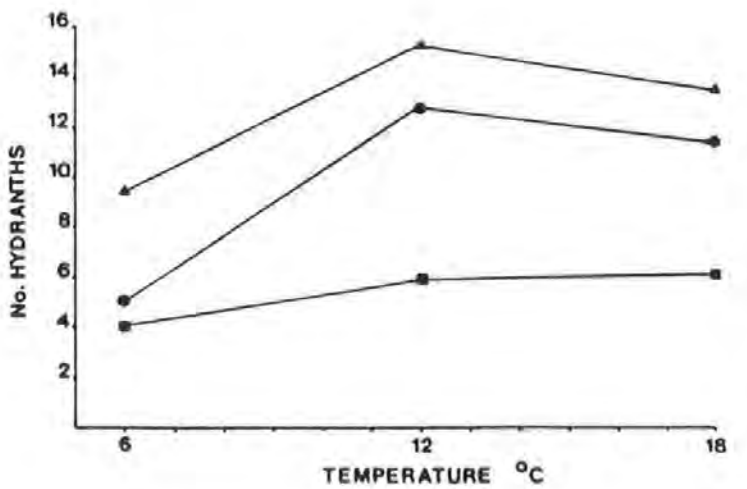
'Colony growth' in Tubularia larynx. The mean number of hydranths per colony, at age 36 days x salinity (^{o}/oo).

Δ = 18^{o}C

O = 12^{o}C

□ = 6^{o}C.

Source of variation	Df	SS	MS	f	P
Temperature	2	512.858	256.429	137.289	0.001
Salinity	2	908.580	454.290	243.221	0.001
Interation	4	128.881	32.220	17.250	0.001
Error	96	179.627	1.868		
Total	104	1729.627	16.631		



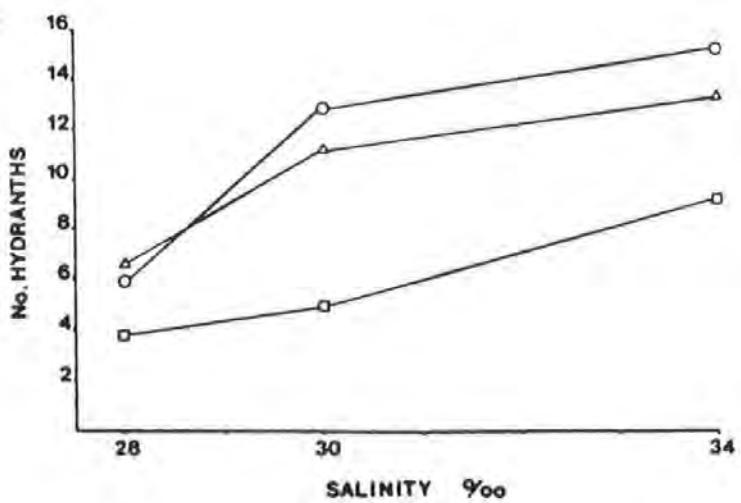


Figure 5.2.7.

'Colony growth' in Tubularia larynx.

The effect of ration on the rate of hydranth production.

(a) 18°C

▲ = 34°/oo salinity (1A) △ = 34°/oo salinity (3A)

● = 30°/oo salinity (1A) ○ = 30°/oo salinity (3A)

■ = 28°/oo salinity (1A) □ = 28°/oo salinity (3A)

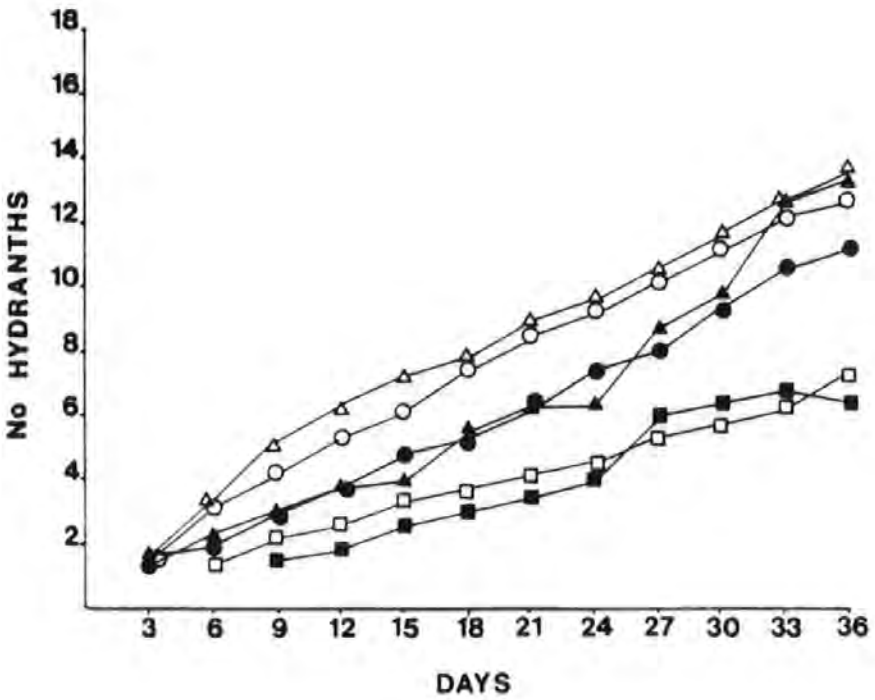
(b) 12°C

▲ = 34°/oo salinity (1A) △ = 34°/oo salinity (3A)

● = 30°/oo salinity (1A) ○ = 30°/oo salinity (3A)

■ = 28°/oo salinity (1A) □ = 28°/oo salinity (3A)

18°C


No HYDRANTHS
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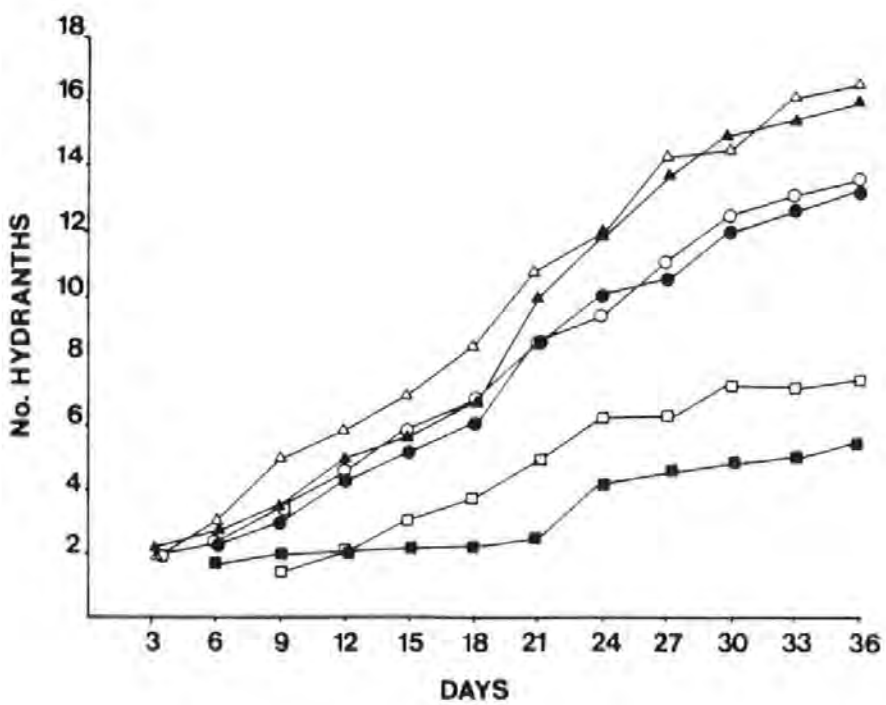
33
36
DAYS

12°C


No. HYDRANTHS
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33
36
DAYS

Table 5.2.8.

'Colony growth' in *Tubularia larynx*. The effect of ration on the rate of hydranth production. Analysis of variance (one-way) tables, for growth at 18^{o}C., and 12^{o}C.

	Source of Variation	Df	SS	MS	f	P
1. 18°C/34°/oo	Ration	1	3.375	3.375	1.336	NS
	Error	22	55.583	2.527		
	Total	23	58.958	2.563		
30°/oo	Ration	1	10.667	10.667	3.520	NS
	Error	22	66.667	3.030		
	Total	23	77.333	3.362		
28°/oo	Ration	1	2.042	2.042	0.741	NS
	Error	22	60.583	2.754		
	Total	23	62.625	2.723		
2. 12°C/34°/oo	Ration	1	5.042	5.042	3.708	NS
	Error	22	29.917	1.360		
	Total	23	34.958	1.520		
30°/oo	Ration	1	0.375	0.375	0.214	NS
	Error	22	38.583	1.754		
	Total	23	38.958	1.694		
28°/oo	Ration	1	12.042	12.042	7.587	<0.05
	Error	22	34.917	1.587		
	Total	23	46.958	2.042		

Figure 5.2.9.

'Colony growth' in Tubularia larynx. The effect of daylength period (light) on the rate of hydranth production, $12^{o}C$ x $34^{o}/oo$ salinity.

▲= 'shaded' ($4x10^{-2}$ lux)

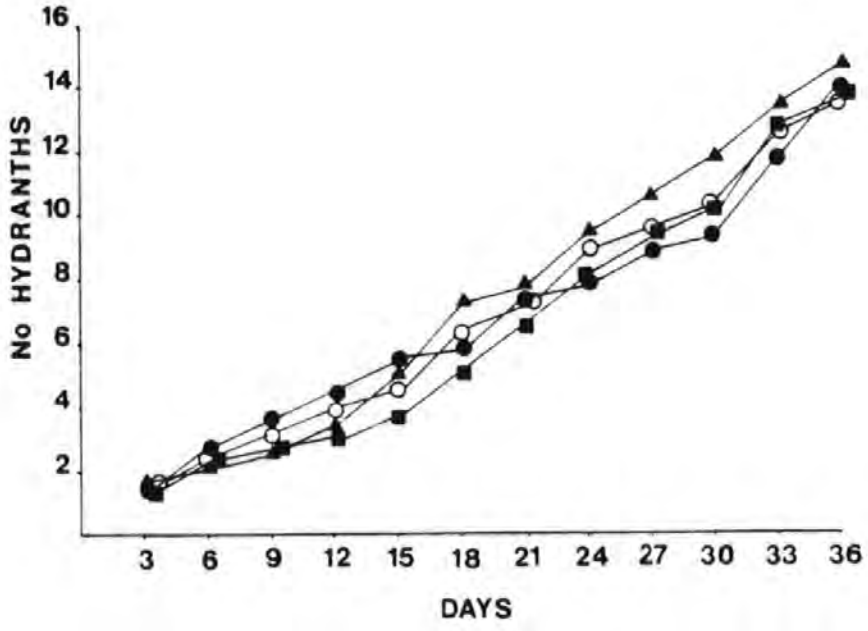
●= continuous light ($4x10^{-3}$ lux)

■= continuous dark

○= 12 hour daylength ($4x10^{-3}$ lux)

Table 5.2.10.

'Colony growth' in Tubularia larynx. The effect of daylength period (light) on the rate of hydranth production. Analysis of variance table (one-way).



Source	Df	SS	MS	f	P
Light	3	3.000	1.000	0.526	NS
Error	44	83.667	1.902		
Total	47	86.667	1.844		

CHAPTER 6

The Mechanism of Settlement in Pomatoceros triqueter (L.)

6.1 *Larval rearing and Development*

The mature eggs were rounded and averaged 70-75 um in diameter, and immediately following fertilisation, underwent rapid division (Plate 6.1.2). Sixteen hours after fertilisation, the first ciliated larval stage swims to the surface and within 2 days the fully formed trochophore larvae (Plates 6.1.3 and 6.1.4) had appeared. By day 4 to day 6 after fertilisation, the trochophore larva had undergone rapid elongation, and, under the most favourable growth conditions, averaged 170 μm to 180 μm long. During this period the larvae were strongly photopositive, and collected at the surface of the culture vessel. If the larvae were subjected to directional light at this stage, they rapidly collected at the surface closest to the source of illumination. Subsequently, the larvae exhibited alternate periods of rapid and slow swimming movements, often spiralling about their longitudinal axis. This phase continued until between 10 and 12 days after fertilisation, under most of the experimental conditions of temperature and salinity. By now, the larvae had reached the stage of late trochophore or metatrochophore. At this time, the swimming movements became slower and the metatrochophores swam deeper, often coming to rest on the sides of the culture vessel and swimming along the bottom. Prior to settlement metatrochophore larvae were seen to swim increasingly, head first along the bottom of the culture beaker or settlement tank, and over the settlement slides (or slates). Frequently, they came to rest and

were sometimes difficult to dislodge even with strong jets of water from a Pasteur pipette. At this time, the larvae were often seen to form small groups of up to 5 or 6 individuals usually at the bottom of the culture vessel. Other larvae may add to the 'cluster' or others swim away, only to come to rest again within a few moments. Larvae that became 'temporarily attached' in this way did so most often at the posterior end, rather than the head region. Close observations under the binocular microscope (x20) showed that nearly always the tip of the abdomen seemed to be 'fixed' to the substrate and the head of the larva, and most of the trunk, remained free. Occasionally, some larvae 'fixed' in this way were seen to rotate about the point of substrate attachment . At no time were any secretions or trailing threads seen, although a 'streaming' effect, around the posterior end, of different refractive properties was sometimes observed.

As a result of these complex swimming and resting behaviours prior to settlement, mortality rates were difficult to determine. Large numbers of larvae (between 600 and 800 per L) were reared under all test conditions of temperature and salinity. The concentration of larvae/L was largely maintained over the 16 day rearing period, under most experimental conditions, and survival rates of 90% and greater were estimated. In one experiment, at $10^{o}C/26^{o}/_{oo}$ salinity, only 116 larvae were available for transfer to the settlement tank after 16 days. This represented only about 14% of the original, approximately, 830 larvae reared after fertilisation.

Across the temperature range, $10^{o}C.$, to $20^{o}C.$, the most rapid rate of development was observed in the *Pomatoceros* larvae held at

at the higher salinity levels of $34^{o}/oo$, $30^{o}/oo$ and $26^{o}/oo$. At these temperature and salinity levels, an initial rapid increase in larval length was recorded by day 4 after fertilisation. This rapid rate of growth was maintained until day 16 (Figures 6.1.6 to 6.1.9). By day 16 at each of the salinity levels, $34^{o}/oo$, $30^{o}/oo$ and $26^{o}/oo$, the mean length of the *Pomatoceros* larvae ranged from 302 μm to 325 μm (at $20^{o}C$); 295 μm to 314 μm (at $15^{o}C$); and 284 μm to 305 μm (at $10^{o}C$).

Within the same temperature range of $10^{o}C$ to $20^{o}C$., a slower overall rate of growth was recorded in those larvae reared at the two lower salinities of $22^{o}/oo$ and $18^{o}/oo$. The initial, rapid increase in length of the early trochophore (to between 160 um and 175 um) by day 4 after fertilisation was again observed, although most larvae developing at $10^{o}C/18^{o}/oo$ salinity did not reach this stage until day 6. Following this initial elongation of the trunk, the subsequent rate of growth to day 16 was, however, reduced in larvae at each salinity ($22^{o}/oo$ and $18^{o}/oo$). On day 16, the *Pomatoceros* larvae held at these two lower salinities ranged in mean length from 263 μm to 266 μm at $20^{o}C$.; 260 μm to 278 μm at $15^{o}C$.; and 245 μm to 260 μm at $10^{o}C$.

A marked difference in the pattern of growth was observed in those larvae growing at $6^{o}C$., throughout the range of test salinities. The initial, rapid increase in length to the trochophore stage (elongated trunk) did not take place, and a slower rate of growth was recorded. Under these conditions of salinity and temperature, most larvae did not reach the fully developed trochophore stage until up to 6 days after fertilisation. This slower rate of growth

continued to day 16 when the mean length of the larvae, across the salinity range, varied from 189 um at $22^{o}/oo$ salinity to 242 um at $34^{o}/oo$ salinity. At this temperature, the larvae appeared to be less active, generally, and swimming activities seemed slower. However, if larvae were fed, they remained alive and continued to migrate through the water column up to 28 days after fertilisation.

Throughout the range of experimental temperature and salinity combinations, there were no significant interaction effects (Table 6.1.10). The rate of growth was principally temperature dependent, and temperature was shown to be significant at $P = < 0.05$. Although no salinity effect could be demonstrated statistically, a linear salinity effect across the temperature range was indicated, with increasing salinity up to $34^{o}/oo$, resulting in higher rates of larval growth. A plot of mean final length of the larvae against temperature at each salinity (Figure 6.1.11) indicated that a higher rate of growth was attained by larvae at the 3 higher salinities, $34^{o}/oo$, $30^{o}/oo$ and $26^{o}/oo$; and a lower rate of growth was associated with the two lower salinities of $22^{o}/oo$ and $18^{o}/oo$. Similarly, (Figure 6.1.12) there was a marked reduction in the rate of growth of larvae reared at $6^{o}C.$, compared to that recorded from *Pomatoceros* developing at the 3 higher temperatures of $20^{o}C$, $15^{o}C$ and $10^{o}C.$, across the salinity range.

Measurements of larval length were the means of 10 random observations in each test combination of salinity and temperature, and are tabulated in Appendix 8.

6.2 *Larval Settlement, the Metatrochophore.*

The fully formed metatrochophore measures between 300 um and 325 μm in length, and a maximum of 160 μm wide (across the prototroch), excluding cilia. A number of well defined bands of cilia cover the surface of the larvae (see Plate 6.2.1). At this stage of development, the main locomotor apparatus of the prototroch cilia and the metatroch cilia remains in good condition, and the feeding cilia are also still present. The mouth is seen as a small depression ventrally, in the mid-line, amongst the feeding cilia. A narrow band of neurotrochal cilia extends posteriorly along the elongated trunk to a point anterior to the anal pore (Plate 6.2.1), or sometimes to the tip of the terminal segment. Often, a pair of elongated cilia are seen, laterally, at the posterior end of the trunk. The head possesses a number of cilia, both singly, and in small groups. Most obvious is a centrally positioned group of long cilia, anterior to the eyes, the so-called apical tuft (Plate 6.2.2). Rarely, single cilia or small groups of fine cilia may be present over the surface of the trunk.

There are four chaetigerous segments, although only 3 pairs of chaetae are visible at this stage. Each of the chaetae is a double structure. The anus opens dorsally as a small ciliated pocket, anterior to the terminal segment (see Plate 6.2.1). The surface of the trunk is marked by numerous openings or pores (Plate 6.2.3). Up to 4 pairs of pores are seen on the ventral surface of the larvae (the ventral gland bulge), close to the neurotroch. These appear either side of the mid-line and extend posteriorly to the terminal

segment. In addition, occasionally, clusters of small pores may open onto the ventral surface, close to the neurotroch (see Plate 6.2.7). A number of lateral pores, dorsal pores and head pores are also seen, although their exact position seems variable (see Plates 6.2.2 and 6.2.3). In the metatrochophore, the posterior tip, typically, bears one or more large pores (up to 15 μm or more in diameter, see Plates 6.2.4 and 6.2.5). These anal pores are rounded and communicate internally with the anal vesicle. Usually, mucus secretions are seen at the opening of the anal pores (Plates 6.2.5 and 6.2.6). The anal pores may be raised, or extend 'tube-like' from the tip of the terminal segment (Plate 6.2.7). The eyes are seen dorsally in the head as paired, darkly pigmented structures, and are a prominent feature of the metatrochophore larva.

Internally, the anterior part of the head contains the head vesicle, which may be partially divided by membranes into a number of sacs. The larval collar is seen as a folded structure, beneath the collar pockets at the level of the prototroch (Plate 6.2.8). The gut is divided into a narrow oesophagus, a large stomach and a short intestine. The alimentary tract is ciliated throughout, and the stomach and intestine are highly glandular and contain numerous darkly staining granules. A prominent feature of the metatrochophore larva is the large anal vesicle (see Plate 6.2.8). This sac-like structure often occupies a large part of the posterior trunk, and displaces the intestine forwards so that the anus opens dorsally, anterior to the terminal segment (see Plate 6.2.10). The architecture of the anal vesicle is variable, and a number of thin membranes may divide the structure into compartments. A pair of

mucous glands (the anal mucous glands, Plates 6.2.8 and 6.2.9) are found in close association with the anal vesicle which opens to the outside through one (or more) anal pores (see Plate 6.2.8). Characteristically, the metatrochophore possesses a number of mucous cell glands. Most obvious are the ventral mucous glands of the ventral gland bulge (Plate 6.2.10). Here, the ventral ectoderm has become thickened and highly glandular, giving rise to the ventral mucous glands. These mucous cells underlie the surface pores around the neurotroch and their contents are intensely staining and coarsely reticulate in nature (Plates 6.2.11 and 6.2.12). Most of the mucous cells in close association with the pores were mature, whilst the innermost gland cells were in various stages of maturation, indicating that mucus may be continually added to the surface of the larva. The ventral ectoderm of the head is also thickened and also contains a number of large mucous glands (h.m.g., see Plate 6.2.8). These cells are intensely stained with both toluidine blue and Mayers haematoxylin, and contain granules of varying electron density. Other mucous glands are scattered in the dorsal ectoderm (d.m.g.) and along the length of the trunk close to the surface in the lateral ectoderm (l.m.g.). In both the dorsal mucous glands and the lateral mucous glands (see Plate 6.2.8), the contents were uniformly lightly stained with toluidine blue and haematoxylin. A similar staining reaction was noted in the pair of anal mucous glands (a.m.g.) found in close association with the anal vesicle. Immediately posterior to the rudimentary collar folds in the ventral ectoderm, a group of highly vacuolated cells of moderate staining intensity with toluidine blue (c.s.g.) are seen (see Plate 6.2.10). These are the paired calcium secretory glands, responsible for the secretion of the inorganic component of the calcareous tube in the adult.

Immediately prior to settlement and metamorphosis, the locomotory cilia of the prototroch and the metatroch are lost (Plate 6.2.13), and the larval collar is protruded (see Plate 6.1.5). At this time the cilia of the apical tuft are also lost (Plate 6.2.13). Subsequently, a primary mucoid tube appeared to be secreted. Scanning electron microscopical investigations showed the remains of a mucoid attachment and mucoid tube at the posterior end of the first calcareous tube (Plate 6.2.14). An X-ray micro-analysis of the first calcareous tube (in Plate 6.2.14), showed that no calcareous deposits were present in the tube from the point of origin to $^1/3$ of it's length (Figure 6.2.15). Subsequently, the mucoid tube is lost, and only the remnants of a mucoid attachment are seen at the posterior end of the newly secreted calcareous tube (Plate 6.2.16).

6.3 *Histochemistry*

All the mucous cell glands of the metatrochophore stained strongly with PAS and lead tetra-acetate-Schiffs indicating a muco-polysaccharide (MPS) secretion (Table 6.3.1). Small amounts of calcium were demonstrated only in the calcium secretory glands behind the collar fold, whilst proteins were found in only the ventral mucous cell glands. The Sudan Black test for lipids was negative in all cases.

(a) Head mucous glands

The thickened ectoderm of the ventral half of the head contained a number of large mucous cell glands (h.m.g.), containing both weakly acidic and neutral MPS. The weakly positive PA-HID-AB and HID-AB

reactions indicated the presence of some sulphated MPS; whilst non-sulphated MPS were also present (AB-AY).

(b) Ventral mucous glands

The prominent ventral mucous cell glands (v.m.g.) of the highly thickened ventral ectoderm underlying the neurotroch, contained a weakly acidic and neutral MPS secretion, and a small amount of glycoprotein material.

(c) Dorsal mucous glands

The mucous cell glands scattered throughout the dorsal ectoderm contained secretions which were alcianophilic at pH 1.0 and 2.5, and stained with Alcian Blue containing 0.5M-$MgCl_2$ (AB-CEC) indicating the presence of a highly sulphated, acidic MPS. Sulphated MPS were also shown by the strong reaction to PA-HID-AB, HID-AB.

(d) Lateral mucous glands

The lateral mucous cell glands were scattered over the trunk of the metatrochophore in the lateral ectoderm. The secretion of these cells was comprised of acidic (AB 1.0, 2.5) and neutral (PA-LID-AB), sulphated and non-sulphated MPS.

(e) Anal mucous glands

The contents of the pair of anal mucous cell glands closely associated with the anal vesicle was strongly alcianophilic at pH 1.0 and 2.5, indicating the presence of an acidic, sulphated MPS. The secretion also stained with Alcian Blue containing up to 0.6M-$MgCl_2$ (AB-CEC) indicating the presence of highly sulphated MPS. Sulphated

MPS were also shown by the reactions to PA-HID-AB and HID-AB; whilst small amounts of neutral MPS (PA-LID-AB, LID-AB) may also have been present.

(f) Anal vesicle

The 'contents' of the anal vesicle were weakly reactive to methods for the demonstration of an acidic, highly sulphated MPS.

6.4 *Laboratory Settlement*

In the laboratory, *Pomatoceros* larvae failed to settle on a number of test substrate materials. These included wood, rough perspex, smooth perspex and 'tufnel', which proved very successful as substrates for larval settlement in the field. In the laboratory, however, few larvae settled on plates or slides made of these materials. Prior exposure of these experimental substrates to sea water for 2 to 3 days (in order to promote a 'slime' film) did not increase the rate of settlement. Old roofing slate (cut to 10 cm x 3 cm 'slides') held in sea water for 2 to 3 days, was found to be the most favourable substrate for the attachment and settlement of *Pomatoceros* larvae.

Large numbers of larvae were reared at all temperature and salinity combinations, and the number of metatrochophores transferred to each settlement tank was limited to 200. In one experimental run, at $10^{o}C/26^{o}/_{oo}$, large mortalities occurred, and only 116 larvae were available on day 16 for transfer to the settlement tank. Observations of the number of larvae settling under each set of experimental conditions, included those larvae which were sometimes found settled in the corners of the settlement tanks.

6.4.1 *The Effect of Temperature and Salinity*

Observations were made at 4 temperature levels: 20°C, 15°C, 10°C and 6°C, and at 3 salinity levels: 34°/oo, 26°/oo and 18°/oo. (Figure 6.4.1 and Table 6.4.2.) In the reduced salinities of 26°/oo and 18°/oo, the calcium concentration was maintained at 430 mg/L., by adjustment with $CaCl_2$. The highest rate of settlement, after 18 days from fertilisation, was observed at 20°C/34°/oo. At this temperature, the mean settlement recorded at 26°/oo salinity was 13%; whilst at 18°/oo salinity, 12% successful settlement was recorded. Similarly, at both 15°C., and 10°C., most *Pomatoceros* larvae settled in the two higher salinities. At both temperatures, the mean settlement recorded in the lowest salinity (18°/oo) was only 6%. Fewer larvae settled at 6°C., throughout the salinity range. At this temperature, the mean settlement recorded by day 18 in full salinity seawater (34°/oo) was reduced to 8% (or 16 individuals).

At most temperature and salinity combinations, further settlement of larvae occurred between day 18 and day 21 after fertilisation. In the settlement tanks held at both 20°C and 15°C, this further settlement was most obvious in the lowest salinity (at 18°/oo). At this salinity level, a further 9% settlement was recorded at 20°C., and a further 6% of *Pomatoceros* settled by day 21 at 15°C. However, at 10°C., a larger proportion of larvae were recorded settling between day 18 and day 21 at both of the higher salinities (34°/oo and 26°/oo). At this temperature, a mean settlement of 16% was recorded by day 18 at 26°/oo salinity, and further settlement of another 24 *Pomatoceros* (12%) was observed by day 21. In the settlement tanks maintained at the lowest test temperature, 6°C., further

settlement of *Pomatoceros* between days 18 and 21 was also observed across the salinity range, but was again minimal in each case (see Table 6.4.2).

6.4.2. *The Effect of hypercalcinated seawater (490 mg/L.)*

A similar pattern of settlement at each of test combination of temperature and salinity was recorded at the higher calcium level (490 mg/L., see figure 6.4.1). At this calcium concentration, a mean settlement of *Pomatoceros* larvae of 22% was observed by day 18 at $20^{o}C/34^{o}/_{oo}$. Overall, the greatest rates of settlement were again recorded in the higher salinity tanks ($34^{o}/_{oo}$ and $26^{o}/_{oo}$) at each of the temperatures: $20^{o}C$, $15^{o}C$ and $10^{o}C$. Similarly, within this temperature range, the lowest mean settlements occurred amongst the *Pomatoceros* held at the lowest experimental salinity, $18^{o}/_{oo}$. At this salinity level the mean larval settlement recorded was 10% ($20^{o}C$), 5% ($15^{o}C$) and 4% ($10^{o}C$), see Table 6.4.3. The addition of calcium, also had no significant effect upon the numbers of larvae settling at the lowest temperature of $6^{o}C$. At this temperature, the lowest mean settlement was again observed in each of the test salinities.

6.4.3 *The Effect of Light*

Each of the groups of larvae in the settlement tanks exposed to each of the test conditions of light was maintained at $20^{o}C/34^{o}/_{oo}$. At each light level, most of the settlement recorded, occurred by day 18, and few larvae settled between day 18 and day 21 (Figure 6.4.4 and Table 6.4.5). Overall, the rates of settlement observed were similar to that recorded in the experiments to test the effect of temperature and salinity, at $20^{o}C/34^{o}/_{oo}$.

Group I.

Under conditions of continuous light (4×10^{-3} lux), 19% of the *Pomatoceros* larvae settled by day 18 after fertilisation, and only 1 more individual had settled by day 21. Notably, almost all of the larvae which had settled did so on the underside of the slates.

Group II.

Under conditions of continuous darkness, 17% of the larvae had settled by day 18, whilst a further 3 *Pomatoceros* had completed settlement by day 21.

Group III

Under conditions of continuous light to day 16, and continuous darkness in the settlement tank, 22% of the larvae had successfully settled by day 18. No further settlement of tubeworms was observed between days 18 and 21.

Group IV

At this light level, the larvae were reared under a 12 hour daylength period (4×10^{-3} lux). Under the conditions in the settlement tank a total of 36% of the tank population had settled by day 18. Of this settlement, nearly all the larvae (31 individuals) had settled on the slates in the shaded half of the tank. The remaining 5 tubeworms had settled in the illuminated half of the tank. Between days 18 and 21 a further 8 larvae had settled in the shaded half of the tank only.

6.4.4 *The Effect of Resident Pomatoceros triqueter*

Settling *Pomatoceros* larvae clearly preferentially colonized the experimental plates having resident members of their own species. Overall, 284 settled individuals were recorded, of which 86% preferred to settle on the experimental slates, whilst only 14% settled on the control slates (Table 6.4.6). Figure 6.4.7 illustrates the results from the 18 pairs of slates used in the experiment. Here the horizontal axis shows the percentage of total settlement that occurred on the experimental slates (having resident *Pomatoceros*) in each pair, and the vertical axis shows the number of times when the indicated percentage of settlement occurred. In many cases, more than 90% of the settling larvae colonized the slates with resident *Pomatoceros* individuals, and on only one occasion did the proportion of settlement on the experimental slates fall below 70%. The non-parametric Mann-Whitney U test using data from the 18 pairs of slates, and values for the number of larvae that settled showed a highly significant difference ($P = <0.001$, Table 6.4.8) between experimental and control slates.

6.5 *Field Settlement Studies*

The general pattern of settlement is illustrated in Figure 6.5.1. There was a peak in the numbers of *Pomatoceros* settling during May (the total settlement on all experimental surfaces was 147), followed by a decline in recruitment over the summer months. A second heavy settlement period was recorded during September, but thereafter the rate of settlement decreased sharply and was minimal during the winter months. The total number of *Pomatoceros* recorded from the three months

period, December to February, from all panels was only 18. The peaks of settlement recorded in May and September, coincided with maximum recorded temperatures of 11.8°C., and 15.8°C., respectively (Table 6.5.2). The monthly salinity level recorded at the raft ranged from 30.6°/oo to 31.9°/oo, during May, and 30.8°/oo to 32.1°/oo during September (see Table 6.5.2). Larger salinity fluctuations and lower temperatures were observed during the winter months, when the rate of settlement was at its lowest. The minimum temperature recorded, of 7.1°C., occurred in February; whilst during January, the salinity level ranged from 27.9°/oo to 33.6°/oo.

In addition to the seasonal variation in *Pomatoceros* settlement, there were marked differences in the numbers of *Pomatoceros* which settled on the panels in each of the light zones A, B and C. (Figure 6.5.3.) The heaviest settlement occurred on the panels attached to the shaded, underside of the 'turtle' raft, in the zone of least incident light (light zone C). By the end of March, four weeks after exposure, all the panels in light zone C were fouled with *Pomatoceros*, and during April large numbers of the tubeworm had settled. During the following 4 weeks, to the end of May, a further 78 tubeworms had settled on the panels in light zone C. This figure represented more than half the total settlement recorded from all panels during the peak settlement period of May. Further settlement during the year followed the seasonal pattern already described with a decline in the rate of settlement during the summer months, a second peak of settlement during September, and minimal settlement in the period, December to February. The total number of *Pomatoceros* settled on all test panels in light zone C, throughout the year was 390.

The seasonal pattern of settlement was also observed in the settlement recorded from the panels in the intermediate light zone B. The May and September peaks in *Pomatoceros* settlement were well defined, although the overall number of tubeworms settling was reduced. During the 12 months period of observations, the total number of *Pomatoceros* recorded from all panels in light zone B was 188. Seasonal features, such as the May and September peaks of settlement, were less obvious in the pattern of settlement observed in light zone A. In light zone A, the least settlement of *Pomatoceros* was recorded, and only 19 tubeworms settled during the peak settlement period of May, compared with 50 individuals in light zone B, and 78 in light zone during the same month. The total number of *Pomatoceros* recorded from light zone A over the 12 months period of observations, was only 89. The effect of light on the settlement rate in *Pomatoceros* was shown to be highly significant at $P = < 0.001$ (Table 6.5.4).

In addition to showing a marked preference to settling on shaded surfaces, *Pomatoceros* larvae also displayed a preference in choice of substrate. A higher proportion of the tubeworm larvae settled on the wood (marine plywood) panels than on any other of the test substrates. (Figure 6.5.5 and Table 6.5.6). During the May period of peak settlement, 61 individuals had settled on the wood panels, compared with 39 on the roughened perspex panels, 32 on the tufnel panels and 15 on the smooth perspex panels, at all locations on the raft. More *Pomatoceros* settled on the wood panels, in almost every month, than any other test surface, and the type of substrate was shown to be significant at $P = < 0.05$ (Table 6.5.7).

6.6 *Discussion.*

The naturally occurring settlement of *Pomatoceros triqueter* around Plymouth, England, followed a seasonal pattern and was characterized by two distinct settlement peaks; the major settlement occurring in May and a less intensive peak being recorded in September. Although the periodicity of breeding in *Pomatoceros* appears to be mainly controlled by temperature, with the maximum settlements occurring at temperatures above 10°C., some larval settlement was recorded throughout the year. The variability of the annual breeding rhythm in *Pomatoceros* was noted by Barnes and Powell (1950) who observed that most *Pomatoceros* settlement in the Firth of Clyde (during 1949) occurred in the late summer and autumn, with a heavy settlement reported at the end of October; whilst in earlier years (1944 to 1948) also at Millport, Pyefinch (1950) had reported that settlement of *Pomatoceros* occurred mainly in the summer months of June, July and August. However, a similar settlement pattern to that observed in the present study was reported in both *Pomatoceros triqueter* and *Pomatoceros lamarckii* around the coast of Brittany (Castric-Fey, 1982). According to Zibrowius (1968), Castric-Fey, (1982) and O'Connor (1983), *P. triqueter* was found predominantly in the sublittoral, whilst *P. lamarckii* was typical of the intertidal zone. Contrary to these views no representatives of the latter species were identified in the field settlement studies, and *P. triqueter* only was the subject of all laboratory and field experiments carried out.

Laboratory observations, here, of the development of *Pomatoceros triqueter* largely confirm the earlier reports of Segrove (1941) and Føyn and Gjøen (1954). Segrove (1941), however, described a number of

late larval stages succeeding the development of the metatrochophore which were not identified in this study. The post-metatrochophore larvae described by Segrove (1941) showed various stages of branchial crown development which, according to Føyn and Gjøen (1954) were probably settled stages which had crawled out of their tubes and were incapable of producing a new tube. Similar larval stages, possessing branchial rudiments were reported, however, prior to mucoid tube formation in *Pomatoleios* (Crisp, M. 1977). Complete metamorphosis and the development of the tube-living worms in *Pomatoceros triqueter* was not directly observed in the present study, although the latest motile or semi-motile larval stage immediately preceding settlement (see Plate 6.1.5), corresponded to Segrove's (1941) 'creeping' stage larva found at the onset of metamorphosis. Subsequently, Segrove's (1941) findings were disputed by Føyn and Gjøen (1954) who suggested that such 'creeping' stage larvae were "abnormal" and "too old" to settle or complete metamorphosis. However, the results obtained here indicate that 'creeping' stage larvae as described by Segrove (1941) do occur immediately prior to metamorphosis and settlement, and appear to confirm Segrove's (1941) view that the course of metamorphosis in *Pomatoceros* is initiated by the regression of the locomotory apparatus of the prototroch and metatroch cilia.

The fastest rate of larval development in *Pomatoceros* from the fertilised egg to metamorphosis and settlement was 16 days at $20^{o}C/34^{o}/_{oo}$, and agrees with the earlier estimates of the laboratory development in *Pomatoceros* of Segrove (1941) and Føyn and Gjøen (1954). A limited series of tolerance tests in this study showed that the trochophore larvae of *Pomatoceros* survived and continued to develop over a wide

range of temperatures from 5°C to 26°C., and within the experimental temperature range, 6°C to 20°C., the rate of larval development increased with increasing temperature. Segrove (1941) had earlier reported the temperature dependent rate of development in *Pomatoceros*, and, similarly, the rate of larval development in other serpulids has also been shown to be temperature dependent (e.g. Wisely 1958; Gaucher, et. al., 1967; Crisp, M. 1977; Hong, 1980; Scheltema, et. al., 1981). In addition, the developing trochophore larvae were also tolerant to a wide range of salinity, from around 16°/oo to 34°/oo, although the mortality rate at the lower salinity meant that the experimental range adopted was 18°/oo to 34°/oo salinity. This degree of tolerance to reduced salinities broadly agrees with the findings of Lyster (1965) who reported that *Pomatoceros* larvae could survive in salinities as low as 10°/oo, if only for a few hours. However, here, a slightly slower rate of laboratory development was typical of the larvae in the reduced salinities of 18°/oo and 22°/oo compared to the rate of growth observed at the three higher salinities of 26°/oo, 30°/oo and 34°/oo. This slower rate of development in dilute salinities, although not statistically significant, occurred throughout the temperature range, and there were no significant temperature and salinity interactions. It appears, therefore, that temperature has a direct linear effect on the rate of development of *Pomatoceros* larvae, whilst salinity, at least over the range studied, had no marked effect even though slower rates of growth were associated with lower salinities. By contrast, Lyster (1965) suggested that 14°C may be the optimum temperature for larval development in *Pomatoceros*, and reported the maximum salinity tolerance at this temperature. Salinity and temperature interactions have been

recorded in the development of other serpulids (e.g. Gaucher et. al., 1967; Gray, 1976), and also in the development of a number of bivalve larvae, (e.g. Davis and Calabrese, 1964).

The rate of laboratory settlement observed in the present study rarely exceeded 30% in any experiment. In the temperature range, 10°C to 20°C., the rate of settlement was similar in the two higher salinities but a marked decrease in settlement was recorded at the lowest salinity of 18°/oo. Notably, most of the settlement recorded in 18°/oo salinity at both 20°C and 15°C., occurred between days 18 and 21, suggesting a slower rate of development at this salinity. Similarly, settlement at the lowest temperature of 6°C., was also minimal, throughout the salinity range. Overall, these results appear to support the earlier findings of larval development rates in *Pomatoceros*, and indicate that temperatures below 10°C., and salinities less than 26°/oo may restrict the survival, metamorphosis and settlement of *Pomatoceros triqueter* larvae. Further evidence of the effect of reduced temperatures on settlement in *P. triqueter* was shown by the field study observations, where settlement was minimal during the winter months when temperatures were low, (e.g. January, 7.1°C). In addition, an increased calcium concentration in the sea water had no significant effect upon the numbers of *Pomatoceros* successfully settling and developing into tube-living worms. Notably, in the hyper-calcinated sea water of the *Pomatoceros* cultures of Føyn and Gjøen (1954) up to 100% settlements occurred. It may be supposed that a greater environmental concentration of calcium may enable settled larvae to more rapidly and successfully construct the calcareous tube; however, no increase in the rate of settlement was recorded in any experiment here, indicating that there

may be a 'threshold' level in the rate of uptake of calcium from the sea water and the ability to produce the calcium carbonate tube. Previously, non-linear relationships between the calcium concentration of sea water and the rate of calcium carbonate production have been reported in the rate of tube formation in adult *Eupomatus* (Neff, 1969) and shell formation in *Ostrea gigas* (Kado, 1960). However, recently settled *Pomatoceros* individuals (see section 7.1.1) exposed to hyper-calcinated sea water (490 mg Ca/L) grew significantly faster than the 'control' populations in each of the test combinations of salinity and temperature. Consequently, it is possible that the successful completion of metamorphosis and settlement in *Pomatoceros* depends principally upon the tenacity and effectiveness of the initial mucoid attachment, prior to the secretion of the calcareous tube.

During the larval development period, the *Pomatoceros* trochophore larvae showed a distinct behavioural pattern seemingly governed mainly by light responses; whilst in comparison, the swimming larvae of the closely related serpulid, *Pomatoleios kraussii* showed no consistent response to light (Crisp, M.1977). During the first few days after fertilisation, the larvae of *Pomatoceros triqueter* were photo-positive and collected at the surface of the culture vessel. Later, during the period of active swimming through the water column this response appeared to diminish and prior to settlement the larvae became photo-negative. At this time, the metatrochophores were undergoing a period of temporary substrate attachments before settling mainly on the bottom slates, or on the underside of the mid-water slates. These observations are contrary to the findings of Segrove (1941) who reported that *Pomatoceros* larvae became "positively phototropic"

immediately prior to settlement, and settled mainly just below the water surface nearest the source of incident light. In the field, a photo-positive response prior to settlement has been postulated by Thorson (1957) and Lewis (1964) to explain the aggregation of *P. triqueter* in the tidal zone. However, laboratory observations, here, not only showed that *Pomatoceros* larvae appeared to be photo-negative during settlement, but that conditions of shade encouraged greater numbers of larvae to settle. These results seem to confirm the earlier observations of Føyn and Gjøen (1954) who reported that late trochophores of *P. triqueter* became photo-negative and settled mainly on the bottom of the culture bowl. Further evidence of a possible photo-negative response during settlement was recorded from the field study in the river Yealm, where large numbers of *Pomatoceros* settled in the zone of least incident light and comparatively few were found in the illuminated zone. Similarly, Klockner (1976) reported that *P. triqueter* settling on experimental panels in the southern North Sea were "negatively phototactic". Other serpulids were also commonly found on the lower surface of experimental surfaces (McDougall, 1943; Wisely, 1958; Gaucher et. al., 1967; Straughan, 1968, 1972; Sentz-Bracconot, 1968; Relini and Sara, 1971). However, the influence of other factors which may cause settlement on the underside of substrata have been cited. These factors included both biotic and abiotic factors (Wilson, 1952; Dybern, 1967; Sentz-Bracconot, 1968; ten Hove, 1979). Dybern (1967) found that the settlement of *Pomatoceros triqueter* was a compromise between a stratified salinity gradient, oxygen content and light conditions, and reported that, as a consequence, *Pomatoceros* formed intertidal belts in Scotland, but was

found at 3-5 m depth in Norwegian fjords where low salinity surface layers restricted settlement in the species. Earlier, Lyster (1965) suggested that *Pomatoceros* larvae usually settled during periods of high salinity and were killed by low salinity fluctuations. More recently, Bosence (1979) has shown that settling larvae of the tube-worm *Serpula*, and algae are in competition in the shallow water zone. In view of these considerations, it would appear that the vertical settlement pattern in *Pomatoceros* recorded at the field study raft, may not be directly due to light conditions. However, algae (and any other 'competing' fouling organisms) were regularly cleared from the the experimental panels of all the raft zones to allow the maximum possible 'free space' for *Pomatoceros* settlement, and algal competition in the illuminated zone was minimized. In addition, all the settlement panels were within the first 1.5 m depth and the distance between the illuminated and shaded panels was only 1 m depth and no significant vertical salinity variations were recorded throughout the period of observations. In the absence of significant algal competition, or a salinity barrier, the vertical distribution of *Pomatoceros* settlement at the raft study may be primarily influenced by light, and larval 'recognition' that the illuminated zone may be an unfavourable habitat, subject to algal overgrowth and 'smothering' or siltation.

Results from the field raft study also showed that the settling larvae of *Pomatoceros triqueter* preferred rough surfaces, and were found in significantly greater numbers on the wood and roughened perspex panels, than on the smooth perspex and tufnel panels. Similarly, laboratory observations, showed that *Pomatoceros* larvae readily settled on rough slate surfaces. Overall, therefore, the pattern of larval

settlement in *Pomatoceros* was similar to that recorded in *Ficopomatus* (as *Mercierella*: Straughan, 1968, 1972), and a preference for shaded surfaces, rough in texture and facing downwards, was shown in the larval settlement in both tubeworms. The preference for rough surfaces shown by settling *Pomatoceros* larvae here, does not agree however with earlier reports that recently settled *Pomatoceros* individuals were commonly found on smooth surfaces, but were absent from rough surfaces (Barnes and Powell, 1950; Pyefinch, 1950); whilst the laboratory observations of Føyn and Gjøen (1954) showed larvae were capable of settling on smooth glass. Barnes and Powell (1950) found that whilst *Pomatoceros* larvae readily settled on 'smooth' fibreglass matting, surfaces that were rough and fibrous were free of *Pomatoceros*. Similarly, Pyefinch (1950) reported that, although *Pomatoceros* larvae could settle on a "wide variety of non toxic substrata", rough (granular) resin surfaces were not colonized. By comparison, more recently, Klockner (1976) found that the surface structure and substrate material of experimental panels had no significant effect upon the settlement of *Pomatoceros triqueter*. Although in the present study, field observations showed settling *Pomatoceros* larvae preferred rough surfaces, of wood and roughened perspex, these materials were not colonized by laboratory populations of *Pomatoceros*, even though some larvae settled, in almost every experiment in the corners of the perspex tanks. Prior exposure in seawater of all materials, to acquire a microbial slime film, was carried out and (in the light of field observations) the inability to obtain settlement on all substrates except slate cannot be easily explained. Possibly, in the early attempts to obtain laboratory settlement using wood, roughened and smooth perspex and tufnel sub-

strates failed largely because of some 'unfavourable' condition in the settlement tanks during early experimental runs, even though subsequent, successful, settlements on slate substrates were achieved. In every case the larval rearing process, and food source and ration remained the same. It is also possible that the physical 'character' of the slate material may provide sufficient stimulus to initial attachment and metamorphosis to overcome any adverse conditions in the settlement tanks. Notably, settlement rates up to 30% only have been achieved here, whilst Føyn and Gjøen (1954) reported regular 100% laboratory settlements from their cultures. However, under the more natural conditions in the field study, the settling larvae of *Pomatoceros triqueter* clearly preferred rough surfaces, even though settlement also occurred on smooth materials. These results are contrary to the findings of Barnes and Powell (1950) and Pyefinch (1950), do not agree with the report of Klockner (1976), and do not substantiate the laboratory findings of Føyn and Gjøen (1954).

Often, *Pomatoceros* larvae were seen to settle in the corners of the settlement tanks quicker than settlement occurred on the slate substrates. Similarly, Føyn and Gjøen (1954) reported that groups of larvae settled "especially in the corner between the bottom and the sides of the jar". Field observations have shown that the settlement of *Pomatoceros triqueter* (Pyefinch, 1950) and *Ficopomatus* (as *Mercierella*: Turpaeva, 1961; Straughan, 1972) occurs mainly during periods of slack water. Although no direct investigation of the influence of water velocity on the settlement of *Pomatoceros* was carried out in this study, the apparent 'preference' for settlement in the corners of the experimental tanks (where the least water flow may be expected) appears to substantiate the view that settlement in serpulid larvae occurs mainly

during periods of slack water.

The larvae of *Pomatoceros triqueter* also preferred to settle upon surfaces already inhabited by members of their own species rather than to colonize empty substrates with no *Pomatoceros* tubes. This apparent gregarious settlement-response in *Pomatoceros* substantiates the observations of Sentz-Bracconot (1968) and Klockner (1976), although no relationship between the density of the resident population and the settling larvae was identified. A similar gregarious settlement-response has been recorded in other serpulids. For example, the searching larvae of *Sabellaria alveolata* were only induced to settle by direct contact with the primary mucoid tube or cemented tube of adult members of their own species (Wilson, 1968; 1970). Straughan (1972) reported that the larvae of *Ficopomatus uschakovi* (as *Mercierella enigmatica*) were attracted to areas already occupied by the species, both by the ridges formed by the tube and the living animal. Subsequently, Crisp M. (1977)showed that *Pomatoleios* larvae settled more readily in the presence of adult shell fragments, whilst a gregarious settlement-response was demonstrated in the larvae of *Hydroides dianthus* to settlement slates having resident *Hydroides* tubes. (Scheltema et. al., 1981). In the present study, laboratory results showed that usually, more than 90% of the larval settlement occurred on the experimental slates having resident *Pomatoceros* individuals. According to Scheltema et. al., (1981) such a gregarious settlement-response enables solitary species, such as many tubeworms, to colonize available space in large numbers and form dense populations. By this mechanism, therefore, *Pomatoceros triqueter* may be able to form large aggregations and pre-empt available space in the competition to rapidly colonize the surfaces of hard substrata.

The larval behaviour pattern observed in *Pomatoceros triqueter* in the laboratory largely confirmed the earlier observations in the species by Føyn and Gjøen (1954). Although there were marked differences in the light-response of the larvae here, and the cultures of Segrove's (1941) study, the pre-settlement 'creeping' stage larva described by Segrove (1941) was identified. This late larval stage was apparently responsible for testing of the substrate immediately prior to attachment and settlement in *Pomatoceros*. Similar larval behavioural patterns have been reported in other serpulids (e.g. Andrews and Anderson, 1962; Sentz-Bracconot, 1964; Gaucher, et. al., 1967; Crisp, M. 1977, Scheltema et. al., 1981) including the well documented sequence of behaviours involved in the site selection mechanism in the sub-family Spirorbinae (e.g. Knight-Jones, 1951, 1953, 1957; Wisely, 1960; Williams, 1964; Gee, 1965; Nott, 1973). Prior to the development of the 'creeping' stage larva, the free swimming demersal metatrochophores undergo a period of 'seeking' behaviours and temporary attachments across the bottom of the culture vessel. At this stage the larvae retain all the ciliary tracts, including the distinctive apical-tuft cilia. The attitude of such larvae during this behavioural phase (i.e. often swimming head first across the surfaces of the settlement slates) suggests that the apical tuft cilia may be directly involved in the initial site selection in *Pomatoceros triqueter*. Scanning electron microscopical observations showed that the apical tuft remained a prominent feature of the metatrochophore larva right up to the appearance of the 'creeping' stage larvae and that, in addition, a number of other (often smaller) cilia, in groups or singly may also be present. Contrary to these findings, in other serpulids the apical tuft may be lost in the demersal larva. For instance, Grant, (1981) and

Marsden and Anderson (1981) reported that the apical tuft in the metatrochophore of *Galeolaria* was lost during the demersel or 'seeking' phase, although Grant (1981) noted the appearance of other apical cilia, which he suggested played a sensory role in substrate selection. Similarly, Lacalli (1981) reported the regression of the apical tuft in the metatrochophore larvae in *Spirobranchus* and *Phyllodoce* "long before metamorphosis begins". Apical cilia, whether organised into an apical tuft or otherwise arranged are a feature of the head of trochophore larvae (e.g. Gravely, 1909; Fuchs, 1911; Segrove, 1941; Føyn and Gjøen 1954; Holborrow, 1971). Holborrow (1971) described a regular pattern of apical cilia on the head of the late trochophore larva in the polychaete, *Harmothoe imbricata*, the rootlets of which appeared to be in close association with underlying nerve bundles. She suggested the apical cilia may play a sensory role in site selection. No direct associations of the ciliary rootlets of the apical tuft with nerve cells were found in the metatrochophore in *Pomatoceros*, here, but the rootlets often extended into the head, close to areas containing nervous tissue. These findings, together with observations of larval 'seeking' behaviours provide circumstantial evidence that the apical tuft in *Pomatoceros* may also be involved in site selection, and to some extent support the views of Schroeder and Hermanns (1975) that the apical tuft may be sensory in function. Earlier, Nott (1973) had described the apical tuft in *Spirorbis spirorbis* as a possible chemo-sensory structure used in the site selection habits peculiar to this genus.

Temporary attachments were a feature of the 'seeking' behavioural phase in *Pomatoceros triqueter*. Metatrochophore larvae were frequently seen coming to rest and swimming away again after a brief substrate

attachment by the posterior end of the larva. Such temporary attachments may be effected by the secretion of an 'adhesive' mucous from the anal mucous cell glands (mainly acidic, sulphomuco-polysaccharides) associated with the anal vesicle, although the secretions of other mucous cell glands may contribute to such attachments. The occurence of these temporary attachments in *Pomatoceros triqueter* confirms the earlier observations of Segrove (1941) and Føyn and Gjøen (1954); whilst temporary attachments by the secretion of an 'adhesive' mucous have been recorded in a number of other serpulids (Straughan, 1972; Nott, 1973; Crisp, M. 1977; Grant, 1981; Marsden and Anderson, 1981; Scheltema et. al., 1981). Wisely (1958), however, found no temporary attachments occurred in his cultures of *Hydroides* where larval settlement was direct and permanent. A mucus-mediated temporary attachment has also been recorded in the settling pediveliger larva of the bivalve mollusc, *Ostrea gigas* (Cranfield, 1973a, 1973b). At no time in the present study were any mucus secretions or trailing threads of mucus observed in the laboratory *Pomatoceros* populations, although occasionally a 'streaming' effect could be discerned at the posterior end of the metatrochophore. However, sometimes mucus material was seen at the opening of the anal pores in scanning electron microscopical observations (see Plate 6.2.5). Previously, a number of reports have stated that free-swimming metatrochophores, in laboratory cultures, are often seen to secrete mucus threads from the posterior parts of their bodies, and to 'trail' these threads behind them (Knight-Jones, 1951; Wisely, 1958; Nott, 1973; Crisp, M. 1977; Marsden and Anderson, 1981). These findings, and the laboratory observations in this study (where the demersal metatrochophore may frequently become attached to the substrate by its

posterior abdomen) indicate that the posterior end of the metatrochophore larva in *Pomatoceros triqueter* may play a role in effecting the temporary attachments typical of the 'seeking' behavioural phase.

A conspicuous feature of the posterior part of the metatrochophore in *Pomatoceros* was the anal vesicle. This structure occupied much of the posterior abdomen in the metatrochophore, and was also present in all the free-swimming stages in *Pomatoceros*, confirming the observations of Segrove (1941). Serial sections indicated that the anal vesicle was at its largest in the metatrochophore, immediately prior to settlement. In addition, thin resin sections (light microscopy) and electron microscopical studies revealed the presence of one or more anal pores which communicated with the environment. These anal pores were usually limited to the tip of the terminal segment, posterior to the neurotroch cilia, ventrally, and the anus dorsally, and were sometimes raised to form a tube-like extension bounded by microvilli. Morphologically, this arrangement suggests that the mucus secretions from the large, paired anal mucous cell glands may pass via the anal vesicle to the anal pores to provide the bulk of mucus material effecting the temporary attachments. The secretion of the anal mucous cell glands was shown to be an acidic, highly sulphated mucopolysaccharide, with similar staining reactions to a highly viscous "adhesive mucus" secreted by certain pedal glands in the foot of the gastropod molluscs, *Patella* and *Acmaea* (Grenon and Walker, 1978).

The proposed function of the anal mucous cell glands - anal vesicle - anal pore apparatus, here, is further supported by the observation that larvae forming temporary attachments appear do so by their posterior ends only. Similar observations of the behaviour of serpulid meta-

trochophore larvae have been reported by Segrove (1941) in *Pomatoceros* (although he also suggested that the ventral mucous cell glands were involved in such attachments), Wisely (1958) in *Hydroides* and Marsden and Anderson (1981) in *Galeolaria*; whilst the structure has also been implicated in the mucus-mediated temporary attachments of late trochophores in *Hydroides dianthus* (Scheltema, et. al., 1981). In addition, Grant (1981) reported that the metatrochophore larvae in *Galeolaria* often swim so that the "anal vesicle is pulled across the bottom of the dish" during the period of temporary attachments, prior to permanent substrate attachment and settlement. From the findings of the present study, it appears that in *Pomatoceros triqueter*, the anal vesicle is directly involved in the mechanism of temporary attachments, (and also in permanent attachments) by providing a 'reservoir' of 'adhesive' mucus, continually added to by the closely associated anal mucous cell glands, which is rapidly released through the anal pores upon substrate contact. Although not confirmed here, according to Segrove (1941), Wisely (1958), Crisp M.(1977);Marsden and Anderson (1981), and Scheltema et. al., (1981) the anal vesicle disappears upon permanent settlement (allowing the anus to assume a terminal position) and its loss during metamorphosis was cited as further evidence of the structure's role in the mechanism of substrate attachments. However, Segrove (1941) was not sure that this was the only role of the anal vesicle and suggested that the head vesicle and the anal vesicle may also function architecturally, by streamlining the larval body, in *Pomatoceros*.

Permanent settlement succeeds the period of temporary attachments, and coincides with metamorphosis of the late trochophore to the sedentary tube-dwelling worm. Both permanent attachment to the substrate

and the production of the primary mucoid tube during permanent settlement in *Pomatoceros triqueter* appear to be brought about by the array of mucous cell glands in the metatrochophore. The 'adhesive' mucus secretion of the anal mucous cell glands, chiefly responsible for temporary attachments in the demersal larva, may also play a major role in permanently securing the animal to the substrate during primary mucoid tube formation. This view confirms the findings of Segrove (1941) who reported that metatrochophore larvae in *Pomatoceros* may become fixed to the substrate during metamorphosis and settlement by the secretion of the "hyaline contents" of the anal vesicle; although he also implicated the secretions of the ventral mucous cell glands in this role. Similarly, mucus secretions from glands closely associated with the anal vesicle (together with secretions from the 'attachment gland' via the anus and associated pores) were responsible for effecting the permanent settlement of *Spirorbis spirorbis* larvae (Nott, 1973). In addition, Marsden and Anderson (1981) observed "sticky masses" of secretory material around the anal vesicle during the course of metamorphosis and settlement in *Galeolaria*. In effecting secure substrate attachments, mucus secretions from the anal vesicle may be added to by acidic sulphated mucosubstances from the dorsal mucous cell glands, similar in composition to the mucus contents of the anal mucous cell glands. This view is supported by the observations of Thomas (1940) and Segrove (1941) who reported that *Pomatoceros* larvae settled on the dorsal surface, although this was not directly observed in the present study. Similarly, Nott (1973) reported that mucus secretions from the dorsal collar glands in *Spirorbis spirorbis*, also contributed to the attachment primarily effected by secretions from the

anal vesicle during settlement. In this way, the settling *Pomatoceros triqueter* larva may form a Stefan-type adhesion (Stefan, 1874) with the substrate, brought about by the secretion of acidic mucopolysaccharides (said to be indicative of high viscosity in aqueous solution, Hunt, 1973) from both the large anal mucous cell glands and the mucous cell glands scattered in the dorsal ectoderm.

Following attachment to the substratum, with the ventral surface uppermost, it is postulated that a massive release of secretion from the large mucocytes of the ventral mucus gland cells (weakly acidic and neutral MPS, together with a glycoprotein) is largely responsible for the formation of the primary mucoid tube. Movements of the extended chaetae and the neurotroch cilia would then distribute the mucus over the body of the larva and into contact with the substratum. A similar mechanism was considered to be responsible for primary mucoid tube formation in *Spirorbis* (Nott, 1973), who also reported that the production of the calcareous tube started within one hour of settlement. In the present study, the initial mucoid tube appeared to be gradually replaced by the secretion of a hard, white calcareous tube. The site of secretion of the calcareous tube appears to be the paired vacuolated glands underlying the fold of the collar the calcium secretory glands (c.s.g.). No other glandular area of the larva gave a reaction to methods demonstrating calcium deposits. The production of a primary, wholly mucoid tube, here, did not agree with the findings of Segrove (1941) who reported that the initial tube in *Pomatoceros triqueter* was "composed of mucus partially impregnated with calcareous matter." X-ray microprobe analysis showed that the first one-third of the newly secreted calcareous tube in recently settled *Pomatoceros* individuals, contained no calcium deposits (see Plate 6.2.14 and Figure 6.2.15).

Subsequently, the mucoid tube was lost, and the posterior end of the calcareous tube closed (see Plate 6.2.16).

In the adult forms of the serpulids *Ficopomatus* (as *Mercierella*) *Pomatoceros*, *Hydroides*, *Serpula* and *Spirorbis* (Hedley, 1956b) the collar bearing segment contains two subepithelial, exocrine, calcium secretory glands, producing material to form the tube. These glands are probably homologous both with the calcium secretory glands underlying the collar fold in the *Pomatoceros* larvae and the ventral collar glands of *Spirorbis* larvae (Nott, 1973). In all cases, the calcium secretory glands in the adult worms open to the exterior through a duct in a glandular area of the body wall, the ventral shield. The ventral shield epithelium in adult serpulids is well known to secrete the organic component of the calcareous tube (e.g. Soulier, 1891; Defretin, 1951) and corresponds to the mass of the ventral mucous cell glands in the larva of *Pomatoceros triqueter*, this study. Subsequently Hedley (1956a) fully described the production of the calcareous tube in the adult *Pomatoceros triqueter*, and identified acid sulphomucopolysaccharides (together with a high concentration of calcium) in the mucous cells of the ventral shield epithelium. He considered this tissue was responsible for secreting the organic component of the tube in *Pomatoceros*, which was mixed with secretions of calcium secretory glands, under the fold of the collar during tube formation. Of the larval glandular masses in *Pomatoceros triqueter* identified in the present study, it appears that the ventral mucus cell glands (mainly responsible for primary mucoid tube formation) and the calcium secretory glands underlying the rudimentary collar folds, remain in the adult and become the active tissues in the production of the calcareous tube in the permanently settled animal. Consequently,

the chief purpose of the anal vesicle and anal mucous cell glands in the metatrochophore larva of *P. triqueter* may be to provide temporary attachments during site selection activities, and to securely fix larvaeto the substrate during metamorphosis and permanent settlement. A firm substrate adhesion, brought about by the acid mucopolysaccharide secretion mainly from the anal mucous cell glands, would enable the rapid formation of firstly a mucoid tube, and subsequently a hard, protective calcareous tube to establish the young *Pomatoceros* individual on the substratum.

Plate 6.1.1.

(x 350)

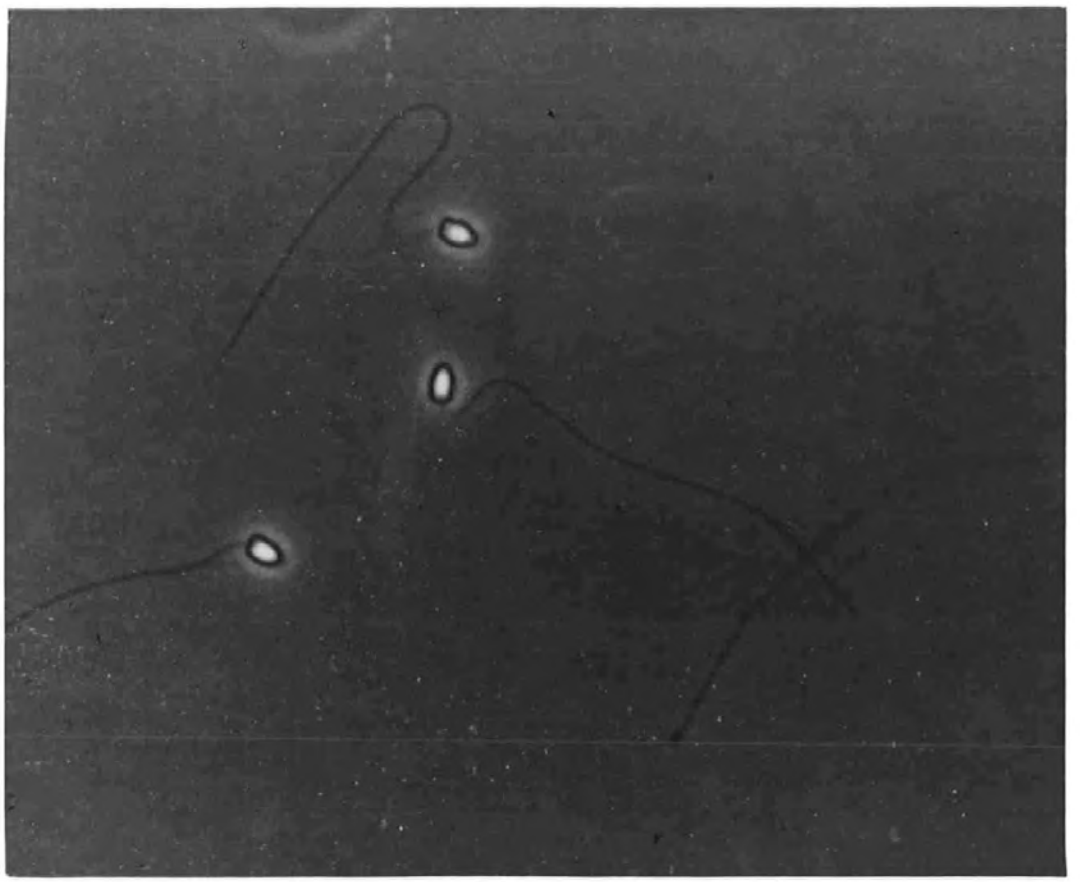
Larval development in *Pomatoceros triqueter*.

Newly released spermatozoa, phase contrast.

Plate 6.1.2.

(x 220)

Larval development in *Pomatoceros triqueter* fertilisation and the early stages of cleavage in the egg.



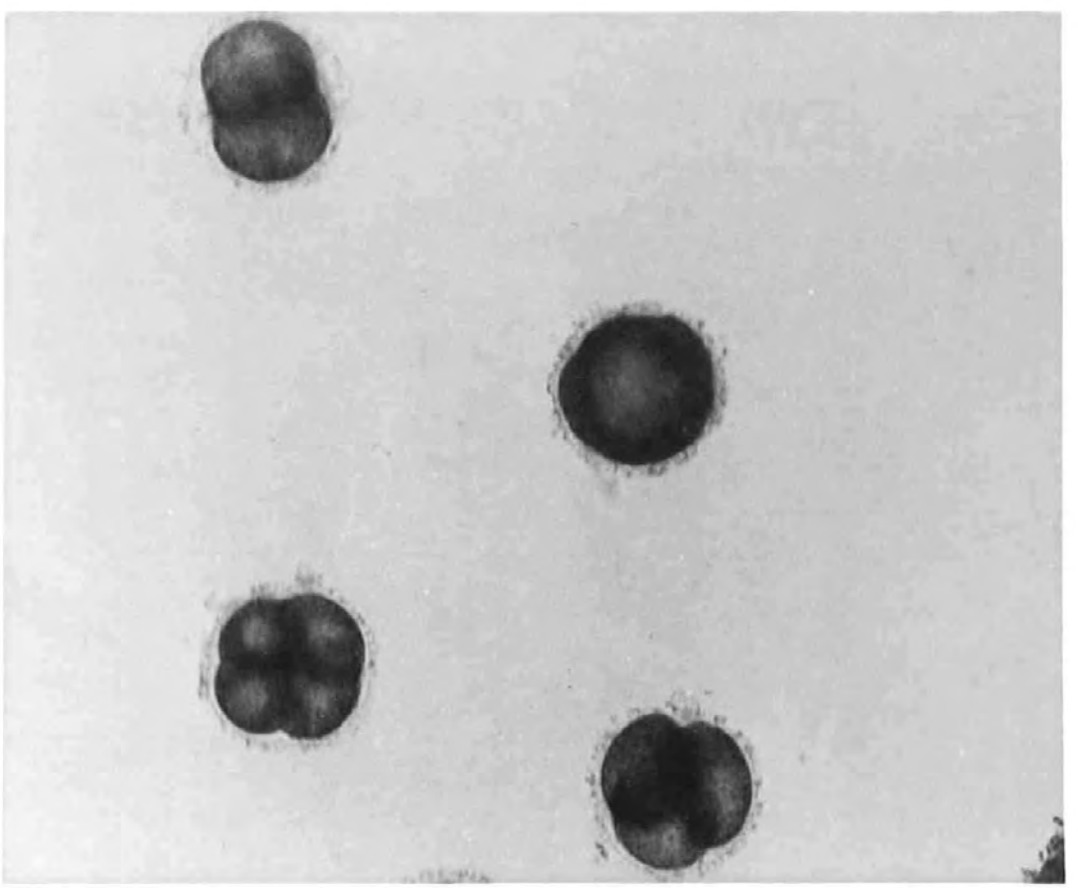


Plate 6.1.3

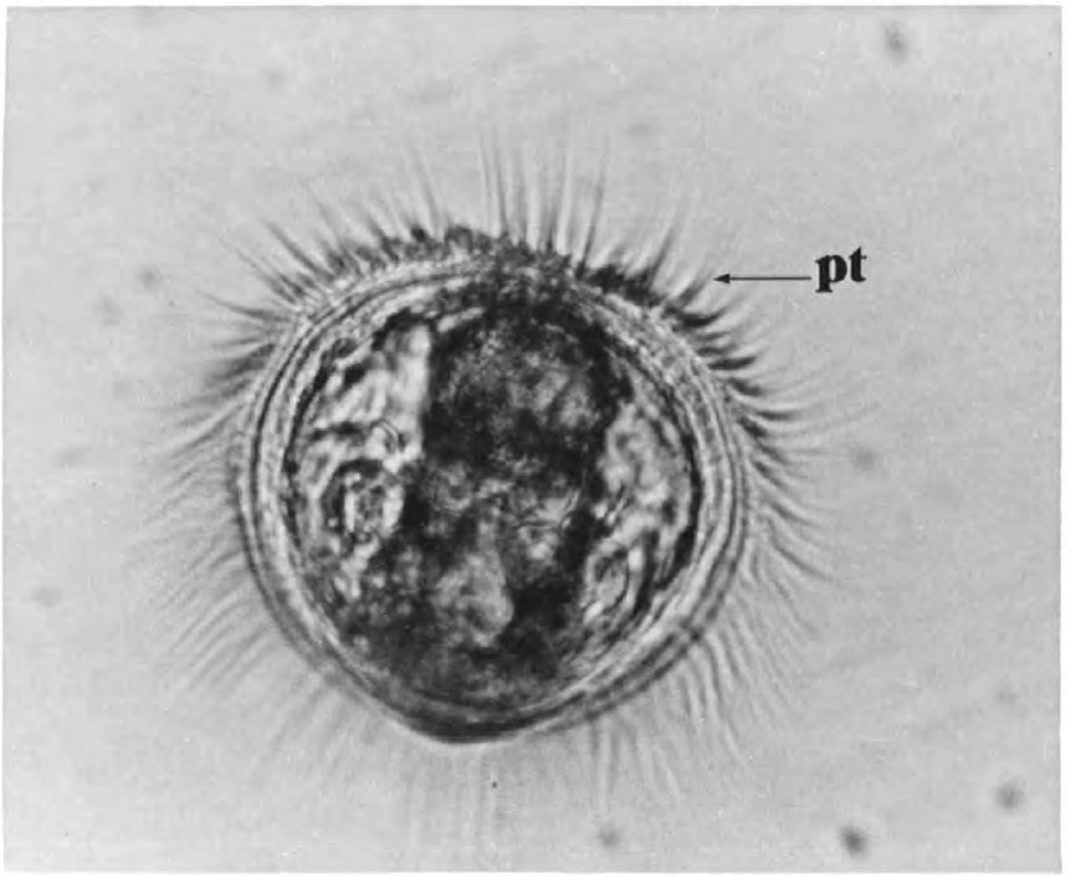
(x 800)

Larval development in *Pomatoceros triqueter*. The early trochophore larva two days after fertilisation. The highly motile larva swims through the water column using the large band of prototroch cilia (pt).

Plate 6.1.4.

(x 800)

Larval development in *Pomatoceros triqueter*. The early trochophore larva, two days after fertilisation. Posterior to the locomotory prototroch cilia (pt), lies the trunk (tr) of the abdomen. Anteriorly, the cilia of the apical tuft (at).


pt

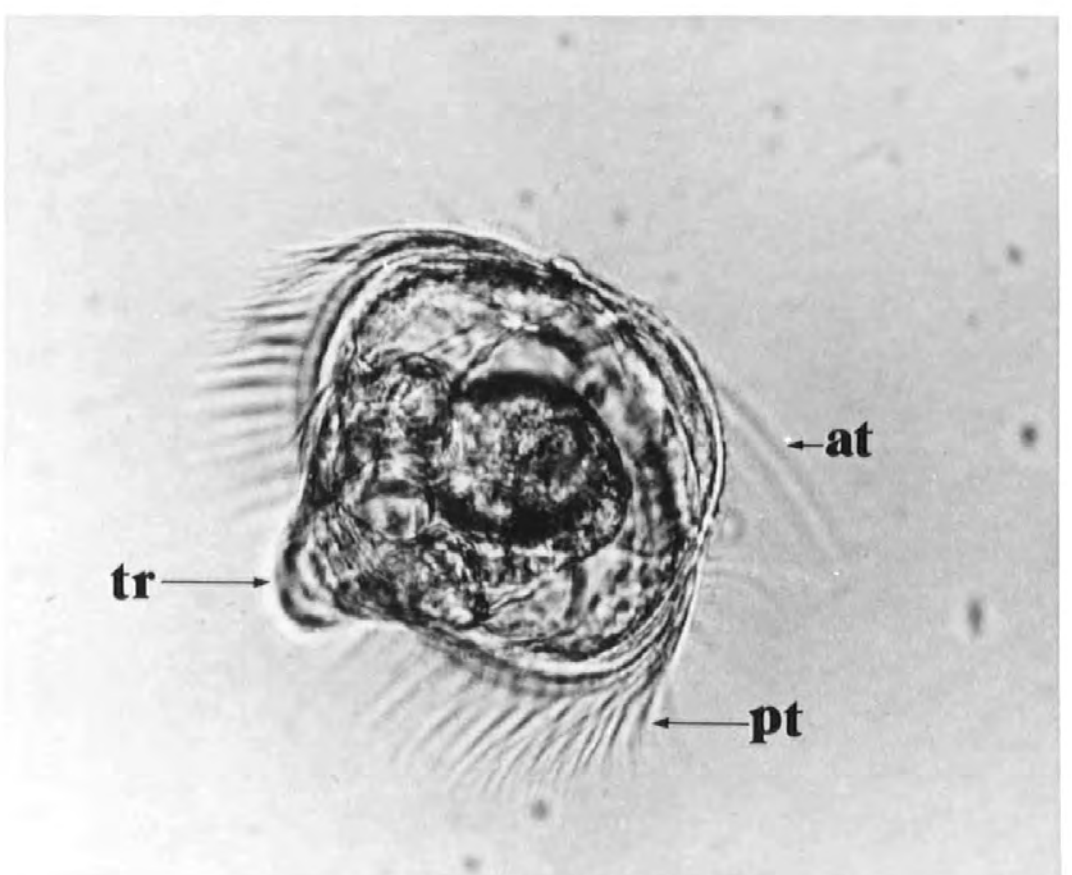

at
tr
pt

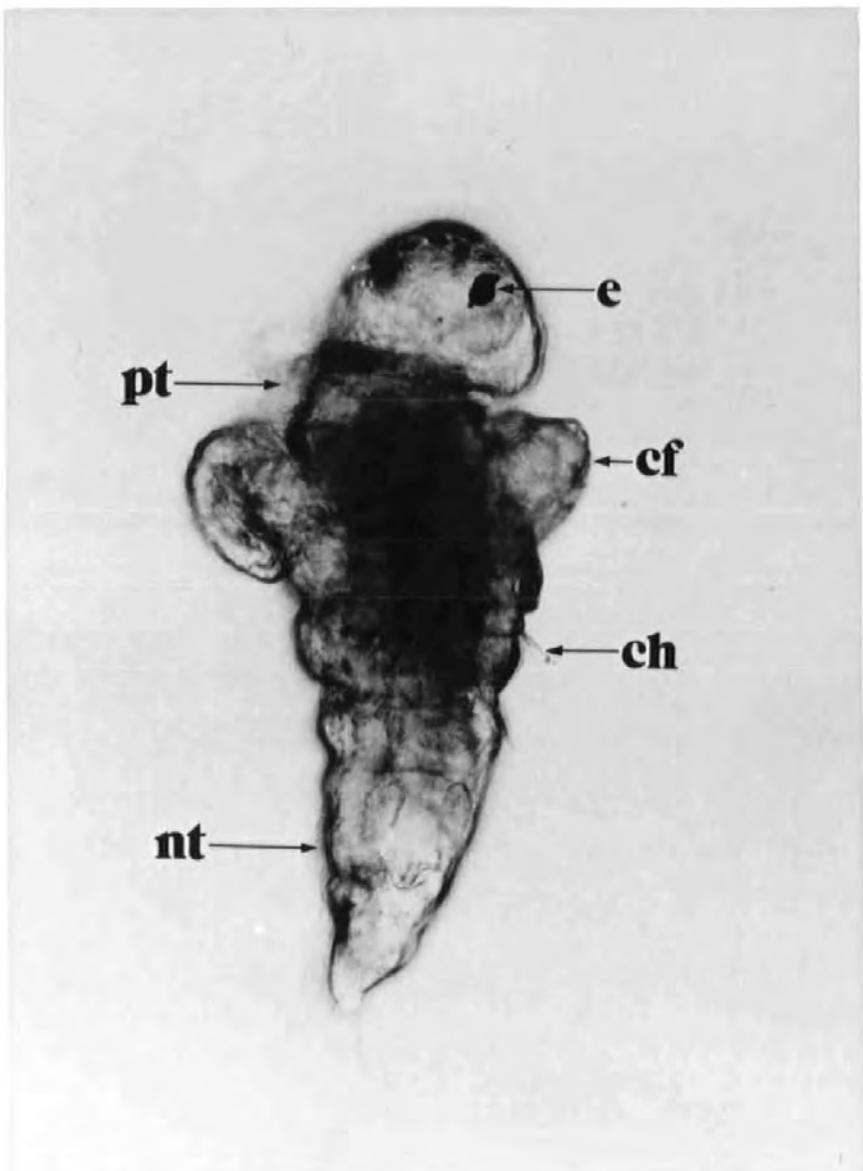
Plate 6.1.5.

(x 340)

Larval development in *Pomatoceros triqueter*. Dorsal view of the 'creeping stage' larva, immediately prior to settlement and metamorphosis. The main locomotory apparatus of the prototroch cilia (pt) is lost, although the neurotroch cilia (nt) remain. The rudimentary cellar folds (cf) are extruded.

e = eye

ch = chaetae


e
pt
cf
ch
nt

Figures 6.1.6 to 6.1.9.

Larval development in Pomatoceros triqueter. The rate of increase in larval length (um) and the effect of temperature, (at 20°C, 15°C, 10°C., and 6°C) and salinity.

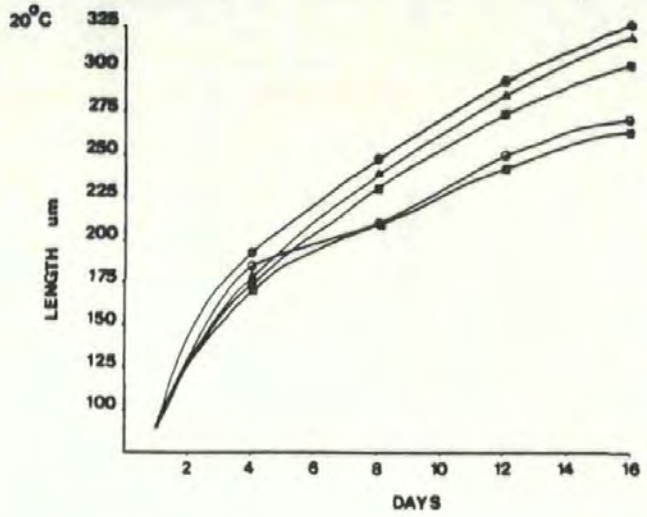
▲ = 34°/oo

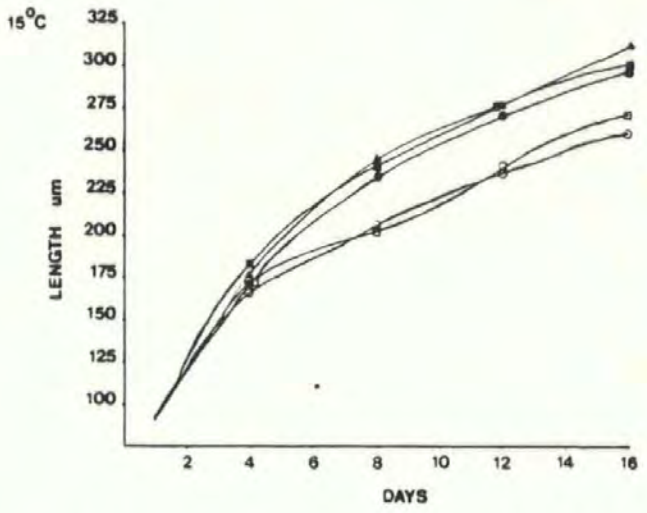
● = 30°/oo

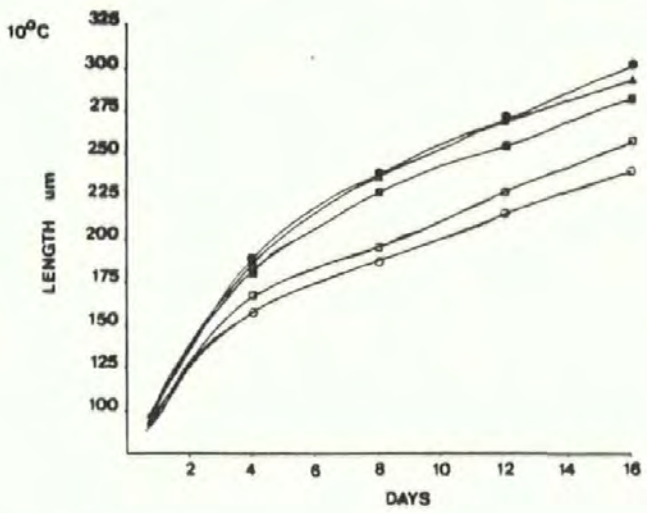
■ = 26°/oo

⊙ = 22°/oo

□ = 18°/oo


20°C
LENGTH um
325
300
275
250
225
200
175
150
125
100
2
4
6
8
10
12
14
16
DAYS


15°C
LENGTH um
325
300
275
250
225
200
175
150
125
100
2
4
6
8
10
12
14
16
DAYS


10°C
LENGTH um
325
300
275
250
225
200
175
150
125
100
2
4
6
8
10
12
14
16
DAYS

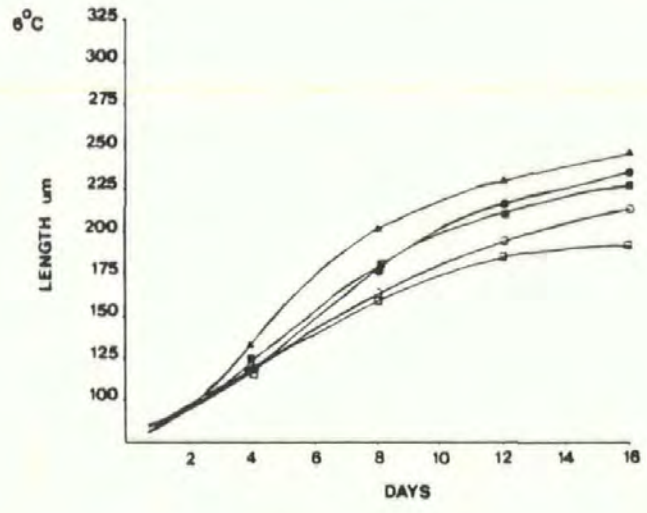

6°C
LENGTH um
325
300
275
250
225
200
175
150
125
100
2
4
6
8
10
12
14
16
DAYS

Table 6.1.10.

Larval development in Pomatoceros triqueter. Analysis of variance (two-way) table, the effects of temperature, salinity and temperature x salinity.

Table 6.1.11.

Larval development in Pomatoceros triqueter. Final mean length (um) at age 16 days, and the effect of temperature (^{o}C).

▲= 34^{o}/oo

●= 30^{o}/oo

■= 26^{o}/oo salinity

□= 22^{o}/oo

○= 18^{o}/oo

Figure 6.1.12

Larval development in Pomatoceros triqueter. Final mean length (um) at age 16 days and the effect of salinity (^{o}/oo).

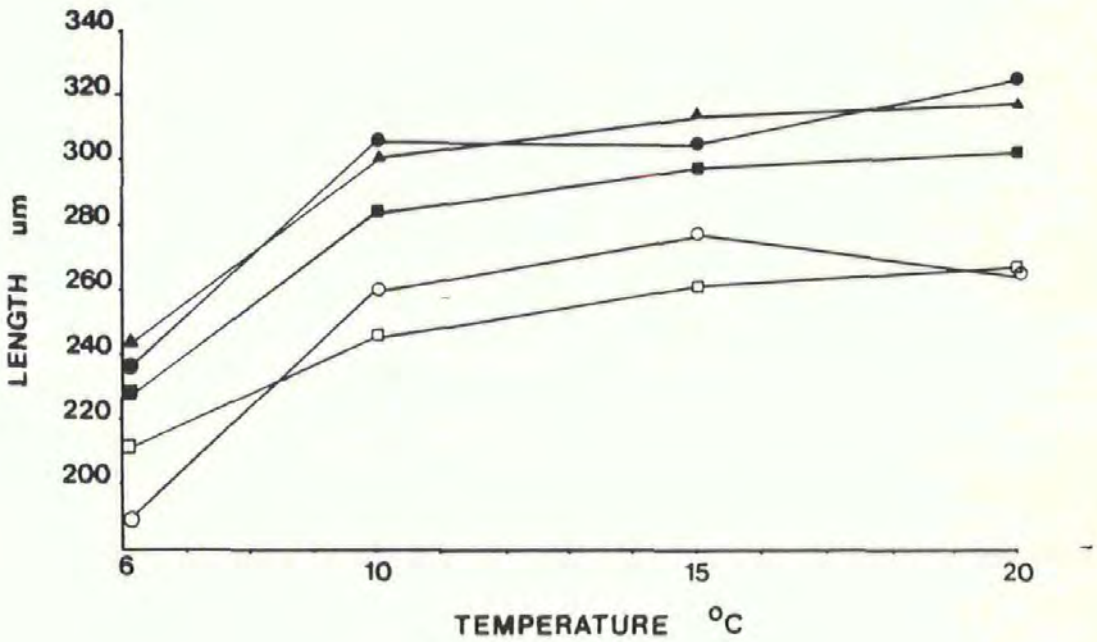
▲= 20^{o}C

●= 15^{o}C

■= 10^{o}C

△= 6^{o}C

Source	Df	SS	MS	f	P
Salinity	4	6980	1745	0.61	NS
Temperature	3	26288	8763	3.10	0.05
Interaction	12	959	80	0.02	NS
Error	80	226988	2837		
Total	99	261215			



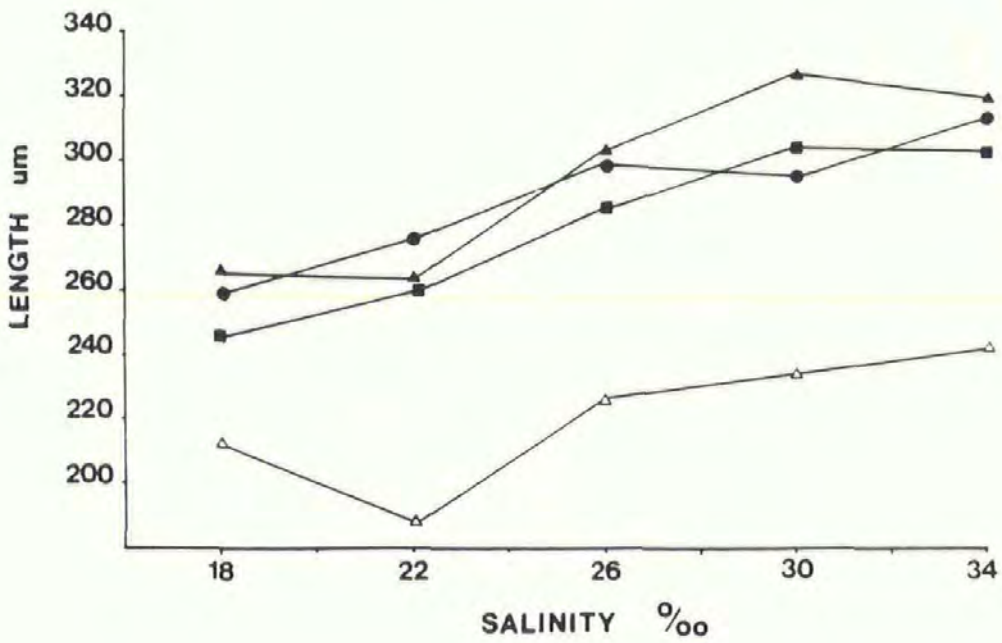


Plate 6.2.1

(x 360)

Scanning electronmicrograph of the metatrochophore larva. Distinct bands of cilia are a prominent feature of the larval body. These include the main locomotory cilia, the prototroch (pt); the apical tuft (at) cilia of the head, and the neurotroch cilia (nt) which runs in a narrow band, ventrally, to the posterior tip of the larva.

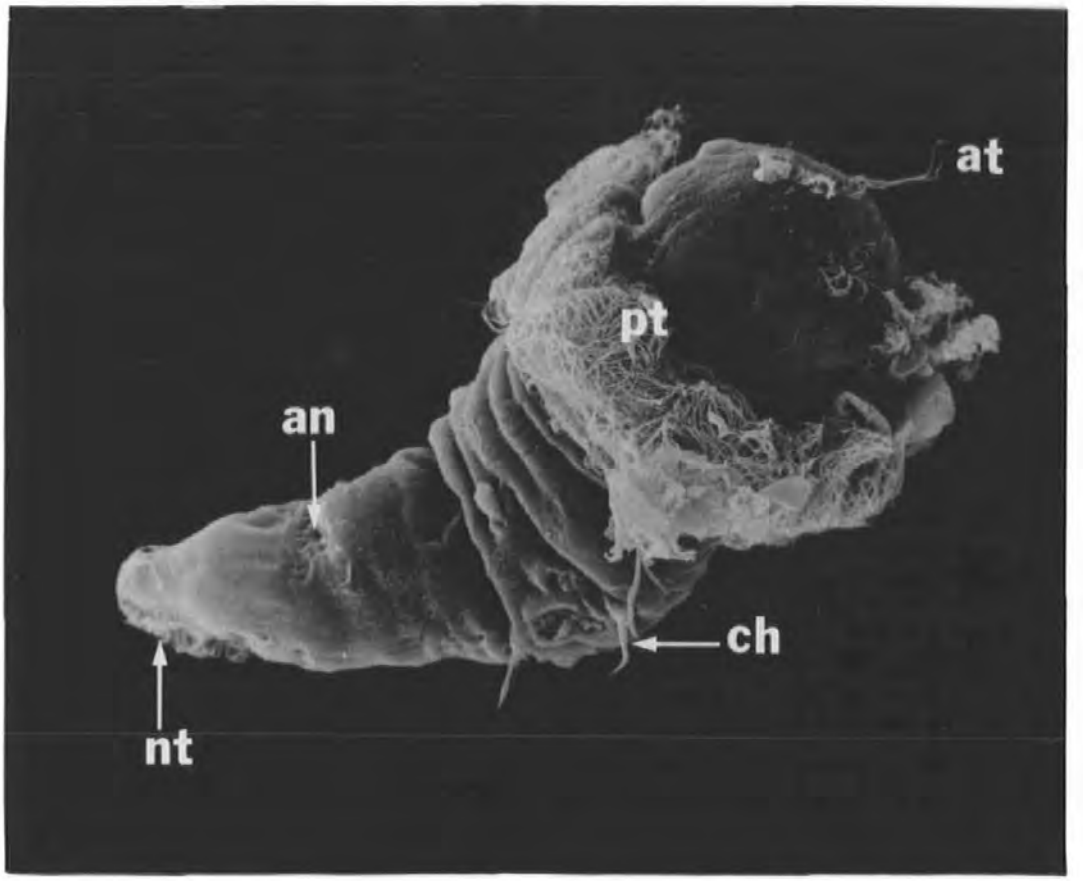
an = anus

ch = chaeta

Plate 6.2.1

(x 700)

The apical tuft (at) is seen as a stiffened bunch of cilia projecting from the head of the metatrochophore. Other cilia, in groups or singly are also present on the head, as well as numerous sunken pores (p).


at
pt
an
ch
nt

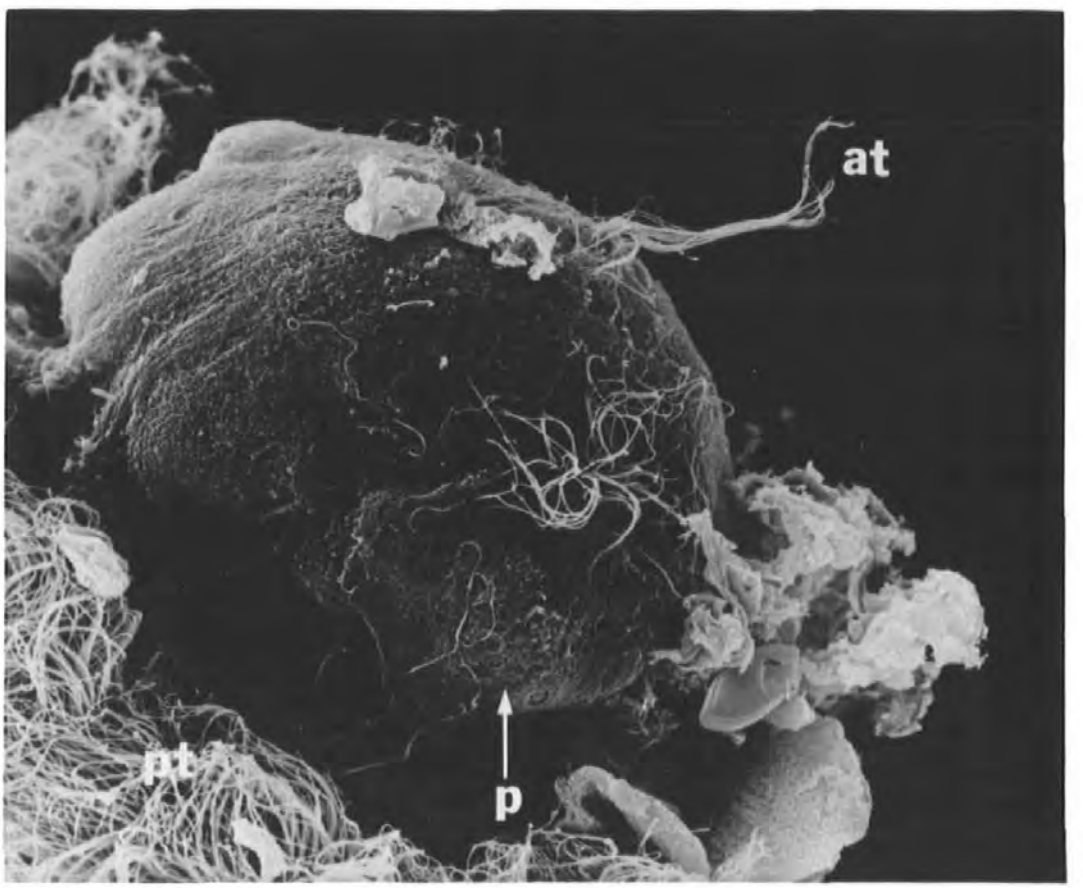

at
pt
p

Plate 6.2.3.

(x 360)

The metatrochophore larva possesses numerous surface pores (p) not only on the head, but also scattered over the trunk.

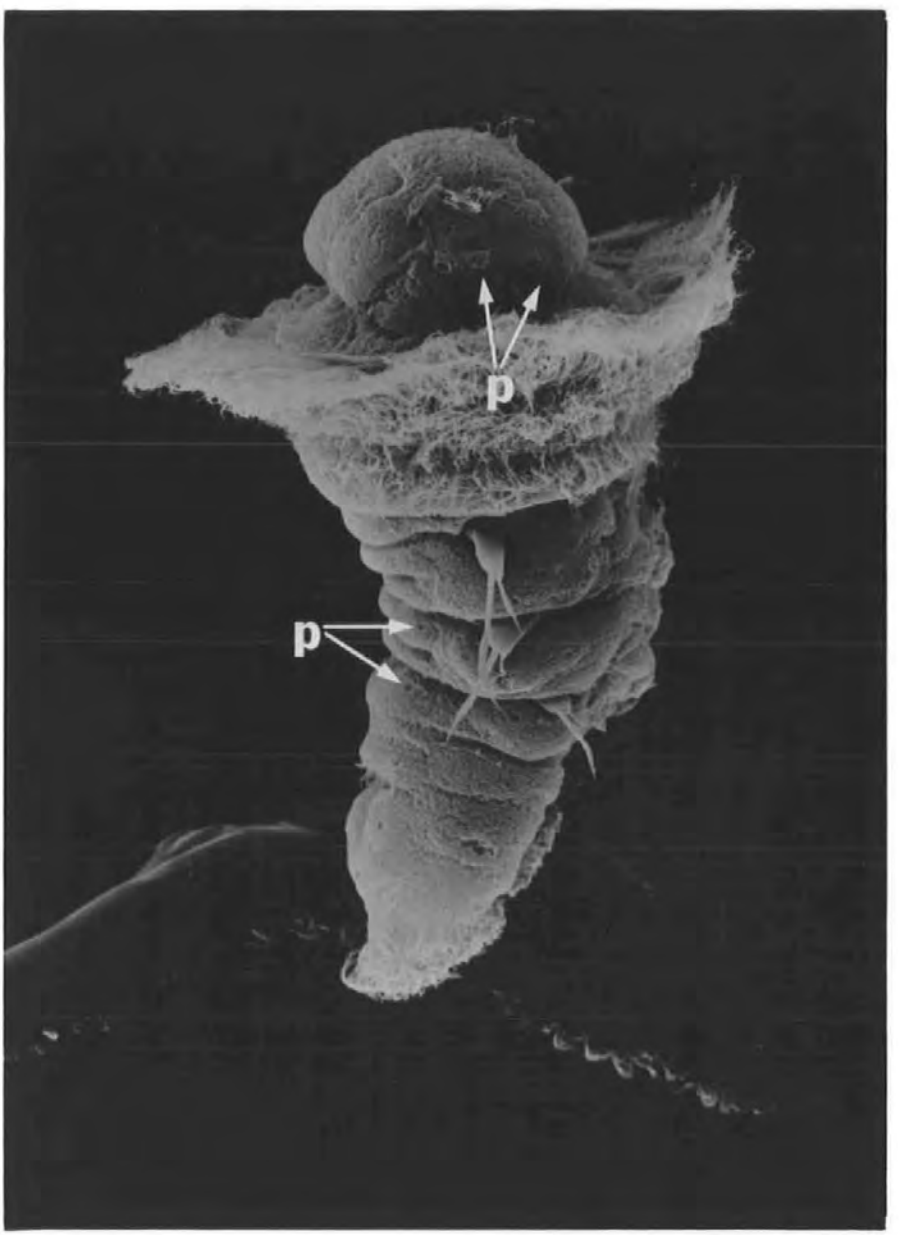

p
p

Plate 6.2.4

(x 360)

A prominent feature of the posterior tip of the abdomen of the metatrochophore larvae, is the presence of one or more anal pores (ap).

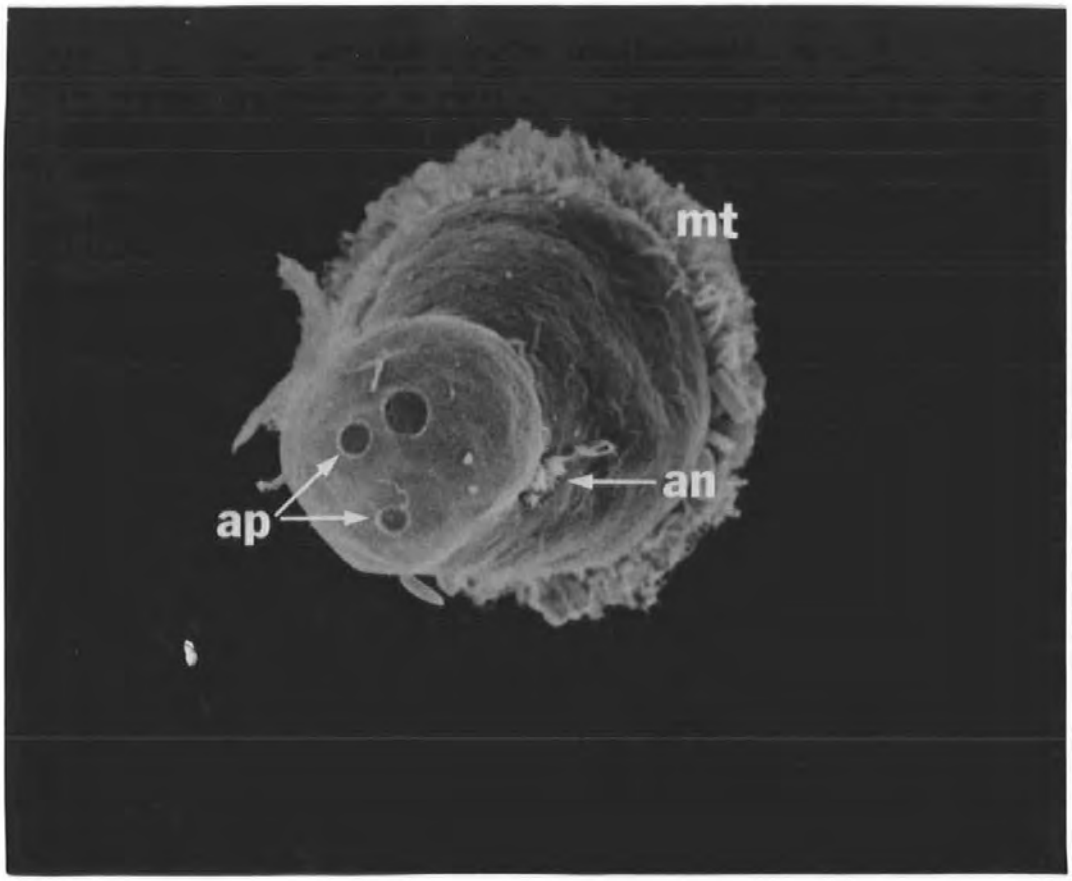
mt = metatroch cilia

an = anus

Plate 6.2.5

(x 740)

The openings of the anal pores (ap) may contain mucus-material. The anus (an) is seen dorsally, as a sunken pit.


mt
an
ap

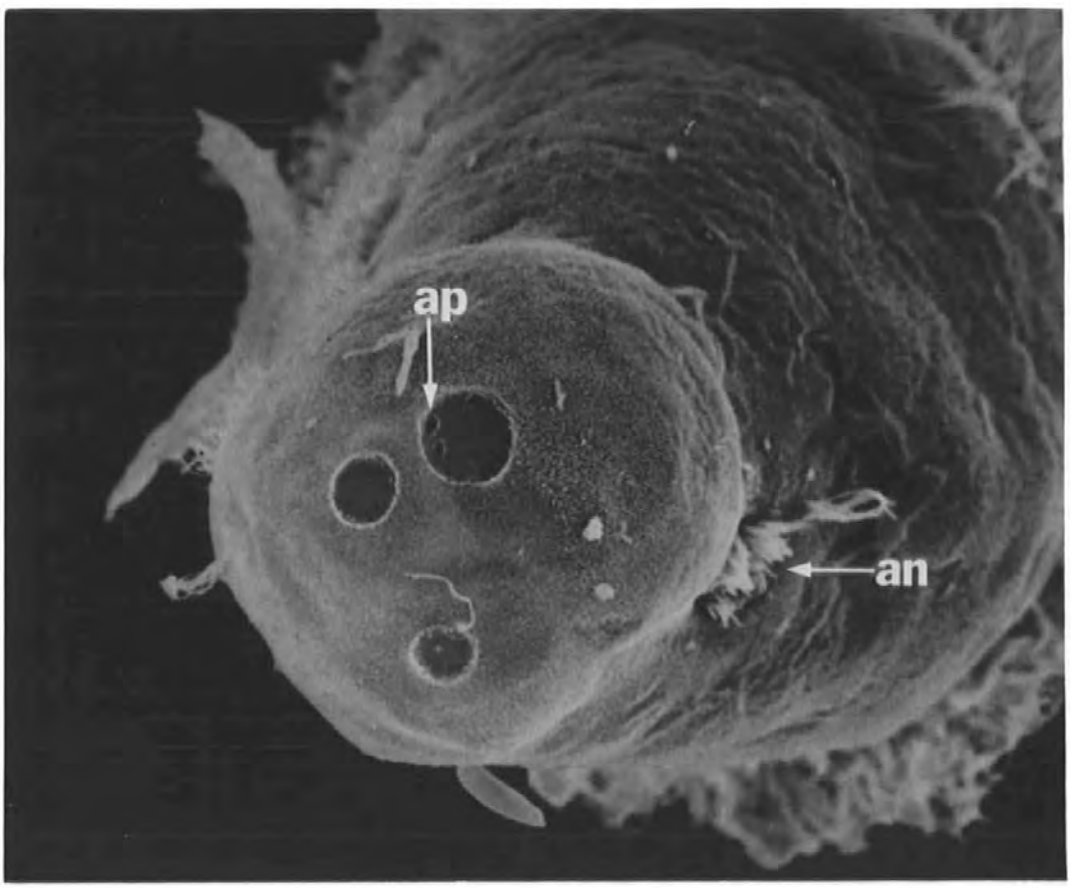

ap
an

Plate 6.2.6

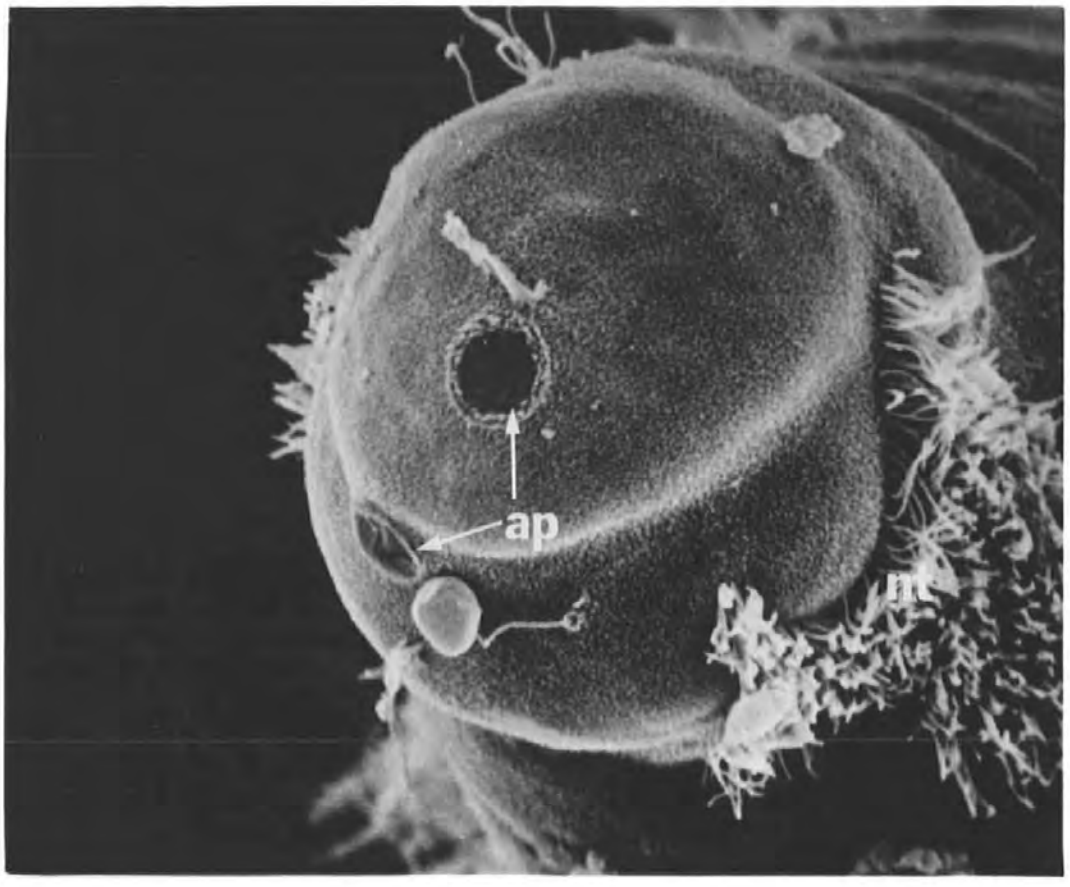
(x 740)

Scanning electronmicrograph of the posterior tip of the abdomen in the metatrochophore larva, showing mucus secretions at the openings of the anal pores (ap).

Plate 6.2.7

(x 740)

The anal pore (ap) may be raised or extend 'tube-like' from the tip of the terminal segment. Other, smaller, pores (p) may frequently be seen, opening on to the ventral surface of the trunk, close to the neurotroch cilia (nt).


ap
nt

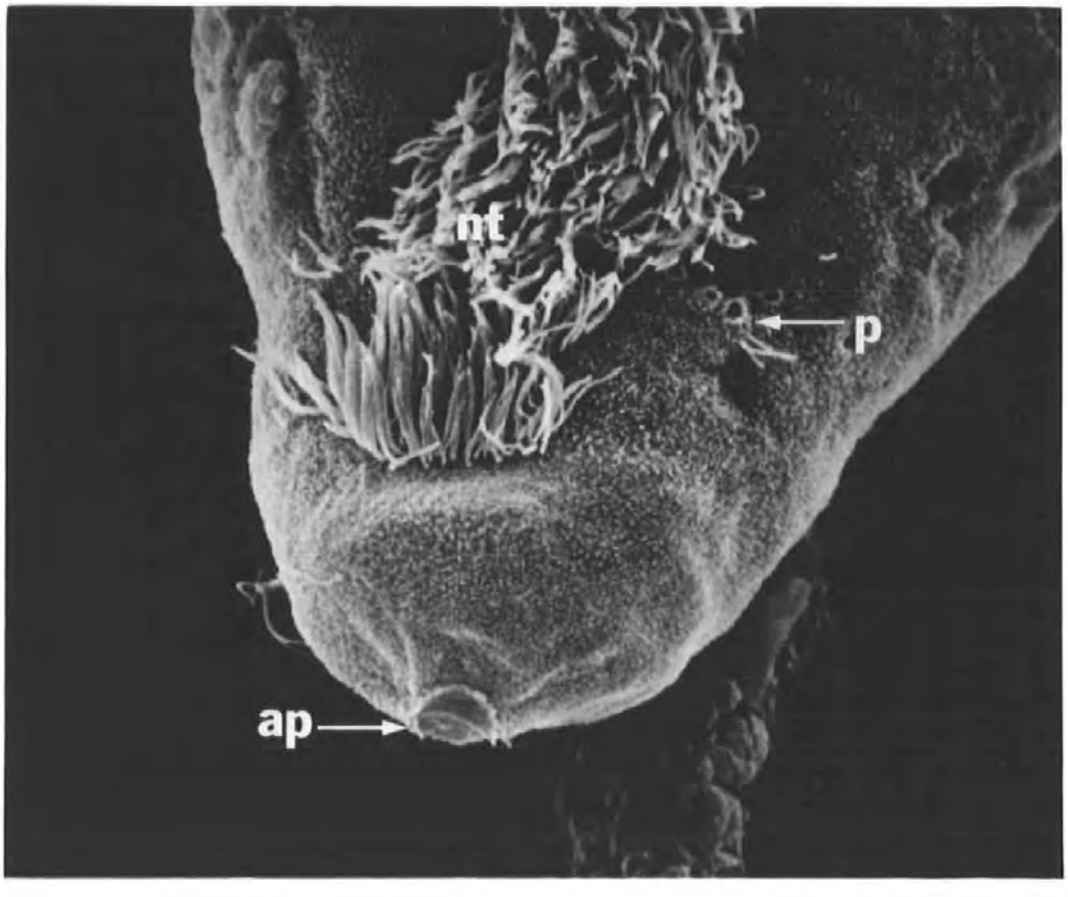

nt
p
ap

Plate 6.2.8

(x 630)

L.S. metatrochophore larva (1 um, toluidine blue), showing the distribution of cilia and mucous cell glands.

hmg = head mucous gland

lmg = lateral mucous gland

amg = anal mucous gland

at = apical tuft cilia

pt = prototroch cilia

mt = metatroch cilia

f = feeding cilia

av = anal vesicle

ap = anal pore

The highly glandular gut (g) terminates through the anus, anterior to the anal vesicle (av). The rudimentary collar folds (cf) are seen lying beneath the level of the prototroch cilia (pt).

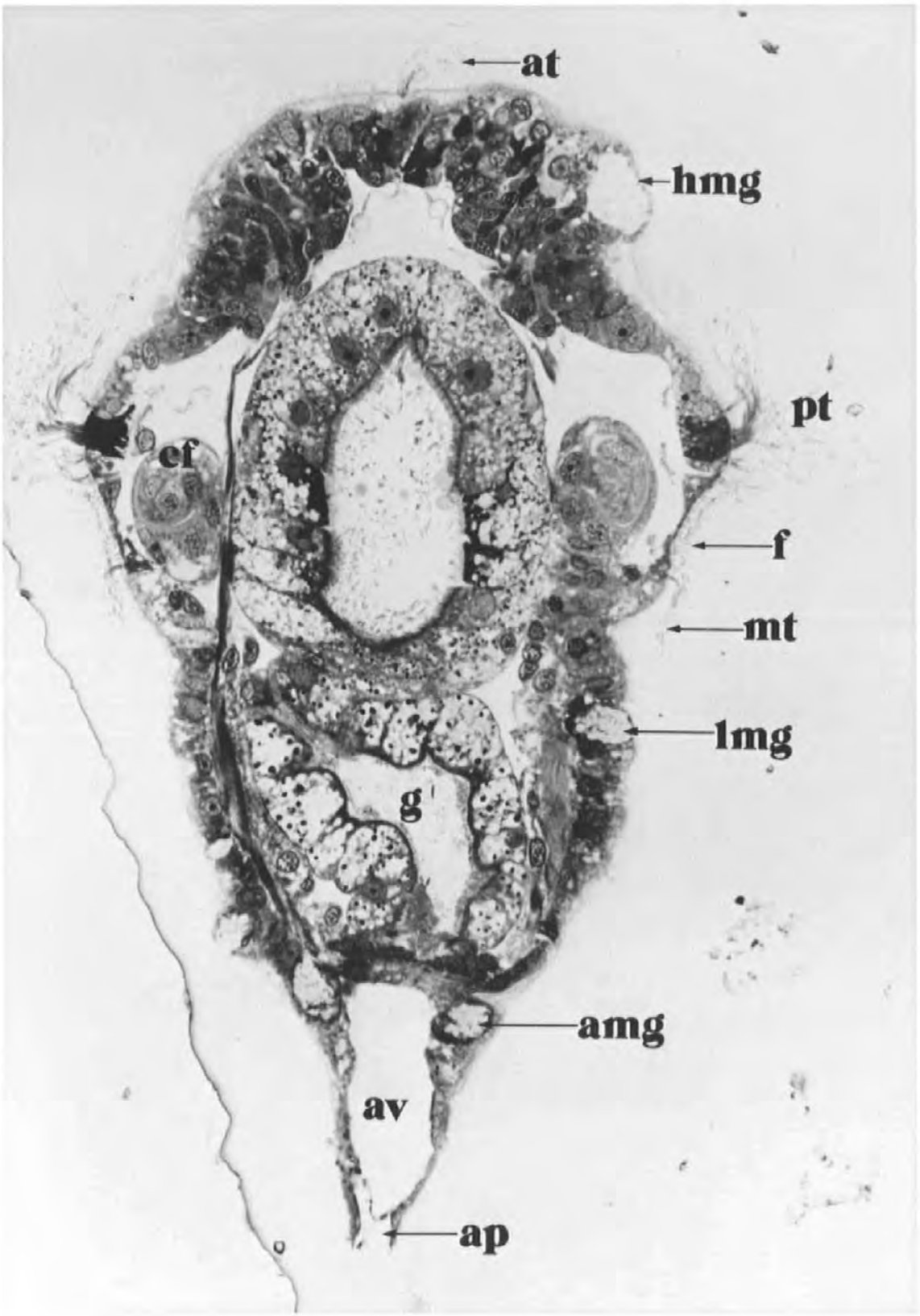

at
hmg
pt
ef
f
mt
lmg
g
amg
av
ap

Plate 6.2.9

(x 5840)

Electronmicrograph of the mature anal mucous gland (amg) in the metatrochophore larva. These large, homogeneous secretions are closely associated with the anal vesicle (av).

m = mitochondrion

ct = cuticle

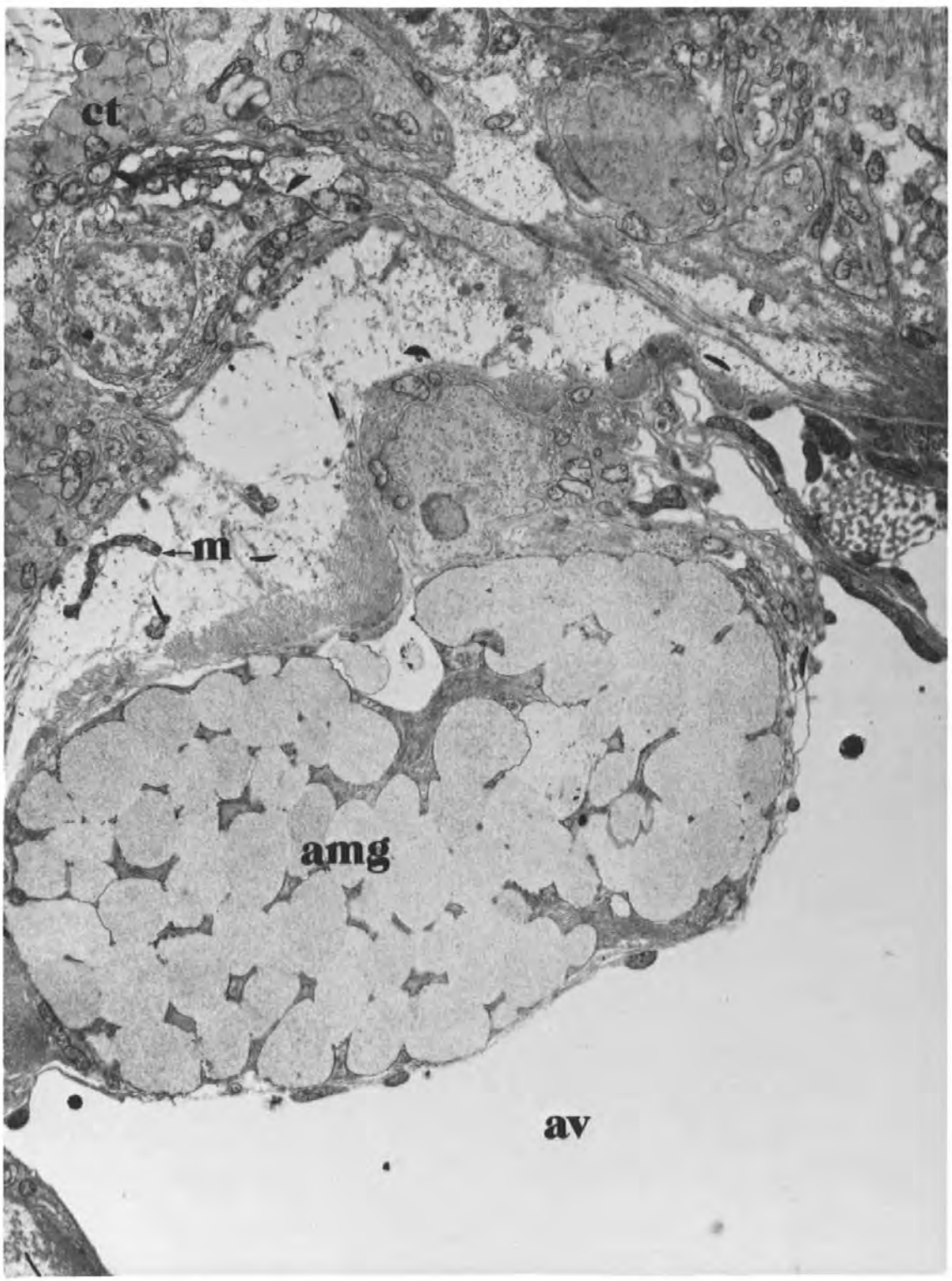

ct
m
amg
av

Plate 6.2.10

(x 470)

L.S. metatrochophore larva (1 um, toluidine blue) showing the intensely staining mass of the ventral mucous cell gland (vmg) of the ventral gland bulge. One of the rudimentary calcium secretory glands (c.s.g.) is seen beneath the level of the prototroch cilia. These paired structures are more lightly stained with toluidine blue, and often appear vacuolated.

hmg = head mucous gland

amg = anal mucous gland

av = anal vesicle

ap = anal pore

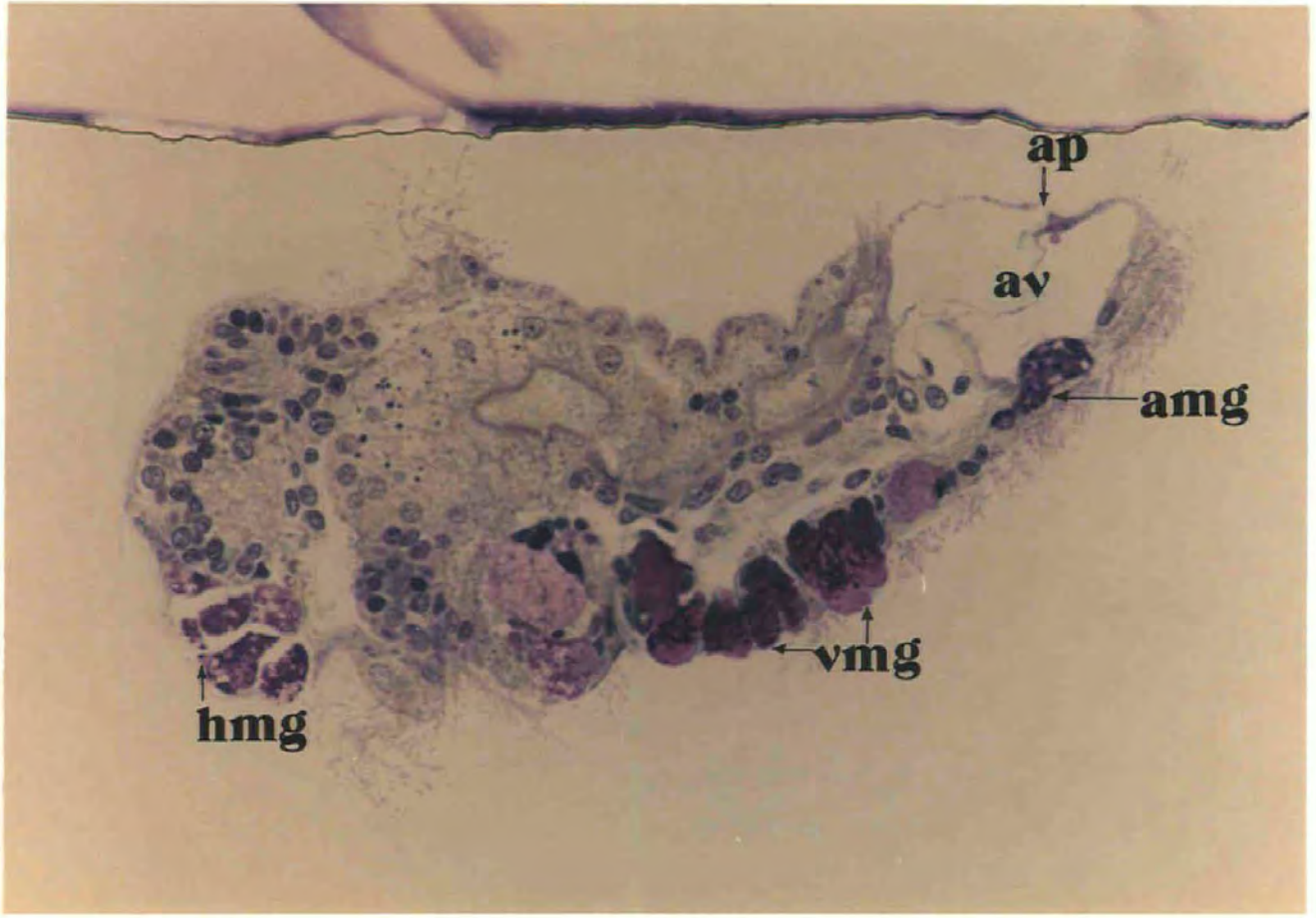

ap
av
amg
vmg
hmg

Plate 6.2.11

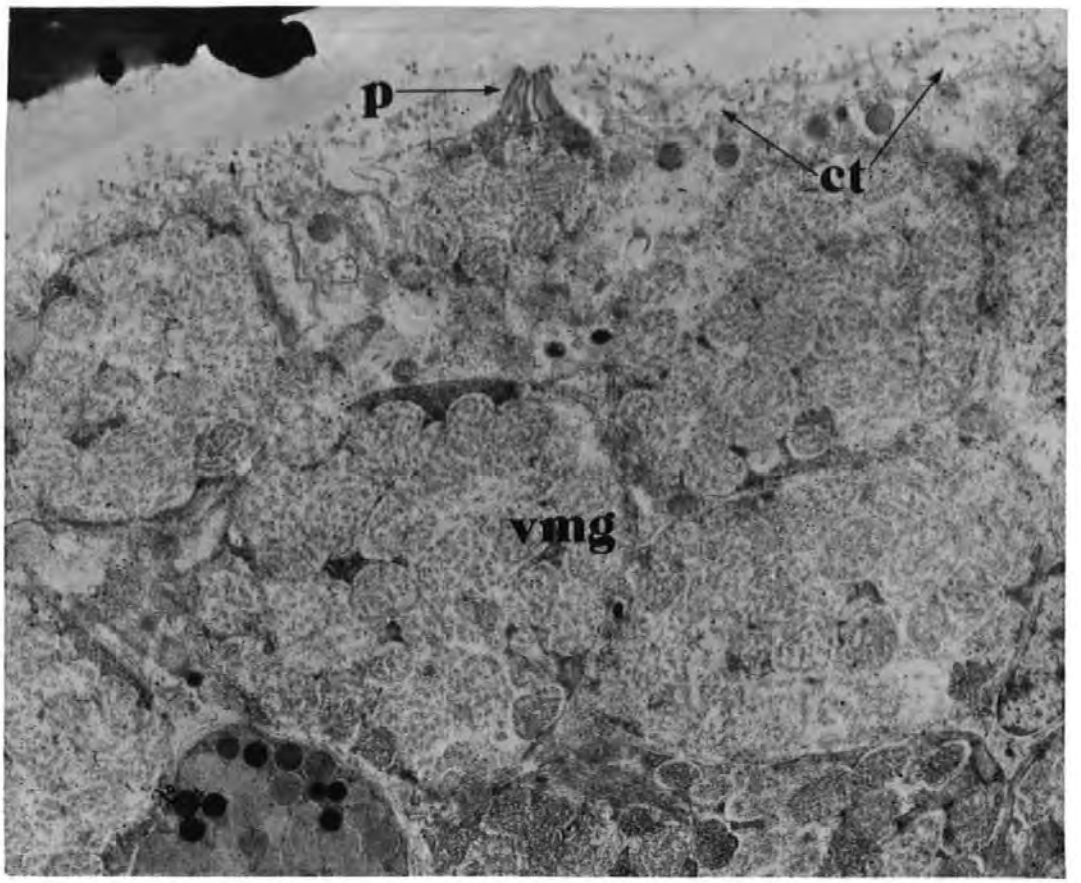
(x 2750) and

Plate 6.2.12

(x 5250)

Electronmicrographs showing the mucous cells of the ventral mucous glands (v.m.g.) The mucous cells are in various stages of maturation, and secretions appear to be liberated to the external surface of the metatrochophore larva via microvilli-bound pores (p).

ct = cuticle.


p
ct
vmg

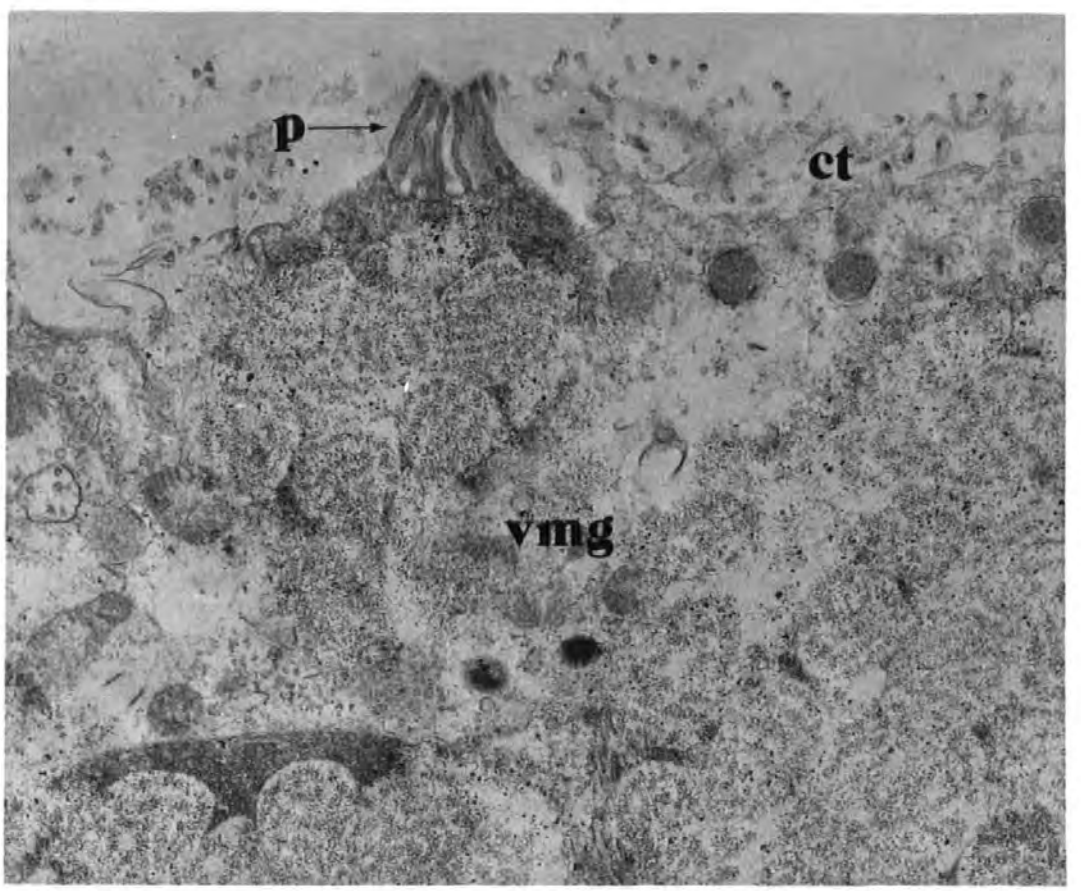

p
ct
vmg

Plate 6.2.13

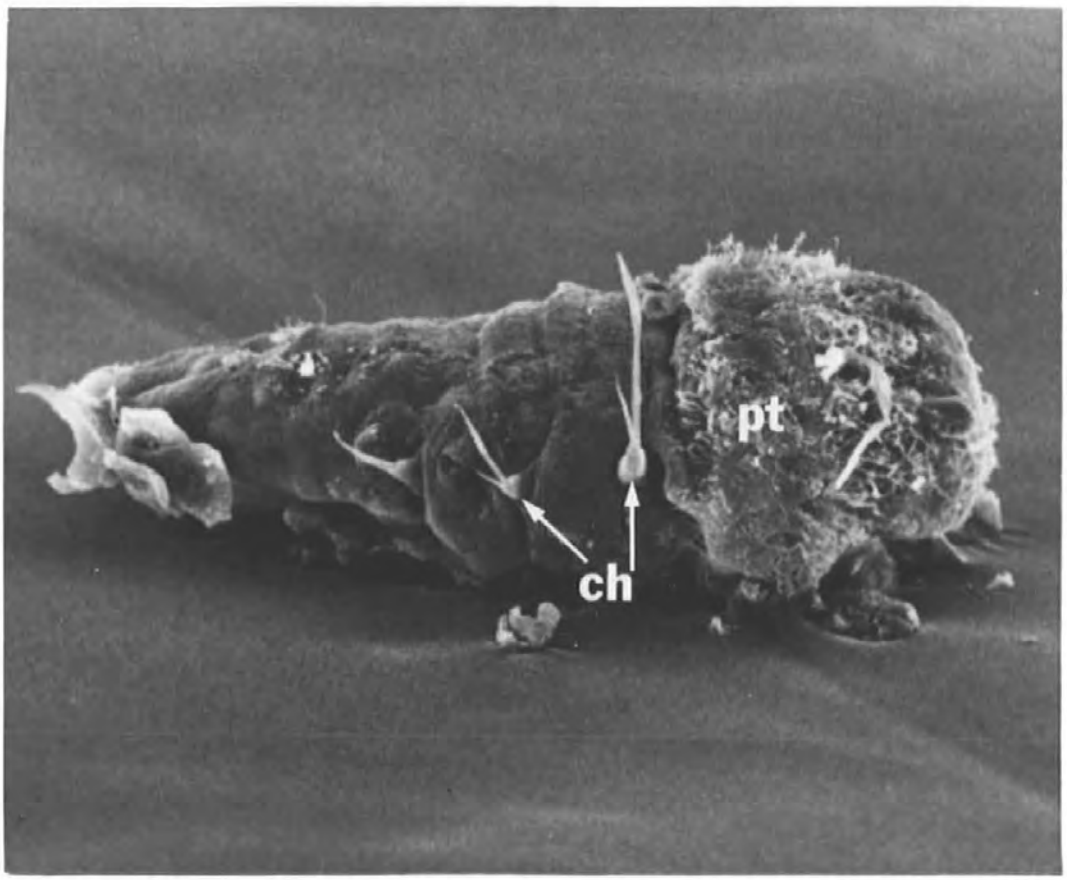
(x 360)

Immediately prior to settlement attachment and metamorphosis, the locomotory prototroch cilia (pt) are lost and the metatrochophore develops into the 'creeping stage' larva.

Plate 6.2.14

(x 120)

Scanning electronmicrograph of the developing tube-stage worm. The remains of the initial mucoid-tube (mt) and its attachment to the substrate can be seen. Anteriorly, the tube becomes calcareous (ct).


pt
ch

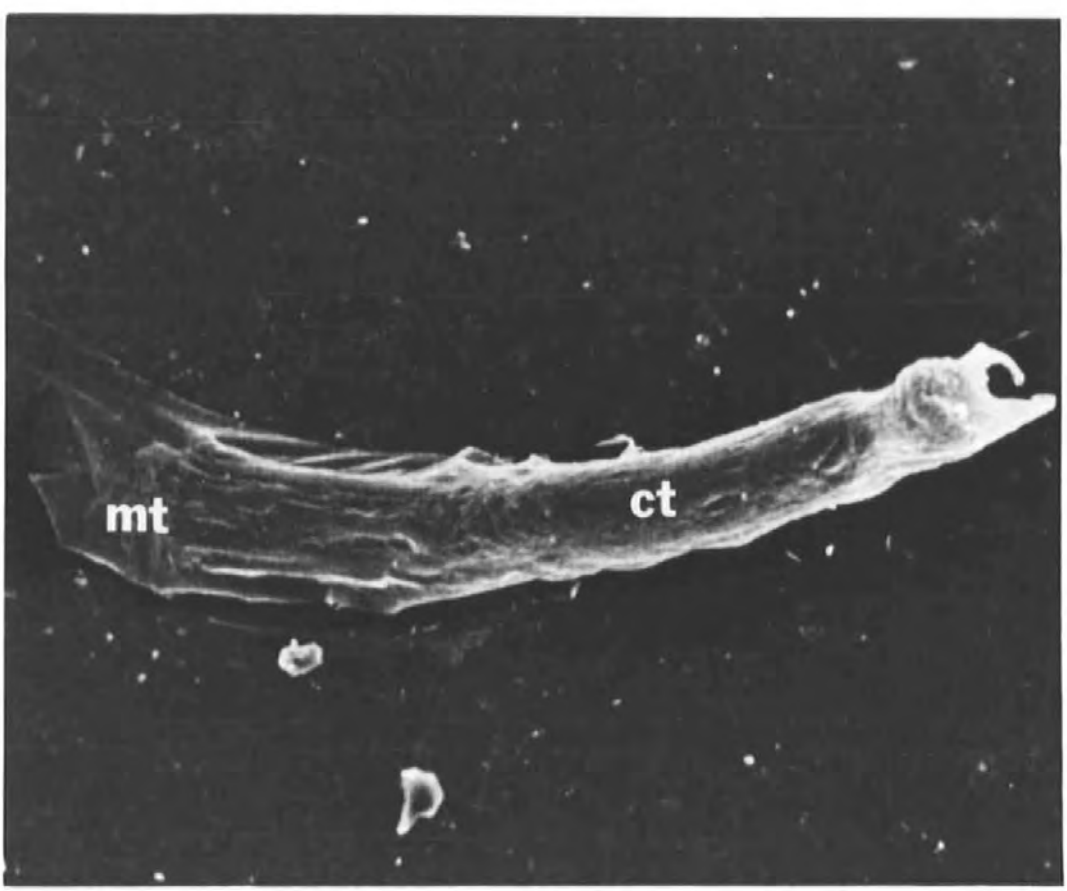
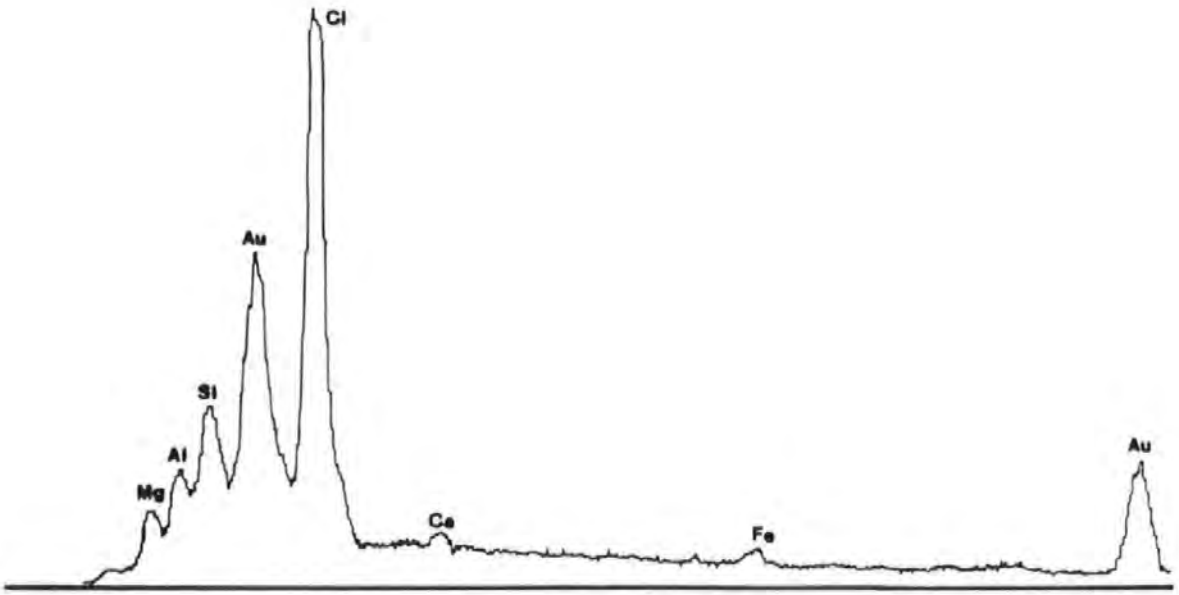

mt
ct

Figure 6.2.15.

Settlement in Pomatoceros triqueter.

(a) X-ray spectrum (0-10 KeV) of the posterior, mucoid, tube (mt) of the recently settled individual in Plate 6.4.14. Note the small Kα calcium peak at 3.68 eV. Peaks for Kα magnesium (1.26 eV), Kα aluminium (1.49 eV), Kα silicon (1.74 eV), and Kα iron (6.41 eV) are also evident.

(b) X-ray spectrum (0-10 KeV) of the anterior, calcareous, tube (ct) of the recently settled individual in Plate 6.4.14. Note the large Kα calcium peak at 3.68 eV and the secondary Kβ peak at 4.01 eV. Peaks for Kα silicon (1.74 eV), Kα iron (6.41 eV), Kα copper (8.90 eV) and Kα zinc (9.12 eV), are also evident.


Cl
Au
Si
Al
Mg
Ca
Fe
Au

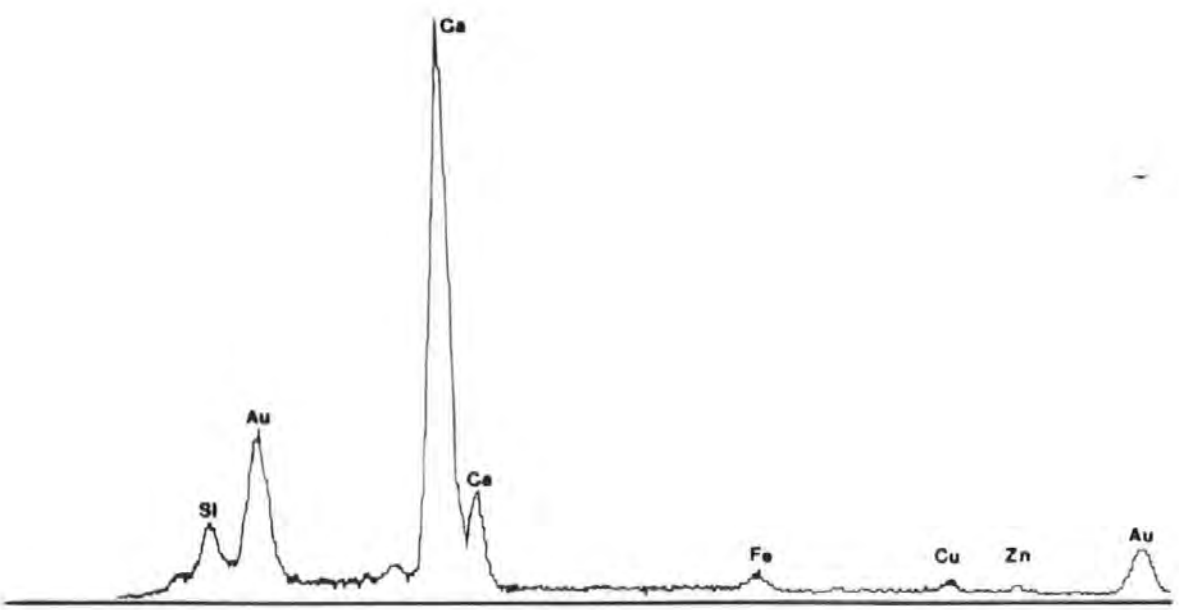

Ca
Au
Si
Ca
Fe
Cu
Zn
Au

Plate 6.2.16

(x 420)

Older tube-stage individual showing the posterior end of the secondary, calcareous tube (ct) which has now almost closed over. The initial, or primary, mucoid tube is lost although the remains of a mucoid attachment (ma) to the substrate can be seen.

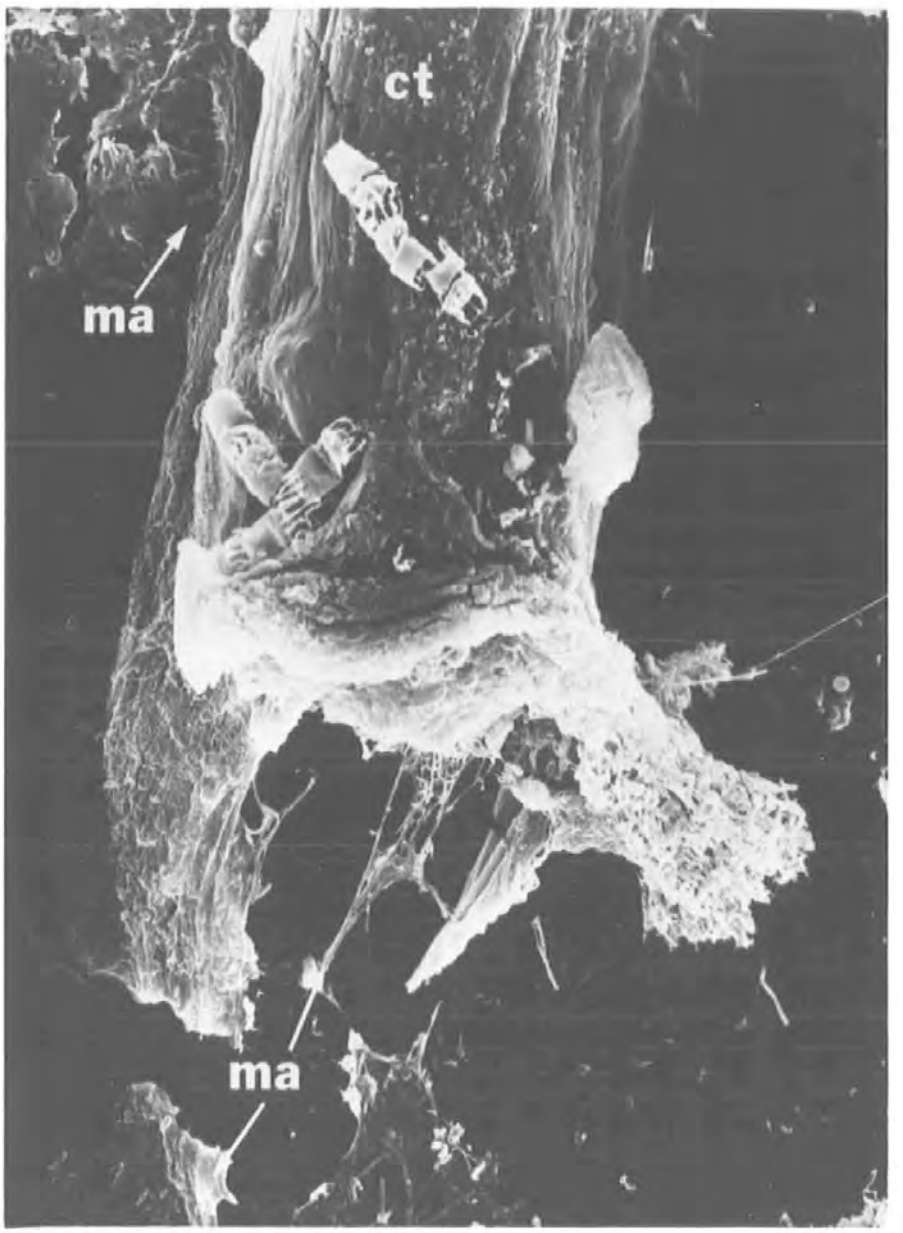

ct
ma
ma

Table 6.3.1.

Settlement in Pomatoceros triqueter.

Staining reactions of the mucous cell glands and anal vesicle in the metatrochophore larva, to histochemical tests for mucins, proteins and lipids.

+++	=	strong reaction
++	=	moderate reaction
+	=	weak reaction
-	=	negative

Tests	Head m.g.	Ventral m.g.	Dorsal m.g.	Lateral m.g.	Anal m.g.	Anal vesicle
Carbohydrates						
PAS	++	+++	+++	+++	+++	+
Lead-tetra acetate Schiff	++	+++	+++	++	+++	+
AB 1.0	+	++	++	++	++	+
AB 2.5	+	+	++	++	+++	+
AB 5.7/C.E.C.						
0.1M $MgCl_2$	++	+	++	++	++	+
0.2M	++	-	++	-	++	+
0.5M	-	-	++	-	++	+
0.6M	-	-	-	-	+	+
0.8M	-	-	-	-	-	-
1.0M	-	-	-	-	-	-
PA-LID-AB	++	++	-	++	+	-
LID-AB	++	++	+	+	+	-
PA-HID-AB	+	-	++	+	++	+
HID-AB	+	-	+++	+	+++	+
AB-AY	++	-	-	+	-	-
Proteins						
Bromophenol blue	-	+	-	-	-	-
Millons	-	-	-	-	-	-
Lipids						
Sudan Black	-	-	-	-	-	-

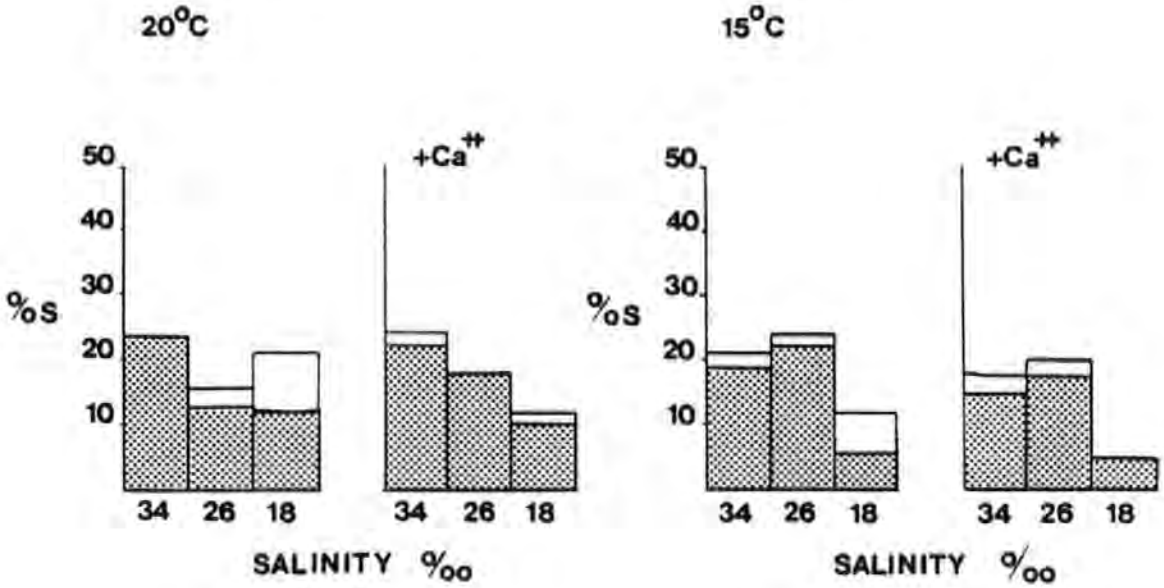
Figure 6.4.1.

Settlement in Pomatoceros triqueter.

Mean % settlement and the effect of temperature, salinity and environmental calcium concentration

▩ = settlement at day 18

□ = settlement at day 21.


20°C
15°C
%S
50
40
30
20
10
+Ca++
34 26 18
SALINITY ‰

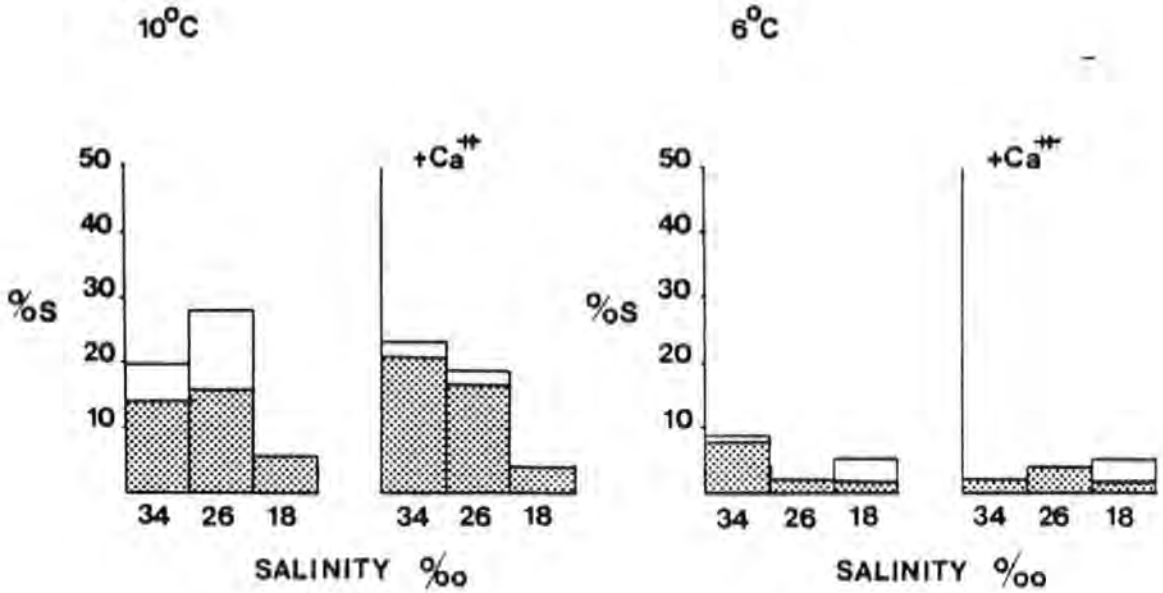

10°C
6°C
%S
50
40
30
20
10
+Ca++
34 26 18
SALINITY ‰

Table 6.4.2.

Settlement in Pomatoceros triqueter.

Mean % settlement and the effect of temperature and salinity. Settlement by day 18 and day 21 after fertilisation.

Table 6.4.3.

Settlement in Pomatoceros triqueter.

Mean % settlement and the effect of temperature, salinity and calcium level. Settlement by day 18 and day 21 after fertilisation.

‰ S

°C	34		26		18	
20	24	-	13	3	12	9
15	19	2	22	2	6	6
10	14	6	16	12	6	-
6	8	1	-	2	2	3

‰ S + Ca^{2+}

°C	34		26		18	
20	22	2	18	-	10	2
15	15	3	18	2	5	-
10	21	2	17	2	4	-
6	2	-	4	-	2	3

Figure 6.4.4.

Laboratory settlement in Pomatoceros triqueter.

Mean % settlement at day 18 and day 21, and the effect of light.

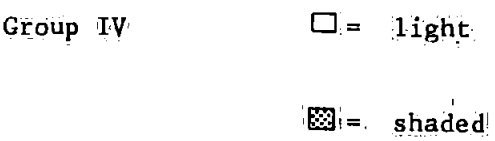
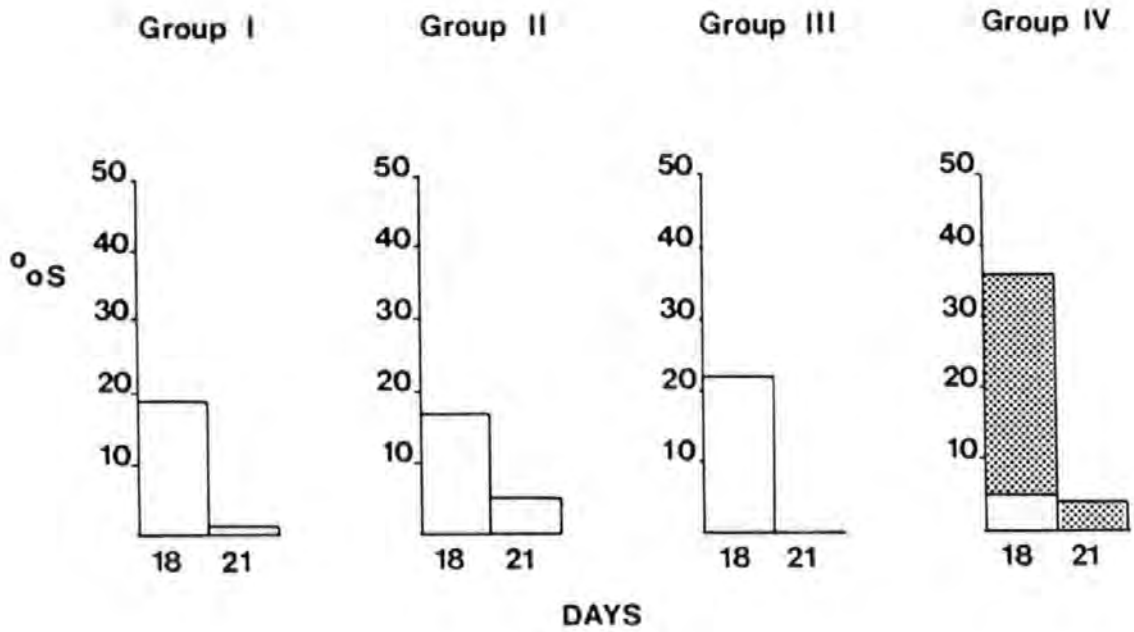


Table 6.4.5.

Laboratory settlement in Pomatoceros triqueter.

Mean % settlement and the effect of light.



	Day 18	Day 21
Group I (continuous light)	19	1 -
Group II (continuous darkness)	17	5
Group III (continuous light continuous dark day 16 - 21)	22	-
Group IV (12 hour daylength to day 16. Settlement tank ½ light; ½ shaded)	5/31	-/4

Table 6.4.6.

Settlement in Pomatoceros triqueter.

The rate of settlement and the effect of resident *Pomatoceros*.

No. settled *Pomatoceros*.

Experimental Slates.	Control slates	Total	% Total on experimental slates	No. Residents
30	2	32	94.0	6
16	-	16	100.0	4
17	-	17	100.0	8
12	3	15	80.0	10
22	6	28	78.5	9
18	-	18	100.0	8
9	1	10	90.0	5
12	4	16	75.0	6
15	1	17	88.0	6
19	-	19	100.0	4
10	4	14	71.5	9
12	-	12	100.0	10
13	3	16	81.0	4
17	1	18	94.0	6
18	6	24	75.0	8
23	12	35	65.5	8
9	-	9	100.0	5
12	4	16	75.0	6
284	47	331		

Figure 6.4.7.

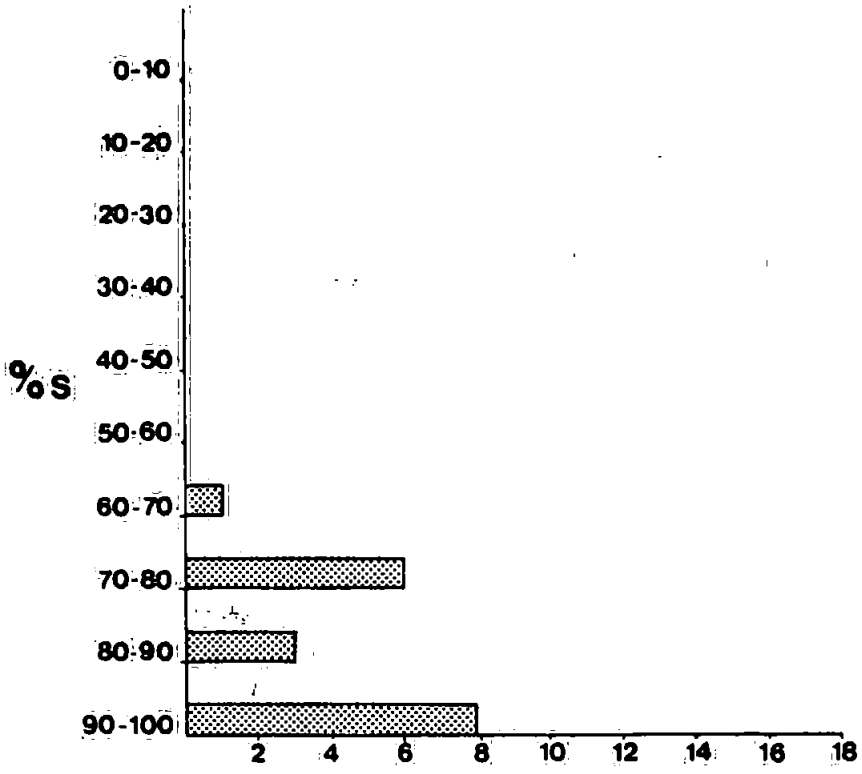
Settlement in Pomatoceros triqueter.

The % successful settlement and the effect of resident *Pomatoceros*. Results from 18 paired slate sets. The vertical axis shows the % of total settlement occurring on the experimental slates, and the horizontal axis shows the number of times the indicated % settlement occurred.

Table 6.4.8.

Settlement in Pomatoceros triqueter.

The Mann-Whitney statistic on data in Table 6.4.6.



Mann-Whitney Statistic

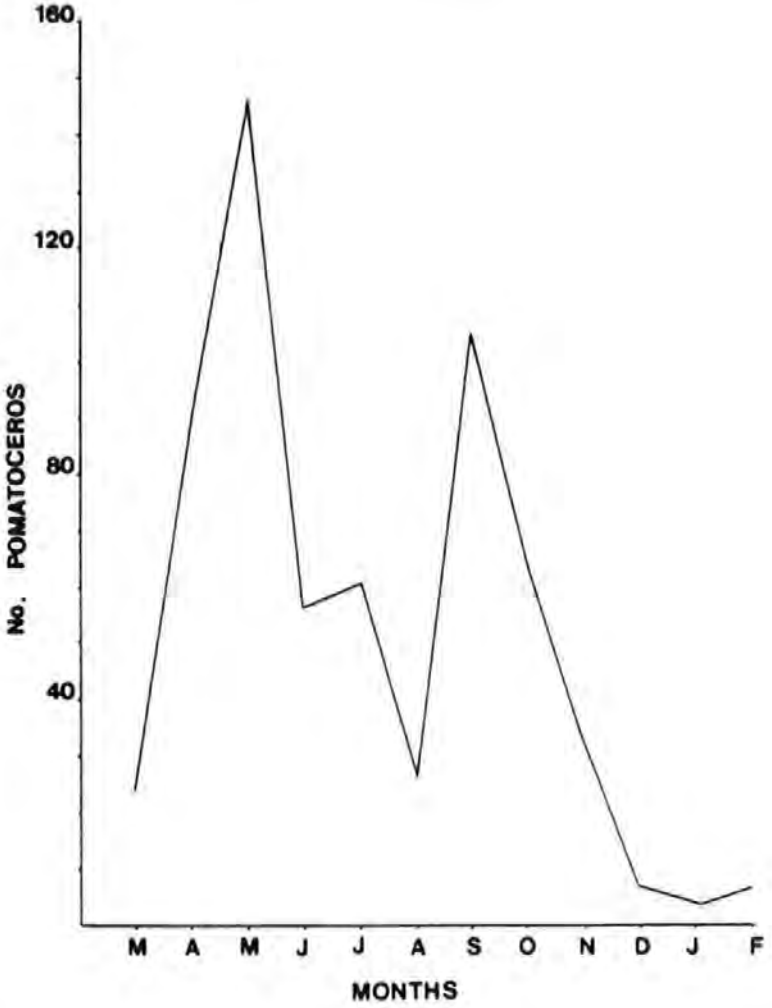
W	P
490.0	< 0.001

Figure 6.5.1.

Field Settlement in Pomatoceros triqueter. The seasonal pattern of settlement at the turtle raft field study.

Table 6.5.2.

Field Settlement in Pomatoceros triqueter. Turtle raft study: physical data, (from March, 1979 to February, 1980) temperature (^{o}C) and salinity (^{o}/oo) at high tide and low tide.



	Salinity (^{o}/oo)		Temperature (^{o}C)	
	High	Low	High	Low
March	33.8	29.6	7.5	7.3
April	32.6	30.1	8.5	7.6
May	31.9	30.6	11.8	10.6
June	33.4	32.0	13.6	12.4
July	33.5	32.8	14.6	13.8
August	34.0	32.9	15.5	15.0
September	32.1	30.8	15.8	14.7
October	33.4	31.6	15.4	14.6
November	32.8	30.1	13.6	13.7
December	32.7	29.4	10.3	10.8
January	33.6	29.9	7.3	7.2
February	33.4	29.2	7.1	7.8

Figure 6.5.3.

Field settlement in Pomatoceros triqueter. Pattern of settlement in light zones A, B and C at the turtle raft field study.

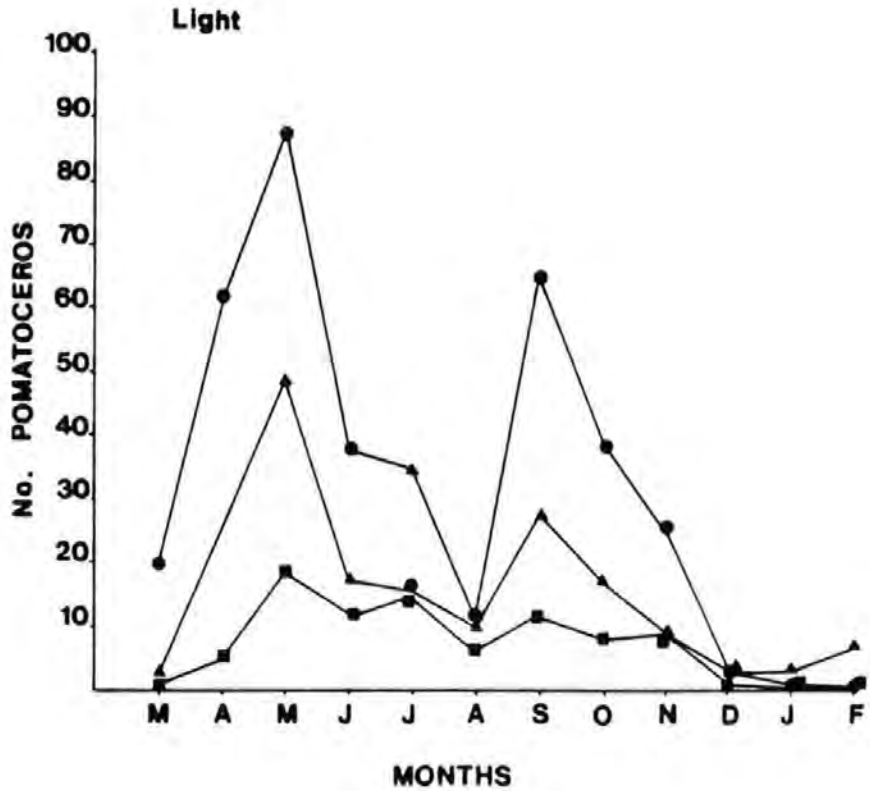
■ = Light zone A

▲ = Light zone B

● = Light zone C

Table 6.5.4.

Field settlement in Pomatoceros triqueter. Analysis of variance table (one-way) and the effect of light.



Source	Df	SS	MS	f	P
Light	2	3568	178	5.80	0.01
Error	33	10152	301		
Total	35	13721			

Figure 6.5.5.

Field settlement in Pomatoceros triqueter. The pattern of settlement on experimental substrates at the turtle raft field study.

▲ = wood

● = rough perspex

■ = smooth perspex

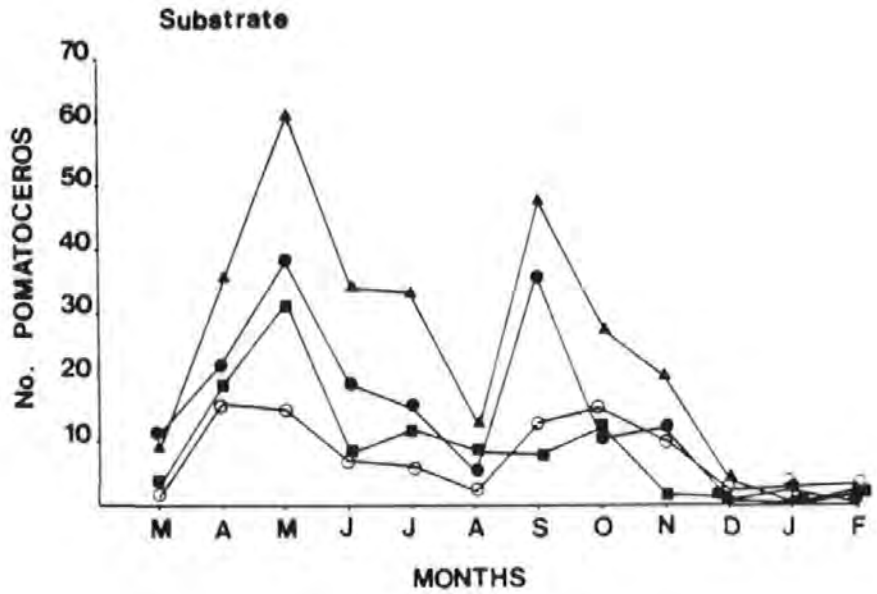
O= Tufnel.

Table 6.5.6.

Field settlement in Pomatoceros triqueter. The % settlement on each experimental substrate within each of the light zones A, B and C.

Table 6.5.7.

Field settlement in Pomatoceros triqueter. Analysis of variance table (one-way) and the effect of substrate.



% settlement — Substrate

	Wood	Rough-perspex	Smooth-perspex	Tufnel
Light zone A(89)	41.5	33.6	6.9	18.0
Light zone B(188)	35.6	26.6	22.3	15.5
Light zone C(390)	49.3	23.8	14.9	12.0

Source	Df	SS	MS	F	P
Substrate	3	1763	588	3.88	<0.05
Error	44	6671	152		
Total	47	8434			

CHAPTER 7

Early 'tubegrowth' in Pomatoceros triqueter (L.)

7.1 *Laboratory Growth*

Following settlement and production of the secondary, calcareous tube the rate of early growth in *Pomatoceros triqueter* was rapid in most of the experimental conditions tested. Generally, an initial exponential period of growth was recorded over the first 8 to 9 weeks (i.e. 9 to 10 weeks after settlement) in most experiments, after which the rate of increase in fresh weight began to slow down with increasing age to week 15. The pattern of growth conformed to the logistic equation (Figures 7.1.1 to 7.1.3). Mean weekly increases in laboratory 'tubegrowth' appear in Appendix 9 and Appendix 10.

7.1.1 *The Effect of temperature, salinity and calcium concentration.*

Experiments to investigate the effects of temperature, salinity and calcium concentration on the rate of 'tubegrowth' in *Pomatoceros* were run as multifactorial experiments or crossed design (Winer, 1970). At each factor level combination of temperature (3 levels), salinity (4 levels) and calcium concentration (2 levels), repeated measurements were taken on the increase in fresh weight of each worm over the 15 week period of observations (Table 7.1.4).

Groups of recently settled *Pomatoceros* maintained at the lowest temperature of 6^{o}C., across the salinity range failed to grow significantly, and did not produce additions to the calcareous tube beyond the first 3 to 4 weeks at each salinity combination. This temperature level, therefore, had many missing values and was omitted from the analysis. The effects of the different factor levels of

temperature, salinity and calcium concentration were variable (Figures 7.1.5 to 7.1.7) but there was a direct relationship between each factor and the rate of growth in *Pomatoceros*. Within the temperature range 10^{o}C., to 20^{o}C., the mean rate of growth of young *Pomatoceros* worms increased with increasing temperature. This direct temperature dependent growth was recorded in tubeworms maintained in both the salinity range with added 'free' calcium (calcium level 1) and the 'ordinary' salinity range (calcium level 0). The effect of temperature was almost linear at the two higher salinities of 34^{o}/oo and 26^{o}/oo. However, at 18^{o}/oo salinity where the rate of growth in *Pomatoceros* increased from 10^{o}C., to 15^{o}C., a further rise in temperature to 20^{o}C., did not result in increased 'tubegrowth'. Temperature was shown to be highly significant at $P = < 0.001$ (Table 7.1.8).

A similar pattern of growth was recorded in response to salinity level, with the rate of growth in *Pomatoceros* generally increasing with increased salinity across the range, 18^{o}/oo to 34^{o}/oo at each temperature level. The highest rates of growth were recorded at the two higher salinity levels (26^{o}/oo and 34^{o}/oo) at both calcium concentrations. Exposure to the lowest salinity levels of 18^{o}/oo and 10^{o}/oo resulted in a marked decrease in the rate of 'tubegrowth'. At 10^{o}/oo salinity the lowest rates of 'tubegrowth' were recorded, and many of the animals maintained at both calcium levels across the temperature range: 10^{o}C., to 20^{o}C., expired within 6 to 8 weeks. When the mortality rate reached 50% or greater in any tank population, fresh weight growth measurements were discontinued. The effect of salinity was shown to be highly significant at $P = < 0.001$ (Table 7.1.8).

Increased calcium availability (calcium level 1; 490 mg Ca/L at 34°/oo salinity and 430 mg Ca/L in each reduced salinity) resulted in an increase in the rate of 'tubegrowth' in *Pomatoceros* across the salinity range at each temperature level. This increase in the rate of growth was most marked in the reduced salinities of 26°/oo and 18°/oo; and at both 15°C and 10°C., the rate of 'tubegrowth' was greatest at the 26°/oo salinity level and decreased in full salinity seawater (34°/oo salinity/490 mg Ca/L). The effect of increased calcium availability was shown to be highly significant at $P = < 0.001$ (Table 7.1.8).

All interaction effects, except that of temperature and calcium, were significant at $P = < 0.05$ or greater (Table 7.1.8).

(a) *'Tubegrowth' at 20°C*

At 20°C., across the salinity range at calcium level 0, the highest rates of growth were recorded in *Pomatoceros* maintained in the two higher salinities, 34°/oo and 26°/oo (Figure 7.1.5). At these salinities an almost identical rate of growth was observed, and the mean fresh weight at age 15 weeks was 0.234g and 0.240g in 34°/oo salinity and 26°/oo salinity sea water respectively. The rate of increase in fresh (tube) weight was markedly slower, however, at 18°/oo salinity. At this salinity level, the initial growth of worms from week 0 to week 4, (0.09g) was comparable to that recorded at 26°/oo (0.102g) and 34°/oo salinity (0.082g); but was markedly slower between week 4 and week 15. At 18°/oo salinity, the final fresh (tube) weight of the worms averaged only 0.126g at age 15 weeks, and was approximately half that recorded in *Pomatoceros* growing in the two higher salinities. Exposure to the

lowest experimental salinity resulted in a much reduced rate of growth and 50% mortalities within 8 weeks.

At calcium level 1 a marked increase in the rate of 'tubegrowth' in the young *Pomatoceros* worms was observed at each salinity level, and the average fresh weight at age 15 weeks was correspondingly higher. Amongst animals held in the two higher salinities of 34°/oo and 26°/oo this increase in the overall growth was most obvious as a more rapid rate of growth from week 0 to week 4 (i.e. to 5 weeks after settlement). At age 4 weeks, the mean fresh (tube) weight of worms in both salinities was 0.148g, compared with *Pomatoceros* at calcium level 0, which weighed an average 0.082g (34°/oo salinity) and 0.102g (26°/oo salinity). At the lower salinity level of 18°/oo, however, no such increase in the initial rate of growth with increased calcium availability was recorded, but instead 'tubegrowth' from week 0 to week 4 was virtually identical to the rate of growth observed in worms at calcium level 0. Here, the effect of increased calcium concentration was observed after week 4, where a higher rate of growth was maintained to week 15.

(b) *'Tubegrowth' at 15°C and 10°C*

Young *Pomatoceros* worms maintained at 15°C., in each test salinity, recorded a slower rate of growth over the 15 week experimental period, than those animals growing at the corresponding salinity levels at 20°C. Similarly, at the lowest temperature, 10°C., the rate of 'tube-growth' was further reduced across the salinity range. A direct relationship was again observed between the rate of growth and salinity, at both temperatures, with the fastest rates of growth being recorded at the two higher experimental salinities, 34°/oo and 26°/oo, and the least growth recorded from *Pomatoceros* growing at 18°/oo salinity.

At both temperatures, the effect of increased calcium concentration (calcium level 1) was seen as a marked increase in the rate of growth in *Pomatoceros* at the reduced salinity levels of 26^{o}/oo and 18^{o}/oo. An increase in salinity to 34^{o}/oo, however, resulted in a smaller increase in the rate of tube formation.

In summary there appeared to be three main influences on the growth response of recently settled *Pomatoceros* in the laboratory, to the environmental variables tested. Firstly, there was a direct temperature effect across the salinity range, and temperature was shown to be highly significant at $P = < 0.001$ (Table 7.1.8). An increase in temperature (across the experimental range, 6^{o}C., to 20^{o}C.,) resulted in an increase in the rate of 'tubegrowth' in *Pomatoceros* at each salinity level. Animals exposed to the lowest temperature, 6^{o}C., failed to grow appreciably (or produce tube) and many did not survive. Secondly, there was a direct relationship between the rate of 'tubegrowth' and salinity. The highest growth rates, at each temperature, were recorded in *Pomatoceros* maintained in the two higher salinities of 34^{o}/oo and 26^{o}/oo. A reduction in environmental salinity level to 18^{o}/oo resulted in a marked decrease in the rate of growth, whilst at the lowest salinity, 10^{o}/oo, the least growth was observed at each temperature level, and significant mortalities were recorded within 6 to 8 weeks in most experiments. The effect of salinity was also highly significant at $P = < 0.001$ (Table 7.1.8). Thirdly, increased calcium concentration (calcium level 1: to 490 mg Ca/L at 34^{o}/oo salinity, and 430 mg Ca/L at 26^{o}/oo, 18^{o}/oo and 10^{o}/oo salinity) resulted in an increase in the rate of growth in each salinity and temperature combination. This increased growth was most marked amongst *Pomatoceros* growing in the reduced salinities of 26^{o}/oo and 18^{o}/oo. The effect of calcium was shown to be highly significant at $P = < 0.001$. (Table 7.1.8 .)

Finally, under all experimental conditions of temperature, salinity and calcium concentration the rate of growth in *Pomatoceros* decreased with age and the effect of age was also highly significant at P = 0.001 (Table 7.1.8).

7.1.2 *The Effect of Light (daylength period)*

As a result of the loss of cultures of the food source, *Isochrysis galbana*, this experiment was terminated after 8 weeks only (Figure 7.1.9). However, during this period of observations, light had no observed effect upon the pattern of growth in *Pomatoceros*, and the rate of 'tubegrowth' under each daylength regime was very similar. The effect of light was shown to be not significant (Table 7.1.10).

7.2 *Field growth studies*

The general pattern of growth in *Pomatoceros triqueter* at each of the field sites, F buoy (Figure 7.1.11) and N1 buoy (Figure 7.1.12), and at each depth level within each site was very similar. The maximum rates of 'tubegrowth' in all test groups of worms was observed during August, September and October. During this period, at F buoy, the temperature ranged from 15.5°C (August) to 14.5°C (October) at 1 m depth and 15.3°C (August) to 13.4°C (October) at 10 m depth (Table 7.1.13). At the more estuarine site, N1 buoy, a similar almost constant vertical temperature profile from month to month was recorded. At this site, the temperature during the late summer/autumn period of rapid 'tubegrowth' ranged from 15.4°C (August) to 13.8°C (October) at 1 m depth, and from 14.9°C (August) to 13.8°C (October) at 20 m depth. (Table 7.1.14). This initial, rapid rate of 'tubegrowth' declined through the winter months of December, January and February,

and the minimum rates of growth at each site were recorded during this period. Slower rates of growth at this time coincided with minimum temperatures (February) of 8.6°C., at F buoy and 8.1°C., N1 buoy. Following the winter period of minimal growth, the rate of increase in the mean fresh weight of *Pomatoceros* at both sites increased with increasing temperature during the spring period, March to May. Throughout the period of observations there were no significant salinity variations between each site or at any depth level.

Overall, the rate of 'tubegrowth' was much less than that observed in laboratory populations of *Pomatoceros*, maintained under constant conditions of temperature and salinity combinations. At the end of the 10 months period of observations, the average final fresh weight of worms at N1 buoy at depths 1m, 5m and 10m, and at F buoy, at 1m and 5m depth was almost identical and ranged from 0.23g to 0.25g. However, a slower rate of growth was recorded in *Pomatoceros* from the deepest cages at each site: 10m depth at F buoy, and 20m depth at N1 buoy. The final fresh weight of worms growing at 10m depth at site F buoy averaged 0.19g; whilst the mean final fresh weight of worms at 20m depth at the site N1 buoy was only 0.155g after 9 months. The effect of depth was shown to be highly significant at $P = <0.01$, F buoy (Table 7.1.15) and $P = <0.001$, N1 buoy (Table 7.1.16). Mean weekly increases in 'tubegrowth' appear in Appendix 11.

7.3 *Discussion*

The rate of early 'tubegrowth' in *Pomatoceros triqueter* immediately following settlement and the production of the first calcareous tube was very rapid, and confirmed the laboratory findings of Segrove (1941). As Hargitt (1912) observed, the formation of tubes by serpulids is not,

strictly speaking, a growth process, and growth of the worm must be considered as only one of several stimuli controlling the rate of tube formation. However, it is in the production of a dense calcareous tube that *Pomatoceros*, and most other serpulids, come to significance as fouling organisms. Consequently, most previous studies have been concerned with the rate of 'growth' in serpulids expressed as increases in the length of the calcareous tube (e.g. Soulier, 1891; Hargitt, 1906, 1909, 1912; Harms, 1912; Dons, 1927, 1943; Hill, 1967). In a comprehensive series of reports of the ecology of *Pomatoceros triqueter*, Klockner (1976, 1978), described the formation of tubes in the serpulidae as "a metabolic function which probably correlated mainly with body growth, and was modified by many exogenous and endogenous factors." Other studies have investigated the rate of mineral regeneration (i.e. the production of calcareous deposits after removal from the tube) in serpulids (e.g. Fauozi, 1931; Thomas, 1940; Swan, 1950; Neff, 1968; 1969; Klockner, 1978), and at least some of the factors affecting the rate of mineral regenerate production will also affect the rate of tube formation. In the present study, the rate of 'tubegrowth' in *Pomatoceros* was measured as increases in the fresh weight of the worms, and their tubes. There was a direct correlation between the amount of 'tubegrowth' (expressed as fresh weight, in mg) and the length of tube (in mm) in *Pomatoceros* (Figure 7.2.1 and Table 7.2.2).

The rate of 'tubegrowth' in recently settled worms was typically exponential and although tube formation (and mineral regenerate production) is not a 'true' growth process, (although a metabolic function) the rate of 'tubegrowth' was dependent to a certain extent upon the relative size (age) of the worms. Thus tube formation (and mineral

regenerate production) has some of the characteristics of a growth process. In most animals the relative rate of growth decreases as the animals grow older (Needham, 1964). The simple growth curve $\frac{dx}{dt}$ against time (or age of individuals) is usually sigmoid or, if extended, becomes bell-shaped. Thus the pattern of growth in *Pomatoceros* expressed as the rate of 'tubegrowth' against age (e.g. Figure 7.1.8) resembles the simple growth curve. Similarly, Hill (1967) reported that the rate of growth in *Ficopomatus uschakovi* (as *Mercierella enigmatica*) was highest during the first two weeks after settlement and the rate of tube formation decreased as the worms grew larger. Hill's (1967) findings were confirmed in the species by the observations of Straughan (1972) and Dixon (1980). Earlier, Gaucher et. al., (1967) reported a similar pattern of growth in laboratory populations of *Hydroides* whilst the relative rate of shell formation by molluscs also decreased with age (Wilbur, 1964).

The rate of 'tubegrowth' in *Pomatoceros triqueter* was also affected by a number of exogenous factors. The rate of growth increased with increasing temperature across the range $6^{o}C.$, to $20^{o}C.$, and was maximal at $20^{o}C.$ The effect of temperature was highly significant at $P = <0.001$ (Table 7.1.8), and the critical temperature appeared to be close to $6^{o}C.$ At $6^{o}C.$, there was little new tube secreted and 'tubegrowth' was minimal. These findings confirm the earlier report of Klockner (1978) in *Pomatoceros*. The laboratory results showing the effect of temperature were further supported to some extent by field observations from the field growth study (at sites F buoy and N1 buoy) where the initial rapid rate of growth in *Pomatoceros* occurred during August, September and October, declined during the winter months and increased again with rising temperature in the Spring. Although consideration of

the age factor should be taken into account when viewing the field growth study data, it appears that the rate of 'tubegrowth' in *Pomatoceros* is directly temperature dependent. Similarly, Castric-Fey (1982) reported that the greatest rate of growth in *Pomatoceros* (both *P. triqueter* and *P. lamarckii*) occurred during the summer months around Brittany, whilst earlier Potts (in Robertson and Pantin, 1938) found that tube formation in *Pomatoceros* occurred only during the period March to August in British waters. Such seasonal variations in growth have also been recorded in other fouling organisms. For instance, Barnes and Powell (1953) found the fastest growth in barnacles was recorded in spring and early summer, and gradually decreased during the winter and with age. The results of the present field growth study confirm earlier reports in several species of tubeworm which failed to produce tube or produced it at a greatly reduced rate during the winter (e.g. Harms, 1912; Dons, 1927; Grave, 1933; Thomas, 1940). Further evidence of reduced 'tubegrowth' during the cooler winter period may be seen in the field settlement study in *Pomatoceros* (see section 6.5) where although colonization occurred throughout the year, settlement and the secretion of a calcareous tube was minimal during the winter and coincided with minimum water temperatures of 7.1°C. Earlier, Føyn and Gjøen (1954) had observed 'tubegrowth' in *Pomatoceros* throughout the year, and similarly found that growth was minimal during the period, December to February. However, they reported increases in tube length occurred at temperatures between 2°C and 4°C. From the laboratory observations in the present study which showed very little 'tubegrowth' occurred in *Pomatoceros* at 6°C; together with the results of field growth studies (sites F buoy and N1 buoy) where minimal growth was coincident with temperatures of 8.1°C., to 8.7°C., it would appear that

at temperatures of 6°C., and lower *Pomatoceros* may not be able to secrete new tube. This view is supported by the findings of Thomas (1940) who reported that *Pomatoceros* was unable to secrete calcium carbonate below 7°C.

The winter temperatures recorded during the field growth study at sites F buoy and N1 buoy, of between 8°C., and 9°C., however, were not particularly low and the reduced 'tubegrowth' associated with the winter period may also be, in part, due to reduced food availability. However, laboratory populations of recently settled *Pomatoceros*, maintained at 6°C., were fed at the same rate as worms exposed to all other experimental temperatures, but did not grow appreciably and often perished within 3 to 4 weeks. Consequently, 6°C., may be close to the critical temperature for 'tubegrowth', in recently settled *Pomatoceros*, below which growth and survival are severely restricted; but at higher temperatures the rate of 'tubegrowth' may also be limited to some extent by the scarcity of food material. Notably, older individuals of *Pomatoceros* may be able to survive lower temperatures than 6°C., (e.g. Føyn and Gjøen, 1954). There is, therefore, a direct relationship between temperature and the rate of 'tubegrowth' in *Pomatoceros*, which may however be influenced to some degree by the availability of food. These findings are contrary to the report of Pyefinch (1950) who considered that food supply was the dominant factor governing the rate of growth in *Pomatoceros*, and that there was no direct correlation between the rate of tube secretion and temperature.

A reduced food supply with depth may also partly explain the slower rate of growth in *Pomatoceros* at 10m depth and 20m depth in the field growth study at sites F buoy and N1 buoy, respectively. There were no

significant temperature or salinity variations within the experimental depth range, and the water column appeared to be well mixed throughout the period of observations. In addition, although light levels were lowest at the deepest cages, laboratory experiments showed the effect of light on 'tubegrowth' to be not significant. The occurrence of *Pomatoceros triqueter* and the ability of the tubeworm to grow into dense masses at depths of up to 200m is well known (e.g. Dons, 1927, 1943; Woods Hole Oceanographic Institute, 1952; Lewis, 1964). Similarly, dense assemblages of *Pomatoceros* have been frequently found at depths of up to 60m, encrusting the anchors of offshore buoys, this study (see Chapter 3). However, such populations were most often made up of large numbers of relatively small tubeworms and few large individuals were regularly recorded. In view of these considerations, and the findings of the field growth study at sites F buoy and N1 buoy, it appears that the rate of 'tubegrowth' in *Pomatoceros triqueter* decreases with increasing depth. This is most likely to be due to a reduction in the food supply, away from surface waters. These findings confirm the study of Klockner (1976) who reported that the rate of growth in *Pomatoceros* was greatest in shallow water zones (to 7m depth) where water temperatures were greater and "the highest levels of food" were found.

The rate of 'tubegrowth' in *Pomatoceros* was also affected by the environmental salinity level. The rate of growth washighest in the higher salinity range between 26^{o}/oo and 34^{o}/oo. Across the temperature range an increase in salinity from 26^{o}/oo to 34^{o}/oo did not result in any significant increased level of 'tubegrowth'. Similarly, Neff (1968) showed the ability of the serpulids, *Hydroides* and *Eupomatus* to produce

mineral regenerate, increased sharply with increasing salinity up to 25°/oo, but there was only a slight increase at higher salinities. Exposure to more dilute salinities, at 18°/oo and 10°/oo, resulted in a markedly slower rate of growth, and mortalities at the lower salinity. The effect of salinity on the 'tubegrowth' of recently settled *Pomatoceros* was shown to be highly significant at $P = < 0.001$. At 18°/oo salinity, an increase in temperature from 15°C., to 20°C., did not result in an increased rate of growth, and this salinity may be close to the critical salinity for 'tubegrowth' in newly settled *Pomatoceros*. A further reduction in salinity level to 10°/oo resulted in only minimal rates of 'tubegrowth' and many young worms expired within 6 to 8 weeks. These findings, together with the results of larval development and laboratory settlement studies (see sections 6.1 and 6.4) which showed that although the rate of larval development was reduced in reduced salinities of 18°/oo to 22°/oo, the larvae were able to settle and secrete a calcareous tube at 18°/oo, salinity, indicating that recently settled *Pomatoceros* tubeworms may be tolerant to fairly wide ranging conditions of salinity. This view agrees with the report of Dybern (1967) and the data of Lyster (1965) in *Pomatoceros*. In addition, the earlier studies of Percival (1929) and Milne (1940) showed that *P. triqueter* was found in a wide range of salinities and further supportedthe findings of the present study. The ability of *Pomatoceros* to grow and produce tube in reduced salinities to 18°/oo, was also reported by Alexander et. al., (1935, in Lyster, 1965), who found that adults may survive in salinities as low as 3°/oo.

Clearly, *Pomatoceros triqueter* may thrive in reduced salinities, and newly settled individuals have been shown, in the present study, to

survive salinities as low as 10°/oo, if only for a short period of time. However, in reduced salinities close to 18°/oo, the rate of 'tubegrowth' in recently settled *Pomatoceros* is restricted. This salinity level may be close to the lower limit of salinity tolerance in *Pomatoceros*, below which the tubeworm may not become established in large numbers. The frequency and occurrence of *Pomatoceros* in areas of low salinity e.g. estuaries may also, of course, be partly limited by larval salinity tolerances, and the reduced growth rate of developing trochophore larvae at salinities in the range 18°/oo to 22°/oo (see section 6.1), may also contribute to the distribution of the species in estuaries and other areas of low environmental salinities. This view confirms the earlier findings of Lyster (1965) in *Pomatoceros*, who also noted that the lack of suitable substrates for settlement in muddy, upstream areas of estuaries should be considered.

Overall, therefore, it appears that exposure to salinities of 18°/oo, and lower, results in markedly reduced 'tubegrowth' in *Pomatoceros* and agrees with previous reports of the detrimental effect of reduced salinities (from 6.4°/oo to 18°/oo) to mussels' growth (Seed, 1976; Almada-villela, 1984). Earlier studies by Schlieper, (1957) and Malone and Dodd (1967), (subsequently confirmed by Almada-villela, 1984), showed that the process of calcification, and shell deposition in *Mytilus* were depressed at salinities around 13°/oo.

However, naturally occurring *Pomatoceros* in areas subject to salinity fluctuations may be able to survive by closing off the relatively impermeable calcareous tube with the opercular plug during unfavourable salinity conditions. Similarly, *Mytilus* may isolate itself from low salinities by closing its shell valves (Milne , 1940; Shumway, 1977; Davenport, 1979). It was demonstrated that the salinity

of the water retained in the mantle cavity of closed mussels was never less than about 21°/oo salinity.

The ability of tubeworms, including *Pomatoceros*, to survive and grow in reduced salinities may be largely influenced by the effect of reduced environmental calcium concentrations in dilute sea water. In the present study, when the calcium concentration of dilute salinities (10, 18 and 26°/oo) was adjusted to 430 mg Ca/L (i.e. the concentration of calcium in 'normal' sea water, at 34°/oo salinity), the rate of 'tubegrowth' increased sharply. These findings appear to support the statement made by Gunter (1961) that "invasion of freshwater by marine organisms is not dependent on the amount of chloride in the water; the important factor is the presence of calcium in freshwater." Further support of this view was shown by Robertson and Pantin (1938) who found that *Pomatoceros triqueter* was unable to produce mineral regenerate in artificial seawater containing less than 50% of the normal amount of calcium. In the laboratory, a further increase in the concentration of calcium to 490 mg Ca/L, in full salinity seawater (34°/oo) also resulted in increased 'tubegrowth' in *Pomatoceros*, although the rate of increase was not as marked as that recorded at the reduced salinities at 430 mg Ca/L. These results do not agree, therefore, with the findings that there was a non-linear relationship between the calcium concentration of sea water and tube formation in adult forms of the serpulids, *Eupomatus* and *Hydroides* (Neff, 1968) and in bivalve shell formation (Kado, 1960). Consequently, it appears that at higher environmental calcium concentrations (and salinities) the rate of 'tubegrowth' in recently settled *Pomatoceros* may not be strictly limited by the capacity to take up calcium from the environment.

Recently settled *Pomatoceros triqueter* individuals, therefore, are able to survive and grow (produce tube) under a wide range of conditions of temperature, salinity and calcium concentration, which may contribute to the success of the species in the colonization of varied habitats.

Figures 7.1.1 to 7.1.3.

Early 'tubegrowth' in Pomatoceros triqueter. The rate of growth of young worms in each treatment combination of temperature and salinity, conformed to the logistic equation.

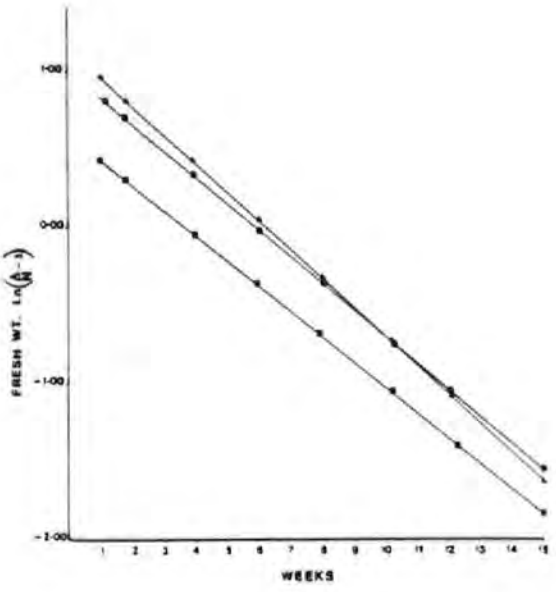
$$N = \frac{A}{1 + ke^{-\lambda At}}$$

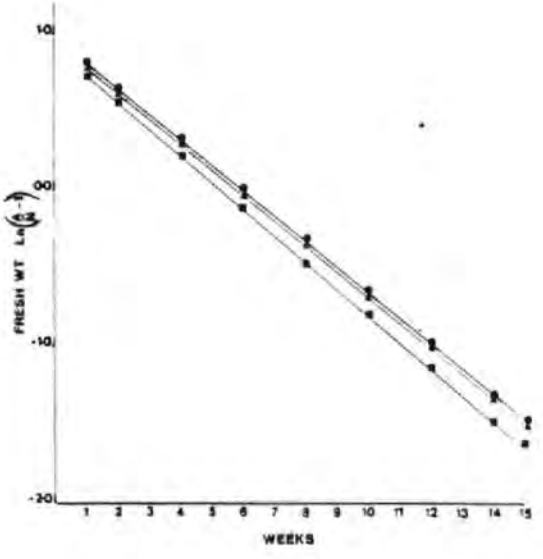
or $$Ln = \left(\frac{A}{N} - 1\right) = Lnk - \lambda At$$

▲= 34°/oo

●= 26°/oo salinity

■= 18°/oo


FRESH WT. Ln(W-1)
WEEKS


FRESH WT Ln(W-1)
WEEKS

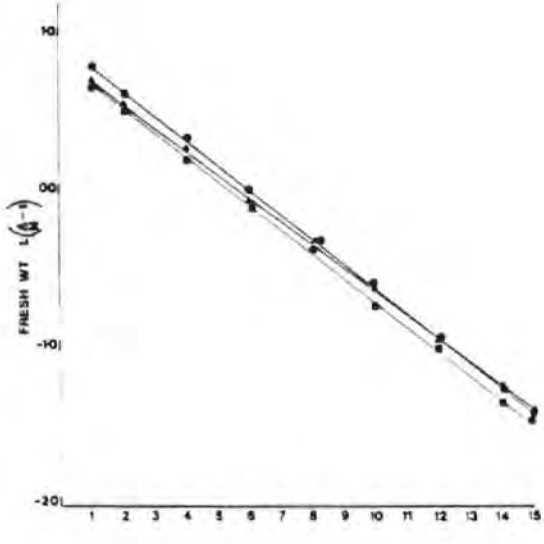

FRESH WT Ln(W-1)

Table 7.1.4.

Early 'tubegrowth' in Pomatoceros triqueter. Experimental design (after Winer, 1970), to investigate the main and interaction effects of salinity, temperature and environmental calcium concentration on the rate of early 'tubegrowth' in *Pomatoceros*, with repeated measures on the age factor.

3 temperature levels

2 calcium levels

4 salinity levels

Temperature	Calcium	Salinity	Weeks 0	1	2	3	. . . . .	15
		1	wl	wl	wl	wl		x10
	1	2	wl	wl				x10
		3	wl					x10
1		4	wl					x10
		1						
	2	2						
		3						
		4						
		1						
	1	2						
		3						
2		4						
		1						
	2	2						
		3						
		4						
		1						
	1	2						
		3						
3		4						
		1						
	2	2						
		3						
		4						

Figures 7.1.5 — 7.1.7.

Early 'tubegrowth' in Pomatoceros triqueter.

The effect of temperature, salinity and calcium level.

Δ = 34°/oo salinity

▲ = 34°/oo salinity + Ca^{++}

O = 26°/oo salinity

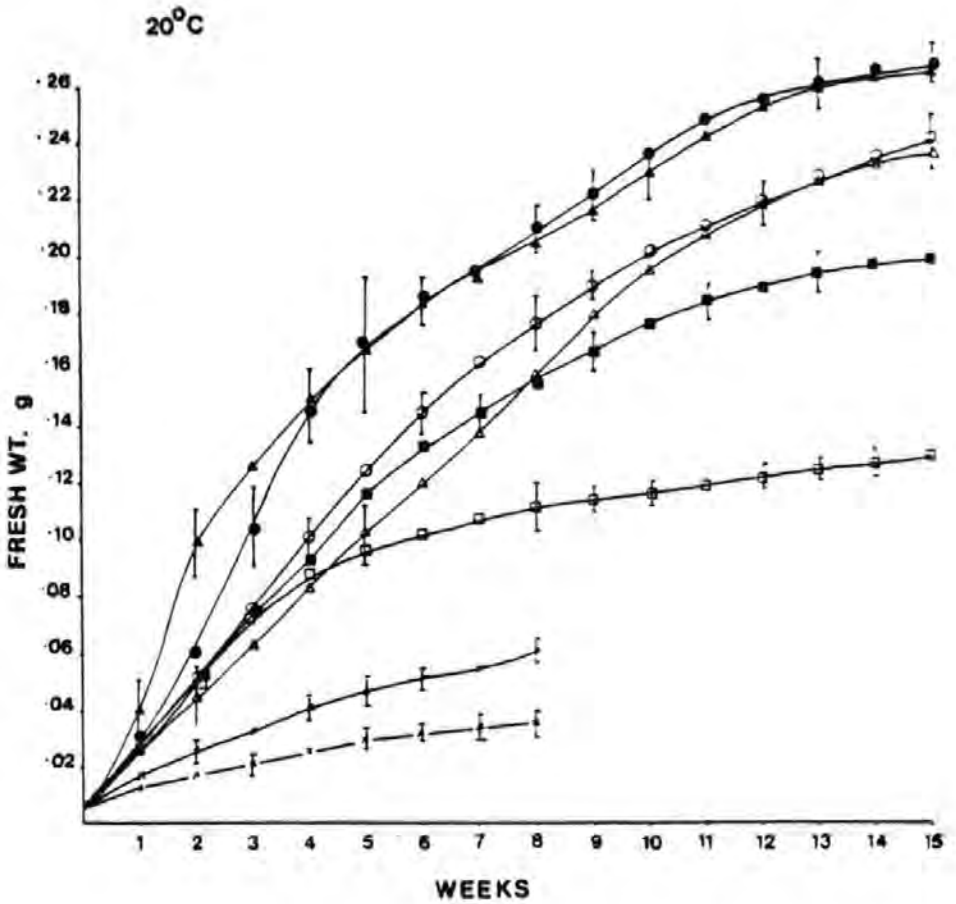
● = 26°/oo salinity + Ca^{++}

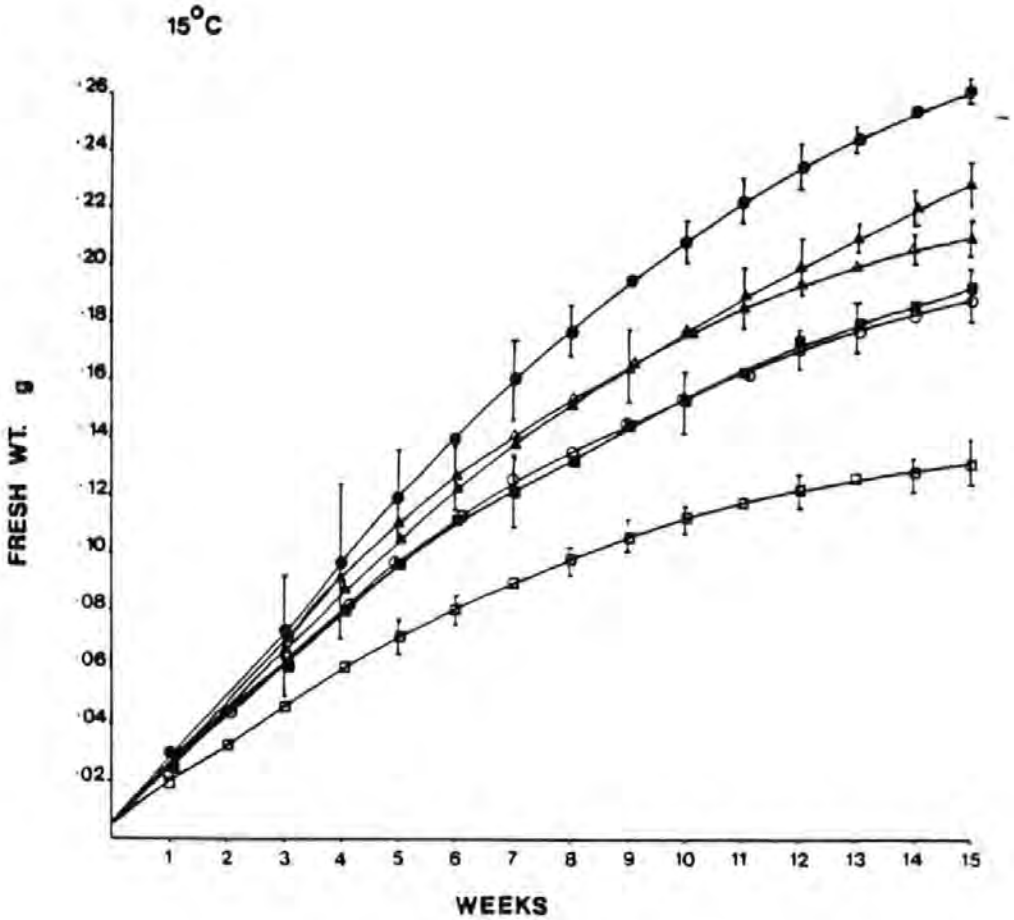
□ = 18°/oo salinity

■ = 18°/oo salinity + Ca^{++}

×—× = 10°/oo salinity

×⟶× = 10°/oo salinity + Ca^{++}


20°C
FRESH WT. g
·26
·24
·22
·20
·18
·16
·14
·12
·10
·08
·06
·04
·02
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
WEEKS


15°C
FRESH WT. g
·26
·24
·22
·20
·18
·16
·14
·12
·10
·08
·06
·04
·02
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
WEEKS

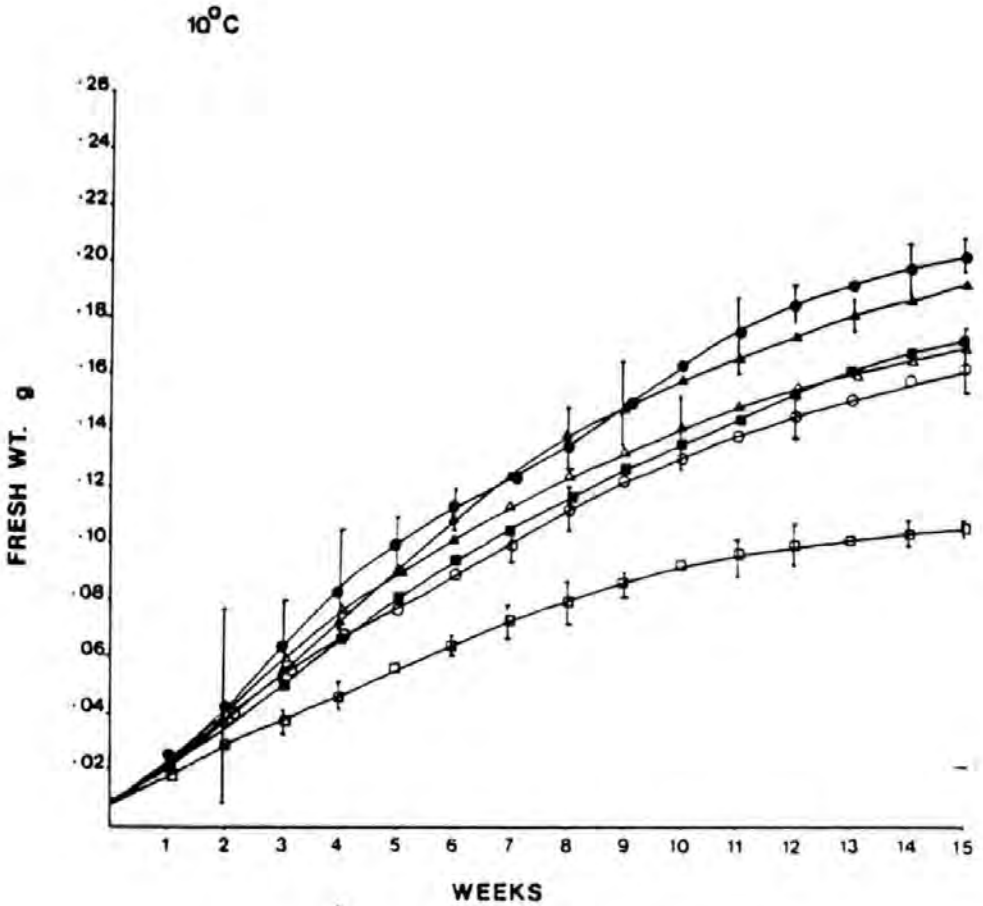

10°C
FRESH WT. g
·26
·24
·22
·20
·18
·16
·14
·12
·10
·08
·06
·04
·02
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
WEEKS

Table 7.1.8.

Early 'tubegrowth' in Pomatoceros triqueter.

The effect of temperature, salinity and calcium level. Analysis of variance table (three-way) together with the effect of age (weeks) on the rate of growth.

Source	Df	SS	MS	f	P
between groups.					
Temperature	2	0.00672	0.00382	50.497	<0.001
Salinity	3	0.08403	0.02805	418.563	<0.001
Calcium	1	0.00265	0.00488	71.132	<0.001
Calcium x Salinity	3	0.00265	0.00086	13.018	<0.001
Temperature x Salinity	6	0.00295	0.00056	7.405	<0.001
Temperature x Calcium	2	0.00233	0.00014	1.711	NS
Temp. x Cal. x Sal.	6	0.00154	0.00034	3.880	<0.001
Temp. x Cal. x Sal. x worm	216	0.01596	0.00013	1.104	<0.01
(groups within treatment combinations)					
Within groups					
Week	15	0.08214	0.00558	81.950	<0.001
Temp. x week	30	0.01035	0.00036	5.125	<0.001
Salinity x week	45	0.02153	0.00055	7.158	<0.001
Calcium x week	15	0.00392	0.00034	3.944	<0.001
Temp.x Sal. x week	90	0.01217	0.00013	2.016	<0.001
Tem. x Cal. x week	30	0.00446	0.00015	2.225	<0.001
Sal. x Cal. x week	45	0.00785	0.00017	2.612	<0.001
Temp. x Cal. x Sal. x week	90	0.01427	0.00016	2.374	<0.001
Error	2997	0.20022	0.00007		
Total	3596	0.47544	0.00013		

Figure 7.1.9.

Early 'tubegrowth' in Pomatoceros triqueter.

The rate of growth and the effect of daylength period (light).

▲ = 12 hours (4×10^{-3} lux)

● = 8 hours (4×10^{-3} lux)

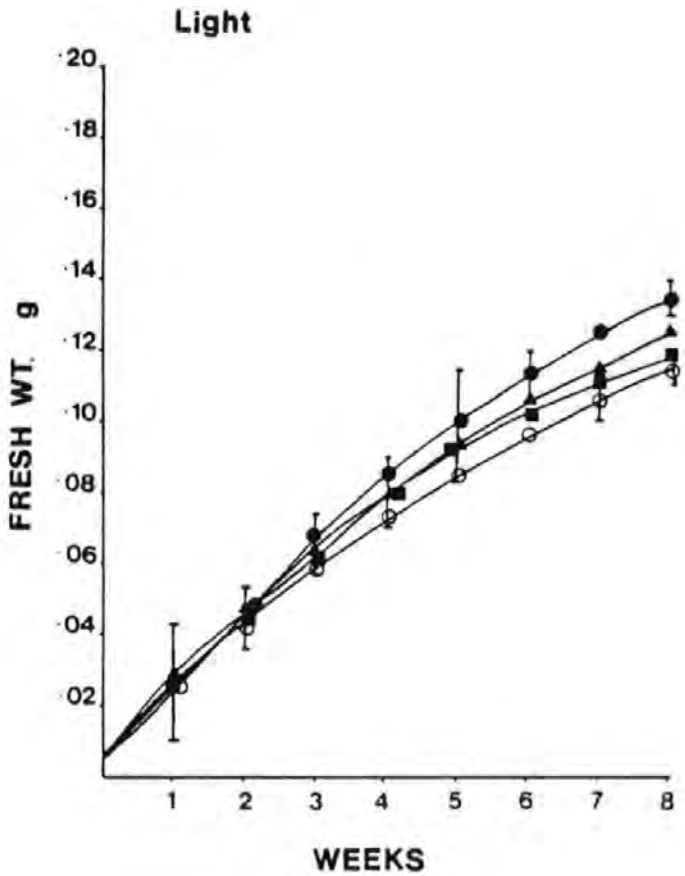
■ = 4 hours (4×10^{-3} lux)

○ = continuous dark

Table 7.1.10.

Early 'tubegrowth' in Pomatoceros triqueter.

The rate of growth, analysis of variance table (one-way), and the effect of daylength period (light).



Source	Df	SS	MS	f	P
Light	3	0.0064	0.0021	2.292	NS
Error	35	0.0169	0.0005		
Total	38	0.0231	0.0006		

Figure 7.1.11.

'Tubegrowth' in Pomatoceros triqueter.

The rate of growth at field station 'F' buoy.

▲= 1m depth

●= 5m depth

■= 10m depth.

Figure 7.1.12.

'Tubegrowth' in Pomatoceros triqueter.

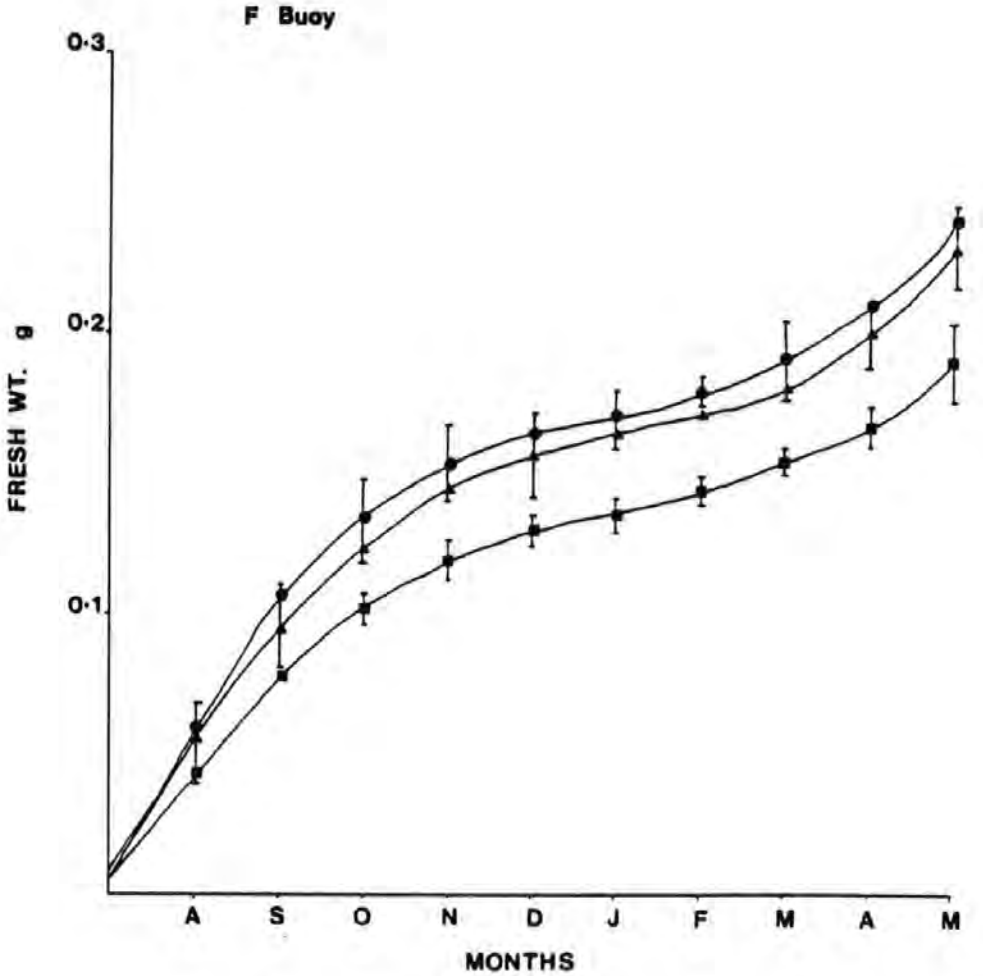
The rate of growth at field station 'N[1]' buoy

▲= 1m depth

●= 5m depth

■= 10m depth

○= 20m depth.


F Buoy
0·3
0·2
0·1
FRESH WT. g
A
S
O
N
D
J
F
M
A
M
MONTHS

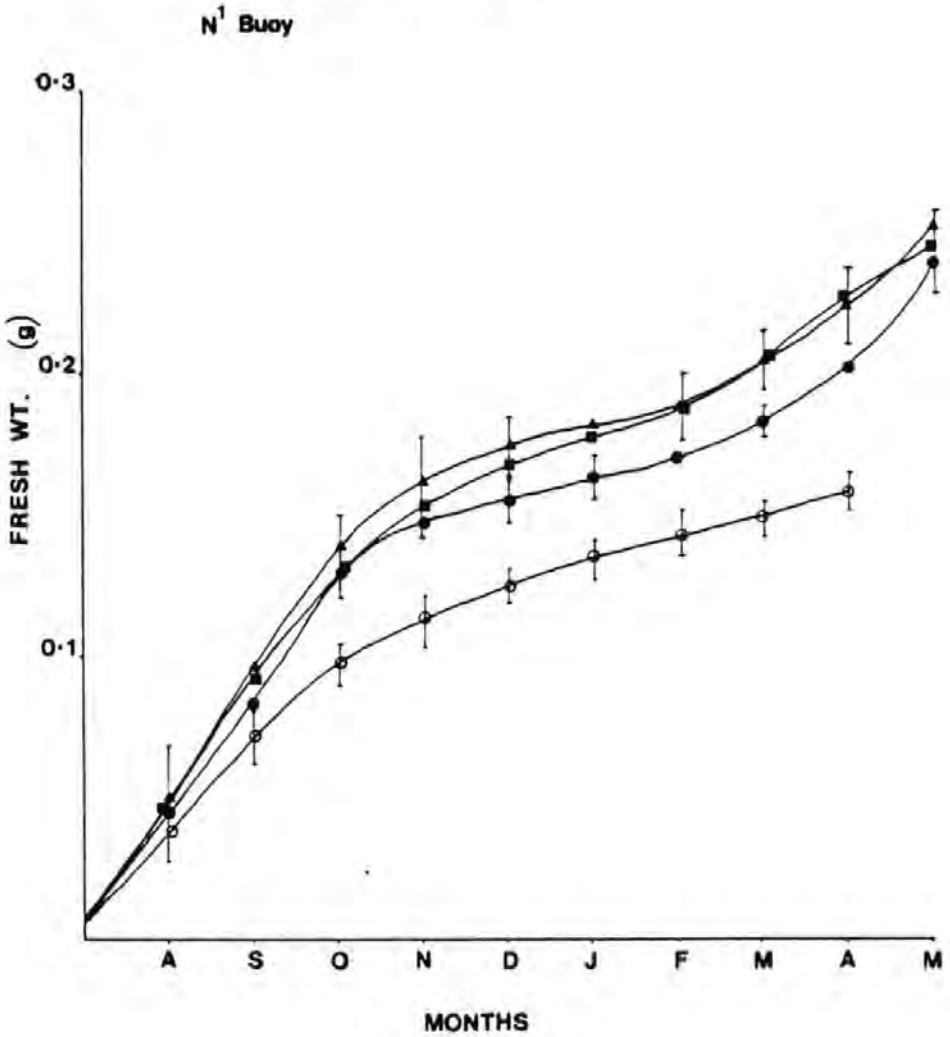

N¹ Buoy
0·3
0·2
0·1
FRESH WT. (g)
A
S
O
N
D
J
F
M
A
M
MONTHS

Table 7.1.13.

'Tubegrowth' in Pomatoceros triqueter.

Field station 'F' buoy, environmental data table.

Table 7.1.14.

'Tubegrowth' in Pomatoceros triqueter.

Field station 'N[1]' buoy, environmental data table.

	Monthly Means									
	A	S	O	N	D	J	F	M	A	M
A. 'F' Buoy : 1 metre										
Temperature (°C)	15.5	14.5	14.5	11.4	10.8	8.6	8.7	8.9	9.3	10.4
Salinity (°/oo)	33.6	34.1	33.9	33.8	32.7	32.8	33.1	31.9	33.4	33.9
Dissolved Oxygen (mg/L)	8.0	7.6	8.4	9.3	7.9	7.7	7.3	8.4	6.2	7.4
Light (% transmitted surface)	68.2	42.0	39.8	57.6	59.2	73.1	65.4	48.7	47.2	53.1
B. 'F' Buoy' : 5 metres										
Temperature (°C)	15.5	14.4	14.5	11.4	10.7	8.6	8.7	8.8	9.2	10.1
Salinity (°/oo)	33.6	34.0	33.9	33.9	33.4	33.4	33.5	33.2	33.4	34.0
Dissolved Oxygen (mg/L)	8.6	8.4	9.3	7.6	8.2	8.5	6.8	8.3	7.5	8.5
Light (% transmitted surface)	51.6	31.3	22.7	34.5	43.6	28.1	38.6	29.2	28.0	31.0
C. 'F' Buoy : 10 metres										
Temperature (°C)	15.3	14.4	14.3	11.3	10.5	8.6	8.7	8.8	9.1	10.1
Salinity (°/oo)	33.8	33.6	34.0	34.3	33.7	33.2	33.6	33.2	33.8	33.9
Dissolved Oxygen (mg/L)	8.5	7.8	7.7	8.3	7.0	9.5	10.3	6.4	7.7	9.5
Light % transmitted surface)	21.2	16.3	10.9	7.4	20.3	6.4	23.5	14.1	9.1	11.3

	Monthly Means									
	A	S	O	N	D	J	F	M	A	M
'N1' Buoy : 1 metre										
Temperature (°C)	15.4	14.6	13.8	11.2	10.4	8.6	8.6	8.7	9.0	10.2
Salinity (°/oo)	32.3	32.6	32.1	32.4	31.1	31.3	30.6	31.2	31.8	32.1
Dissolved Oxygen (mg/L)	8.3	8.1	8.4	9.4	7.8	7.7	8.5	8.2	9.7	9.2
Light (% transmitted surface)	72.6	45.1	48.3	69.2	58.1	63.4	77.9	51.4	50.3	48.6
'N1' Buoy : 5 metres										
Temperature (°C)	15.3	14.6	14.0	11.3	10.5	8.6	8.7	8.8	8.9	10.1
Salinity (°/oo)	32.4	32.7	32.8	33.0	32.8	32.4	31.4	32.5	32.7	32.3
Dissolved Oxygen (mg/L)	8.4	9.3	10.1	7.9	7.6	6.3	7.2	8.0	8.2	8.9
Light (% transmitted surface)	70.3	38.1	36.4	22.9	43.4	46.1	48.3	40.1	44.4	30.0
'N1' Buoy : 10 metres										
Temperature (°C)	15.3	14.4	14.0	11.0	10.4	8.5	8.3	8.4	9.0	9.6
Salinity (°/oo)	32.4	32.8	33.0	33.0	32.8	32.5	31.4	32.6	32.7	32.9
Dissolved Oxygen (mg/L)	9.8	8.1	7.8	8.4	7.6	8.3	6.1	9.2	9.3	7.2
Light (% transmitted surface)	51.2	23.1	18.4	10.1	14.6	20.3	19.1	13.7	17.2	8.3
'N1' Buoy : 20 metres										
Temperature (°C)	14.9	14.3	13.8	11.0	10.3	8.2	8.1	8.3	8.7	
Salinity (°/oo)	32.4	33.0	32.9	33.0	32.9	32.5	33.0	33.1	33.1	
Dissolved Oxygen (mg/L)	6.9	7.4	8.5	7.5	7.9	8.3	8.4	9.0	6.9	
Light (% transmitted surface)	31.0	6.3	8.4	5.0	3.2	8.9	10.2	7.4	6.2	

Table 7.1.15.

'Tubegrowth' in Pomatoceros triqueter.

The rate of growth at field station 'F' buoy. Analysis of variance (one-way) and the effect of depth.

Table 7.1.16.

'Tubegrowth' in Pomatoceros triqueter.

The rate of growth at field station 'N^1' buoy. Analysis of variance (one-way) table, and the effect of depth.

Source	Df	SS	MS	f	P
Depth	2	0.0124	0.0062	5.801	<0.01
Error	52	0.0555	0.0011		
Total	54	0.0679	0.0013		

Source	Df	SS	MS	f	P
Depth	3	0.1097	0.0366	36.147	<0.001
Error	74	0.0748	0.0010		
Total	77	0.1846	0.0024		

Figure 7.2.1.

'Tubegrowth' in Pomatoceros triqueter.

Correlation between tubelength (mm) and tube weight (fresh weight, g).

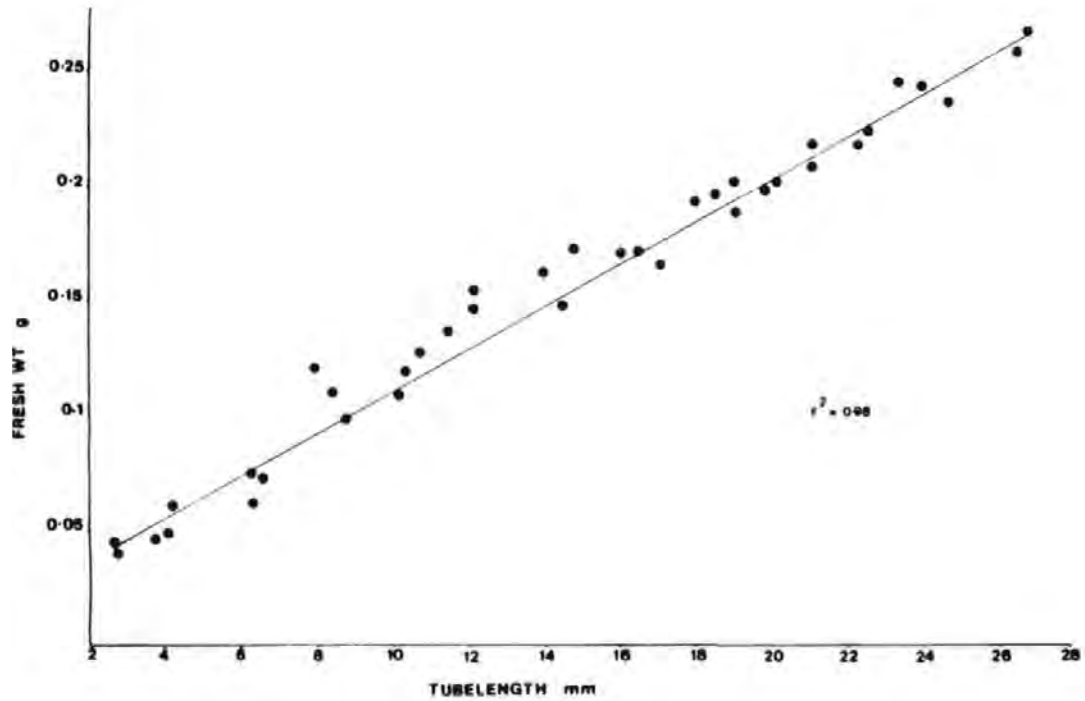
$$r^2 = 0.98$$

$$y = 0.0261 + 0.00912x$$

Table 7.2.2.

'Tubegrowth' in Pomatoceros triqueter.

Correlation between tubelength (mm) and tube weight (fresh weight, g).
Regression analysis on 38 paired data set.



Tubelength(mm)	Tubeweight (g)	Tubelength (mm)	Tubeweight (g)
2.04	0.045	13.99	0.163
2.13	0.051	17.04	0.168
3.86	0.048	16.06	0.173
4.10	0.049	16.20	0.178
4.08	0.065	17.98	0.185
6.30	0.078	18.93	0.201
6.51	0.074	19.01	0.192
6.32	0.120	18.32	0.197
7.95	0.098	20.58	0.207
8.68	0.112	21.02	0.213
8.34	0.123	19.80	0.201
10.12	0.115	21.96	0.226
10.03	0.129	22.15	0.224
10.70	0.157	22.64	0.238
12.04	0.143	23.64	0.248
11.09	0.149	23.40	0.243
12.02	0.154	24.08	0.259
14.38	0.126	24.67	0.262
14.60	0.174	26.78	0.238

CHAPTER 8

General Discussion and Conclusions.

The present study commenced in the paucity of information concerning the distribution of offshore fouling organisms around the whole of Britain and Norway and to add to the wealth of information from coastal sites (e.g. Lewis, 1964; Crisp, 1976) and to new information currently being gathered from the North Sea (e.g. Freeman, 1977; Leitch 1980; Ralph and Troake, 1980). The accessibility of offshore buoys and the types of associated fouling communities, opens up a new and useful structural resource for the study of patterns and interactions among animal and plant populations (i.e. the advancement of ecological theory). Simple cluster analysis techniques applied to the fouling communities divided the U.K. sites (and the Norway sites) into a number of groups based on species composition and the relative abundance of the fouling species. The resulting classification was substantiated by the results of a precise computational program for the identification of indicator species (TWINSPAN, Hill, 1979), although the conclusions and interpretation of the cluster analysis results could be somewhat subjective. For example, the large site grouping D of the U.K. sites (including buoy stations in areas from Cargidan Bay around the north of Britain to the North Sea) could be interpreted as either one group and as a number of sub-clusters depending upon the level of classification desired.

Overall, in British waters there was a West to East, and North to South compression of the depth penetration of algal species, together with a general decrease in fouling community productivity. This trend

may result from the exposure of northern and western sites to clear Oceanic waters, whilst other sites in the North Sea and further east in the English Channel may be exposed to turbid, mixed, coastal waters. Clearly, the reduction in light levels coincident with increasing depth was a major factor governing the vertical distribution of plant and animal species, and a distinct zonation of fouling organisms was found at each buoy site. The study provided the first major overview of offshore fouling communities around Britain and Norway, and, given the limitations imposed by the size of the region, provides an adequate database for future study. The lack of seasonal data is an obvious disadvantage, and a long term objective would be to acquire sufficient information throughout each year to permit a clearer understanding of the composition, distribution and ecology of offshore fouling communities.

Offshore buoys in the present study were seen as transitional between strictly coastal sites and the deep water oil and gas production platform in the North Sea, and as such were subject to factors determining the structure of both inshore epibenthic communities (see Crisp, 1976) and the fouling assemblages associated with the North Sea platforms (Freeman, 1977; Oldfield, 1980; Kingsbury, 1981). The fouling communities found on the deeper water buoys of this study appeared to be more influenced by settlement from local (seabed) populations than coastal organisms. In particular, buoy sites in the North Sea were subject to rapid colonisation by the gymnoblastic hydroid *Tubularia larynx*, most probably recruited from the dense populations of the bydroid commonly found over large areas of the sea bed in that region (e.g. Goodman and Ralph, 1981). A number of other fouling species including *Pomatoceros triqueter* were also commonly found in dense settlements often occupying large parts of the buoys and cables throughout the water column. These

principal fouling organisms corresponded to Meadows (1969) 'pool' species of potentially important fouling organisms.

The occurrence of these rapidly colonising species together with the density of settlement often observed appeared to determine to some extent the type of fouling community found. It may be that the first arriving species may pre-empt the limited available space on the buoys and cables, and influence the subsequent development of the fouling community. These findings agree with the observations of Dean and Hurd (1980), who showed that the rapid settlement of mussels on experimental panels may also pre-empt other species from settling. In this way, the hydroid canopy (usually almost entirely *Tubularia larynx*) was shown to have a possible interference effect on the recruitment of barnacles, substantiating the previous work of MacDougall (1943) and Standing (1976).

The frequency and abundance of both *T. larynx* (as a representative of Freemans, 1977, 'soft' fouling category) and *P. triqueter*, a 'hard' fouler, included both organisms in the 'principal fouling species' classification. The settlement process in both animals was divided into 2 phases. A period of temporary attachments was initially observed, followed by 'settlement attachment' or permanent settlement to the substratum. However, larval activity prior to substrate attachment in each species, and the means by which each organism was seen to effect rapid and successful colonisation was markedly different but in each case maximised the survival and development of the adult form.

No site selection behaviours were displayed by the released actinula larva in *Tubularia*, and the hydroid was capable of rapid settlement and the colonisation of newly immersed substrates regardless of surface texture, colouration or orientation. The settlement response to light, however, was not obvious. Although the greatest densities of *Tubularia* growth at the field study raft were found on the lower panels (agreeing with the observations of MacDougall, 1943; Harris and Irons, 1982; and

Schmidt, 1983) it seems likely that the vertical distribution of *Tubularia* may be influenced by a number of factors (e.g.algal growths in the illuminated zone, Stubbings and Houghton, 1964) including Light. No true 'pelagic' phase exists in *Tubularia* and the actinula larvae are dispersed via water currents. In this respect the surface areas provided by the stiffly radiating aboral tentacles may aid in both buoyancy and dispersal of the larva. However, the rapidity with which settlement in *Tubularia* may be accomplished: n.b. the bulk of settlement occurred within 12 hours after release in laboratory experiments, (this study), and Goodman and Ralph, 1981, determines that new colonies may be found in areas close to parent populations. In addition, the complete life cycle of *Tubularia* may occur within 30 days (laboratory populations) and several generations may be produced within one season, indicating the rapid spreading potential of the species.

Temporary attachments in *T. larynx* were accomplished by means of the nematocysts held in the swollen tips of the aboral tentacles, confirming previous observations in the species by Pyefinch and Downing (1949) and Hawes (1958). Permanent settlement was effected by the hardening of recently secreted chitin at the aboral pole and 'primary' stolon tip, brought about by a phenolic tanning mechanism involving the formation of a quinone, which might then cross-link structural proteins to stabilise the 'semi-liquid' chitin and secure the polyp to the substrate. For the first time electron microscopical techniques were employed, to investigate the mechanism of settlement, which together with histochemical methods, showed that no extra-chitinous cement was present or necessary, confirming the view of Hawes (1958). More research is needed to precisely detail the sequence of events involved in the phenolic tanning mechanism during

attachment at the aboral pole and the 'primary' stolon tip.

In contrast, the larvae of the tubeworm, *Pomatoceros triqueter* underwent a developmental period of up to 3 weeks (16 days at the earliest, at $20^{o}C/34^{o}/oo$ salinity) during which time their behaviour was largely influenced by light. Prior to settlement, the metatrochophore followed a discrete behavioural pattern involving the production of mucus and 'temporary attachments' agreeing with the findings of Segrove (1941) and Føyn and Gjøen (1954) in *Pomatoceros*, and Wisely (1958) Crisp (1977) Marsden and Anderson (1981) and Scheltema et. al., (1981) in other serpulids. Permanent substrate attachment in *Pomatoceros* was mediated by the secretion of an acidic mucopolysaccharide (principally from the anal mucous cell glands and applied to the substrate via the anal vesicle-anal pore apparatus) and the production of a primary mucoid tube. The subequent secretion of the calcareous tube resulted in the permanent settlement of the juvenile to the substrate. The view that acidic mucosubstances may play a role in providing a firm 'temporary' adhesion during the secretion of the calcareous tube is supported by the observations of Pujol (1967) that acidic sulphomucopolysaccharides effect a 'temporary' adhesion in *Mytilus* during byssus formation. Adhesion to the substrate may also be aided by the viscoelastic microbial film covering marine surfaces (Characklis, 1981) although its properties, both chemical and physical, may vary depending on local environmental conditions (Corpe, 1970). According to Characklis (1981) the exterior layer of by-products from the microbial film is ultimately the surface that settling macroorganisms must contend with.

Whereas *Tubularia* larvae readily settled on almost any hard surface (which may result, however, in settlement occurring in habitats ultimately unsuitable for colony growth, Hawes, 1958) settlement in *Pomatoceros* was significantly reduced on smooth and illuminated substrates. However, the gregarious settlement-response in the serpulid may largely 'offset' this disadvantage and enable the animal to occupy large areas of suitable substratum. In addition, the fairly wide range of salinity tolerance displayed by both the larval and recently settled forms of *Pomatoceros* (i.e. the ability to develop rapidly in reduced salinities at $18^{o}/oo$ to $22^{o}/oo$) indicates that the tubeworm may readily occupy estuarine habitats; whilst *Tubularia* was more fully marine and was found to be unable to survive in conditions of reduced salinity below $28^{o}/oo$. On the other hand, the ability of *Tubularia* colonies to flourish at relatively low temperatures (at $6^{o}C$) may be advantageous to the early colonisation of newly immersed hard substrata, whilst other forms capable of development only at warmer temperatures may mature too slowly for their offspring to similarly pre-empt available space. Following settlement the rapid 'tubegrowth' in *Pomatoceros*, and vegetative growth of developing *Tubularia* colonies soon form dense growths and enable both species to rapidly occupy large areas of available free space.

The mechanisms of larval settlement in both *Tubularia* and *Pomatoceros*, then, together with the rapid rate of post settlement growth displayed in the marine environment, may partly explain the frequency and levels of abundance of each animal in offshore fouling communities and contribute to the success of both organisms as fouling species.

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Appendix 1.

Keys and Identification.

APPENDIX I

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Appendix 2.

Staining schedules for wax. embedded *Tubularia* actinulae (sections, 5 µm thick), fixed for 1 hour at room temperature in:

(a) Bouin's (alcoholic)

(b) Formol - saline.

A. Mercury Bromophenol Blue Method (for proteins)

(1% HgCl + 0.05. Bromophenol Blue, in 2% aqueous acetic acid).

(i) Bring sections to water.

(ii) Stain for 1 hour at room temperature.

(iii) Rinse for 10 mins., in 0.5% acetic acid.

(iv) Transfer to tertiary butyl alcohol.

(v) Clear, and mount in Canada balsam.

B. Millon Reaction (Baker modification; proteins, tyrosine).

(i) Bring sections through 50% alcohol to water.

(ii) Place sections in small beaker containing reagent and gently boil for 4 mins.

(iii) Stop heating and allow solution to come to room temperature.

(iv) Remove sections and wash 3 times in distilled water - 2 mins. each.

(v) Dehydrate, clear and mount in Canada balsam.

Appendix 3.

Species - sites distribution data. Values are means of each species calculated over the maximum total number of depth zones (DZ) occupied.

A = Species List

B = Sites List

C = Depth Zone List.

Spp	DZ	UK1	UK2	UK3	UK4	UK5	UK6	UK7	UK8	UK9	UK10	UK11	UK12	UK13	UK14	UK15	UK16	UK17	UK18	UK19	UK20	UK21	UK22	UK23	UK24
1	1-3																0.6								
2	1-3																						0.6	3.3	3.2
3	2-3																								
4	2-4																						0.5		
5	2-5																								
6	1-4																0.3								
7	1-4							0.3																	
8	2-7	1.8	1.2																						
9	2-3	1.9																							
10	2-6					1.2										3.2	1.7	1.4					3.1		
11	2-4																								
12	1-8		0.5			3.7	0.8		1.4	1.8				0.5	2.1	0.9	0.3	1.8							
13	2																								
14	1-2																								
15	1-3	4.0	2.2				2.9																		
16	1-2			1.5																					
17	1-3		1.8																				1.9		
18	2-5																				1.4		1.8		2.8
19	1-3							9.1	3.8	6.5	4.5						6.4	5.1	7.1	1.8			2.7		
20	1-4	2.3	3.9	11.8	16.2	4.3	7.1	8.5	12.6	3.9	3.8	6.8	7.3	5.2	9.6			3.4	4.9	6.2	3.		6.4	5.1	3.7
21	1-3		1.8	4.1	3.2	20.6	6.8	3.1		1.7	2.6	1.3	1.2	2.3	0.5		1.3	1.5			3.3	1.8	1.7	0.8	
22	1-3	0.5	0.7	1.6		2.4																			
23	2-6																		1.0					1.6	
24	2-4				0.9																				
25	2-6														0.8					0.5					
26	3-6							0.2												0.3					
27	2-7							1.7				1.3					0.4								
28	1-3							0.2				0.1	0.1									0.1			0.1
29	1-2				0.2																				
30	1-3														0.2		0.1		0.1						
31	3-10	0.2	2.1	1.7		1.8	1.4	0.8	3.1	0.6	0.9	0.3	0.8	1.5	1.9	1.5	0.7	0.9	3.7	2.9	1.7	1.4	2.8	5.2	2.6
32	2-4	0.4																							
33	1-2							0.2																	
34	2																								
35	4-8																5.4			8.7					1.8
36	3-6		4.2								1.5													3.6	4.8
37	1-3	0.5							1.3																
38	2-5																								
39	3-9					0.8												0.6					4.3	0.8	
40	2-10														0.5										
41	4-10					0.5	0.3										0.4	1.9					2.0		1.9
42	2-3			0.5																					
43	2-3	0.8																							
44	2-5	0.3	1.8		1.4																				
45	3																		0.8						
46	3-6																							2.1	
47	4-11										1.4				0.7		0.8	0.6							
48	6-8					0.4									0.9	1.2									
49	1-4	0.4						0.3	0.1	0.1		0.1	0.1		0.1	0.1			0.1						
50	1-5		0.2		0.2	0.5	0.1	0.1		0.1	0.2	0.2		0.1			0.1	0.1				0.1		0.1	0.1
51	1-4							0.2																	
52	1-3			0.6													0.1				0.1		0.2	0.2	
53	3-7					1.3																			
54	4-9																0.7						0.8		
55	4-13		7.1			5.6	6.3	2.7	1.2								2.6	4.2	5.9	1.7	5.7	1.8	6.4	3.8	2.2
56	4-9							1.6							3.1		1.4			6.6	1.5				
57	3-12	2.9	5.4	2.3	3.1	7.3	2.8				3.2	1.9	1.2		7.7			1.1	10.2	1.4	2.7	3.2	4.2	3.9	1.8
58	1-3			1.3																					
59	5-7																							0.8	
60	4-9												1.0				1.3	0.9						1.3	1.7
61	4-6		0.3																						
62	1-2						1.8										0.3								
63	4-9																				1.9	1.2	8.5	2.6	1.4
64	4-9																1.1		2.3			0.9			
65	4-12				2.4												21.4	18.6	8.4	6.9	7.5	6.2			
66	4-7										1.8						3.4						2.5		
67	5-8									1.3															
68	1-2	0.2	0.9						0.4																
69	1							0.3							0.1			0.1							
70	1-2	0.1	0.2	0.2	0.1	0.2			0.1	0.1			0.1		0.1										
71	1-2																0.1								
72	1-2																								
73	1-3																								
74	4					0.3																			
75	2-5	3.9						0.2		0.4							1.3								
76	2-7							1.0		1.6	2.3														
77	3-7	1.3			0.8	0.5		1.4																	

SPP	DZ	UK1	UK2	UK3	UK4	UK5	UK6	UK7	UK8	UK9	UK10	UK11	UK12	UK13	UK14	UK15	UK16	UK17	UK18	UK19	UK20	UK21	UK22	UK23	UK24
78	4-5						2.0																		
79	2-5		0.1		0.1					0.1					0.1		0.1	0.1	0.1	0.1					
80	2-4	0.1									0.1						0.2		0.1	0.1			0.1	0.1	0.1
81	1-4																0.1								
82	2-4																				0.1		0.1		
83	4-8																								
84	3-12																								
85	4-6																								
86	3-11	3.6	2.5	0.8	2.2	1.3	2.1		0.5	2.3	1.1	4.1	1.8	1.7			0.5	0.9	5.3	1.8	1.7	2.3	0.5	1.8	1.1
87	4-9																					0.3			
88	3-8	1.2	1.8			0.9	1.6	1.3	0.3	0.8		0.3						0.2							
89	1-4																					0.6			
90	4-6			0.8													0.7	1.1							
91	3-8							1.0	0.4	0.9									1.1	2.1	0.8	1.5	4.1	0.7	
92	4-9							2.8														0.4	0.9	1.8	
93	6-8																								
94	4-7										0.8											0.6		1.4	
95	1-2																					0.1			
96	3-4																								
97	4-8					0.8		3.1	2.2	0.6										1.4			2.7		
98	3-6	0.6	0.3		1.1																				
99	3-6										0.3		1.0												
100	4-5																1.6						0.8	1.4	
101	3-8																								
102	4-6																								
103	5-6							0.5																	
104	4-7																								
105	4-7			0.7		1.2	0.4	0.7	1.3	0.9				1.4	2.5	0.9	4.8	1.3	0.5	0.8			0.8		1.2
106	2-4														0.4										
107	3-8										1.6														
108	3-5										0.4			0.5											
109	4-7										0.2														
110	2-5																						0.2		
111	1-5	0.1		0.1	0.1														0.2				0.4		0.3
112	2-6																					0.3			
113	1-5	0.1	0.1	0.1						0.1															
114	1-4	0.1		0.1	0.1			0.1											0.1						
115	2-6																								
116	5-7										1.4						2.2	0.8							
117	4-9								0.7	0.3															
118	5-7																								
119	6-7																					0.4			
120	8-9																0.7								
121	4-8							0.4	0.3		0.4														
122	2-4																		0.2				0.5		
123	3-5																								
124	3-7						1.5	4.6															1.9		3.3
125	5-6																								
126	3-6																								
127	4-5																								
128	2-11	5.4	2.2		1.3	1.6			0.8	1.5	2.1												1.4		
129	4-7	0.4	0.6	0.8	0.2																		0.3		
130	4-8							1.9	0.8														2.3	1.4	1.9
131	3-7																					0.5	0.8	0.7	
132	3-10		1.0						2.3	1.7	1.5	2.1		1.4	0.8	1.9	0.4	0.3	0.7			1.3	0.8	0.7	1.1
133	4-7					0.8		0.5	0.2																
134	5-8														0.7		0.9								
135	4-7										0.4														
136	2-12	2.2	1.9	4.1	1.8	1.9	1.2	0.4	0.8	1.6	0.4	1.1	2.4	0.9	5.6	1.3	0.8		0.7		1.4	3.7	1.1	2.3	4.0
137	2-8			0.3		0.8															0.5		0.3	0.3	
138	4-8																						1.6	1.2	0.7
139	4-6	0.8				0.5																			
140	5-9					0.8											2.0								
141	4-10	1.6		2.2		0.8	0.5		1.1	0.7	1.4			1.1	0.8		1.3		1.5	1.9	3.1	4.2	1.4	1.6	0.7
142	4-5																								
143	5-8																								
144	3-6																								
145	4-7	1.4																							
146	5-6																								
147	4-5							0.8		1.1															
148	3-8	2.8		0.9	1.4	2.2	1.3		0.8	1.3													1.3		
149	4-5										0.3														
150	3-7					1.4								0.9	1.4	0.5	0.2	0.5	0.8	1.2		1.5	0.4	0.8	
151	2-4	0.2	0.2																						
152	2-5		0.1						0.2																
153	2-5						0.1					0.3													
154	2-4							0.1			0.1														

SPP	DZ	UK1	UK2	UK3	UK4	UK5	UK6	UK7	UK8	UK9	UK10	UK11	UK12	UK13	UK14	UK15	UK16	UK17	UK18	UK19	UK20	UK21	UK22	UK23	UK24
155	3-5																								
156	3-4							0.2																	
157	3-6													0.1	0.2	0.2	0.5	0.1					0.6	0.2	1.4
158	4																	0.1							
159	2-4																	0.1	0.1						
160	2-6	0.2	0.2		0.1		0.1			0.1	0.1	0.1							0.1	0.2	0.4	0.2	0.2	0.3	
161	4										0.1														
162	4																		0.1					0.4	
163	3-4													0.1					0.1						
164	2-6	0.3	0.5						0.6	0.5				0.4											
165	2-7	1.4	0.8	1.7		0.8	0.3	1.5		1.4	0.5		1.2	0.5	0.8		0.7	0.2	1.1	1.3	2.5	2.0	0.6	1.3	1.7
166	4-7	1.5	1.8	0.5	0.8	1.6	0.4	0.7		1.2	0.2	0.4											0.8		
167	4-6										1.6			0.8	1.7										
168	4-6							1.5									1.1								
169	5-6																								
170	4-5															0.1									
171	4						0.1	0.1																	
172	3-4																					0.1			
173	4-7							0.1	0.1	0.1					0.1		0.1	0.1		0.1			0.1		0.1
174	4																								0.1
175	4-5																								
176	4-5																0.1								
177	4-7			0.2			0.1						0.1	0.1	0.1										
178	4-5																								
179	4-6							0.1																	
180	4-5																								
181	4																		0.1						
182	4															0.1			0.1						
183	3																								0.5
184	19																								
185	4															0.1									
186	5-7					0.8	1.3		1.4	2.0				1.5											
187	4-8	0.1	0.1	0.2	0.1	0.1	0.1			0.1	0.1	0.1					0.1			0.1	0.1				
188	4-8			0.1	0.1		0.1				0.1				0.1	0.1			0.1	0.1	0.1	0.1	0.1	0.1	
189	10	3.7			0.6	0.8		1.4	0.2	0.5	1.1	1.3													
190	9-12	2.6	0.4	0.5	0.5	1.6	0.4	0.5		0.3	0.4														
191	11														1.3	0.9				0.8					
192	8-9															0.8									
193	4-5																0.2								
194	4-5					0.2					0.1						0.1								
195	4																								
196	4-6																								
197	4																								
198	15																								
199	8-9						0.8																		
200	10																								
201	4-17																		0.2	0.6			1.1		
202	3-18	0.1	0.1	0.1	0.1	0.2		0.1	0.2	0.2	0.1	0.1		0.1	0.1	0.3	0.2	0.3	0.4	0.3	0.1		0.1	0.2	0.2
203	2-6							0.1	0.2																
204	2-4												0.1	0.1						0.1					
205	3-6														0.1										
206	2-3												0.1	0.1	0.1										
207	3-5																								
208	1-4																0.1								
209	2-3	0.2		0.1																					
210	3-5																						0.1		
211	1-3											0.1		0.1											
212	4-10				1.3			0.8	1.1	1.5	0.8	1.7								1.4		0.7			
213	4-15													2.1	2.3	1.5		0.8	1.6				1.1	1.7	0.9
214	9-14				0.5	0.7		1.3		0.4															
215	2-7	0.3	0.4	0.2																				0.4	
216	2-5		0.4		0.2			0.2		0.1		0.3	0.2	0.1			0.1	0.1							
217	2-9						0.1					0.1	0.2	0.2				0.1	0.1						
218	2-3										0.1														
219	3-4										0.1														
220	4-16																								
221	3-7					0.4																			
222	2-19	1.7	4.3	4.1	2.2	2.4	3.9	4.6	2.9	1.7	3.5	4.8	1.6	6.7	3.2	1.8	2.4	3.0	5.9	1.5	1.7	2.3	8.1	3.1	3.5
223	9-14							0.6		0.5	0.4									0.6					
224	14							0.2	0.5																
225	4-6																	0.2							
226	3-6																								
227	3-5																								
228	4-12				0.8	1.3		0.2		1.4			0.4												0.8
229	1-3										0.1			0.1											
230	4-9											0.6			0.8										
231	1-7	1.9																0.8	1.1	1.9	3.2	1.6	1.8	5.7	2.6

SPP	DZ	UK1	UK2	UK3	UK4	UK5	UK6	UK7	UK8	UK9	UK10	UK11	UK12	UK13	UK14	UK15	UK16	UK17	UK18	UK19	UK20	UK21	UK22	UK23	UK24
232	2-8	10.8		2.3	1.3								0.9	1.3	0.7		1.5								
233	1-17	4.6	6.5	4.4	4.8	2.7	5.2	6.3	2.1	3.0	1.8	4.1	2.4	1.3	1.9	5.6	4.0	1.5	1.7	3.1	1.4	2.9	3.8	8.9	4.7
234	4-9					0.9															1.1				
235	3-8																0.8		0.4					1.2	
236	4-6					0.5		0.4			0.6														
237	1-3	0.1	0.1			0.1	0.1	0.1	0.1	0.1	0.1												0.1	0.1	0.1
238	1-2								0.1																
239	1-2												0.1												
240	1-2															0.1								0.1	
241	1-3													0.1		0.1									
242	1-2								0.1	0.1									0.1	0.1		0.1		0.1	0.1
243	1-2							0.1																	0.1
244	1-3					0.1	0.1																		
245	2	0.1		0.1										0.1	0.1	0.1				0.1					
246	1-2																								
247	1-2																							0.1	
248	2-17	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
249	4-8	21.1	12.4	30.1	10.6																				
250	4-5	4.6																							
251	1-2								0.1	0.1															
252	4-6										0.3														
253	1-2			0.1			0.1																		
254	1-2																					0.1			
255	5																							0.1	
256	7-8				0.3				0.2	0.6	1.1														
257	4-7		0.5											0.6											
258	4-6	0.7														0.9									
259	2-5	0.1		0.1	0.1																	0.1	0.1		
260	19			0.8			1.8		3.7	4.2	3.8	8.7													
261	8-12																								
262	5-8																								
263	3-7			0.1		0.4												0.5							
264	4-8					2.4			0.5																
265	3-8		0.8		0.8			1.2																	
266	4																						0.9		
267	5-14												0.6	1.4						1.6					
268	9-11					0.3											2.3								
269	3-9																						0.3		0.4
270	4-16			0.1																					
271	15									1.1	3.2							2.4							
272	3-6																								
273	19																								
274	10									2.5	19.4	6.5	9.3												
275	3-9					0.4	0.8		0.3																
276	4-6	0.3			1.0									0.4		0.2						0.4			
277	4-5										0.3														
278	4-7		0.4																						
279	3-7			0.3															0.5	0.3			0.3	0.1	
280	5-17	1.9	0.8	0.7	2.3				1.3	0.7					1.4			3.8							1.6
281	6-9																								
282	2-5																								
283	5-7				0.3																				
284	3-6				0.4						0.7														
285	5-16				2.8																			1.7	1.4
286	14																								0.5
287	6-16	1.8		3.2	3.1			1.3	0.7														0.4		1.5
288	3-6																								
289	4-5													0.5											
290	5-17	2.8	1.7	1.2	0.8	0.9	1.9											2.1				1.5			
291	3-19	3.6	3.1	1.4	1.9	3.8	2.3	5.4	3.1	2.1	10.4	2.6	6.1	2.9	2.7	8.2	3.6	11.5	4.7	2.9	6.7	3.4	2.2	2.6	5.8
292	2-5		0.3								0.3														
293	6-13			0.7	0.3	0.2	1.4		0.8	0.4										0.2			0.4		
294	3-7											0.2			0.1	0.1									
295	4-6		0.6			1.5	1.1	2.0		0.5				0.4											
296	2-5																								
297	3-5							1.5																	
298	4-9																			0.3	0.5				
299	4-5																							0.1	
300	4																							0.1	0.1
301	5-7										0.8						0.7	0.4							
302	15																								
303	16							0.5																	
304	19		0.2	0.2								0.2													
305	19				0.6																				
306	19	0.7	2.4	1.3	1.6	0.8	0.2		0.2											0.3				0.1	
307	9-19	0.8	0.9	1.4	1.5	1.1	2.6				1.4														
308	19	0.4			0.5		1.1	0.3																	

SPP	DZ	UK1	UK2	UK3	UK4	UK5	UK6	UK7	UK8	UK9	UK10	UK11	UK12	UK13	UK14	UK15	UK16	UK17	UK18	UK19	UK20	UK21	UK22	UK23	UK24
309	19																								
310	19																								
311	19																								
312	19																								
313	4-10							1.4																	
314	4-14				0.9																				
315	19																								
316	7-10																		0.1					0.1	0.1
317	19																								
318	4-13							0.1		0.1					0.1	0.1					0.1	0.1		0.1	
319	19																								
320	5-8																								
321	3-16					0.2					0.1														
322	5-10																					0.1			
323	4-19		0.1	0.1				0.1		0.1			0.1	0.1	0.1	0.1	0.1	0.1			0.1			0.1	0.1
324	19							0.1																	
325	19	0.1	0.1																						
326	19		0.1																						
327	19	0.3	0.4																						
328	6-19																								
329	19																								
330	9-19	0.1		0.1																				0.1	
331	19																								
332	19																								
333	19																								
334	15							0.6																	
335	9-16						0.8	1.4	0.4	0.5	1.3				0.8	1.5		1.9							2.6
336	19																								
337	10													0.1		0.1									
338	19																								
339	19																								
340	3-19	0.3	0.2	0.3	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1		0.1		0.1	0.1			0.1		0.1	0.1
341	8-9																					0.1			
342	8-9																					0.1			
343	4-8													0.1	0.1	0.1									
344	6-7																		0.1						
345	19																								
346	19																								
347	9-16		0.2															0.1							0.1
348	3-10																								0.1
349	4-13																0.1		0.1	0.1				0.1	0.1
350	6-9														0.1			0.1							
351	19	0.3																							
352	19																								
353	3-10																						0.1		0.1
354	19																								
355	6																					0.1		_	
356	19		0.2																						
357	19	0.1			0.1																				
358	17																								
359	19																								
360	19		1.4			0.8																			
361	19																								
362	19				0.8	2.6	1.7	1.2															2.2		
363	19	4.8			1.3		3.0	0.4																	
364	19																								
365	19				1.6																				
366	19	5.1			2.0									0.8	1.5										
367	19	3.9																							
368	17																								
369	6-9	1.4		0.9																					
370	4-16												0.8												
371	4-15		2.3	0.9		3.8	2.3		0.6	0.8	1.5						1.4								
372	13													0.8			2.5								
373	3-8											1.8	0.5										3.7	4.6	5.8
374	4-8																	0.8	9.7	2.3			8.1	2.4	5.3
375	4-7																						3.1		
376	3-17	6.3	2.6			3.1			0.5	0.7	1.8										1.4			2.0	
377	12		0.9	0.4																					
378	4-7																								
379	8-13																								
380	6-7			0.8	2.3													0.5							0.7
381	8-15	2.8			0.9	1.1									0.7	0.6							0.6		
382	5-9					0.8																			1.2
383	4-17		0.7					1.5																	2.9

SPP	DI	UK25	UK26	UK27	UK28	UK29	UK30	UK31	UK32	UK33	UK34	UK35	UK36	UK37	UK38	NO1	NO2	NO3	NO4	NO5	NO6	NO7	NO8	NO9	NO10
1	1-3																								
2	1-3			1.8																	0.3		0.8		
3	2-3											1.4	0.2												
4	2-4														0.1	0.1		0.2		0.1	0.1		0.3		
5	2-5											0.2								0.1					
6	1-4																				0.2				
7	1-4										0.3	0.1	0.1			0.1				0.1					
8	2-7							0.4					0.3						0.8						
9	2-3												0.4						0.3	2.1	0.8		0.6		
10	2-6			2.2				0.7										1.2	0.4						
11	2-4																				0.6	0.7			
12	1-8			2.2						2.9		1.2						1.3		1.7		0.9	0.4		0.8
13	2									1.8					1.2					0.8					
14	1-2							0.5																	
15	1-3						1.4										3.7	2.8				3.1		0.5	7.9
16	1-2																					2.9			
17	1-3															3.6			1.6			6.8		1.1	
18	2-5																								
19	1-3				3.4			0.6	1.4		0.8	0.7		0.5	0.5						0.7	0.8			
20	1-4	10.1	8.3	11.2	5.1	1.8	0.5	3.2	1.8	2.1	1.4	1.6	0.4	0.7	0.7	1.6	5.3	6.2	0.9	2.7	2.8	1.7		1.1	1.3
21	1-3	3.6	1.4	1.8		3.7	1.7		2.6	0.6					0.4		0.5		10.2				0.5	1.8	
22	1-3												0.3			2.1	0.8								
23	2-6											0.3		0.2											
24	2-4													0.3						0.2					
25	2-6																				0.4				
26	3-6				0.2			0.3		0.1	0.1														
27	2-7									0.3	0.2														
28	1-3			0.2	0.1	0.1	0.1			0.1				0.1									0.1		
29	1-2							0.1												0.1	0.1		0.1		
30	1-3						0.1																		
31	3-10	1.7	0.9	1.1	1.3	1.6	1.4	0.8	3.4		1.1	0.4		0.3		1.5	0.8	1.3	2.2		2.5	4.5	0.8	0.9	2.3
32	2-4									0.1	0.1			0.1											
33	1-2							0.1							0.1					0.1					
34	2								0.1											0.1	0.3				
35	4-8								6.8													5.0	15.2	24.1	5.3
36	3-6		6.4																						
37	1-3																								
38	2-5										0.1		0.2	0.1											
39	3-9																								0.3
40	2-10				0.3									0.2											
41	4-10																0.2			0.8		0.6		2.4	0.6
42	2-3															1.1				0.8	3.1				
43	2-3				0.3								0.2												
44	2-5																	0.9							
45	3							0.2																	
46	3-6																								
47	4-11							0.3																	
48	6-8																								
49	1-4				0.1			0.1		0.1	0.1	0.1	0.1	0.1		0.1		0.1	0.1	0.1			0.1		0.1
50	1-5		0.5										0.1					0.1	0.1	0.1		0.1	0.1		0.1
51	1-4					0.1																			
52	1-3					0.1		0.1				0.1					0.1							0.1	
53	3-7						6.3		1.3													22.6	1.9		
54	4-9						0.3													0.5				0.4	
55	4-13			3.8													3.1	1.4		8.7					
56	4-9								0.9				1.4												1.4
57	3-12	8.4	6.4	3.3	5.3	8.5	2.2	3.0	1.6	2.8	1.3	2.6	0.4	1.3		3.4	1.2	2.9	3.1	1.5	0.9	3.8	1.6		2.7
58	1-3		4.8									0.2		0.3											
59	3-7											0.4	1.8												
60	4-9	2.3	1.4	2.6	0.4	1.2	1.4	2.2	0.8	0.6	0.5			0.3	0.7										
61	4-6				0.4	1.1		0.8					0.4												1.4
62	1-2				0.3					0.3															
63	4-9	2.1	1.8						3.0	2.4		0.8													
64	4-9																				0.4				
65	4-12																							0.6	
66	4-7			1.3															0.8						0.6
67	5-8													0.4											
68	1-2			0.4																			0.2		
69	1														0.1										
70	1-2				0.1																				
71	1-2		0.2			0.1							0.1												
72	1-2																					0.1			
73	1-3										0.1											0.1			
74	4			0.4													0.8								
75	2-5	0.8							0.3																
76	2-7																								
77	3-7								0.1																

SPP	DI	UK25	UK26	UK27	UK28	UK29	UK30	UK31	UK32	UK33	UK34	UK35	UK36	UK37	UK38	NO1	NO2	NO3	NO4	NO5	NO6	NO7	NO8	NO9	NO10
78	4-5						0.2																		
79	2-5																								
80	2-4												0.1												
81	1-4											0.1		0.1											
82	2-4																								
83	4-8		2.8																						
84	3-12			0.4				0.5							0.4		1.1								
85	4-6							0.2												1.6					
86	3-11	2.3	4.2	1.3	0.8		1.9	0.9	1.4		1.0	1.7		2.4	1.7	2.8	1.9		1.3	1.8	1.9	3.6	1.3		2.2
87	4-9								0.4		0.2		0.8			0.7	1.1	3.4	1.7		0.6	2.2		0.4	0.5
88	3-8	1.1	1.2				2.4							0.4					0.2			0.8			
89	1-4																	0.1							
90	4-6																								
91	3-8		2.2																				1.1		
92	4-9				0.4																				
93	6-8															0.9									
94	4-7														0.8										
95	1-2			0.1						0.1											0.1	0.1			
96	3-4						0.6				0.8														
97	4-8	0.6	1.5			0.3																			
98	3-6																								
99	3-6																								
100	4-5																								
101	3-8			0.3												1.2	1.1								
102	4-6									0.5															
103	5-6												1.3												
104	4-7											0.5	1.1												
105	4-7	0.8	1.7	2.4														1.2					1.3		
106	2-4																			1.1					
107	3-8			0.3																					
108	3-5																								
109	4-7	1.2																							
110	2-5							0.3									0.1							0.1	
111	1-5		0.2											0.1			0.1								
112	2-6																								
113	1-5	0.3																						0.1	
114	1-4													0.1											
115	2-6		0.2											0.1											
116	5-7			0.6																1.7					
117	4-9							1.8																	
118	5-7											0.2									0.3				
119	6-7														0.1										
120	8-9																								
121	4-8							1.4				0.2			0.8										
122	2-4								0.1																
123	3-5																								
124	3-7																								
125	5-6															0.6									
126	3-6		1.6																						
127	4-5																								
128	2-11	1.4	1.8	1.6																		2.0			0.7
129	4-7		1.2																						
130	4-8			0.8																					
131	3-7							0.5					0.8			1.1	0.8								
132	3-10				0.8			0.4	0.7		0.6		1.2			1.7	2.6	0.2	0.3		1.7	0.3	1.6	1.6	1.8
133	4-7		3.5		0.3																				
134	5-8												2.3				0.3					1.5			
135	4-7																								
136	2-12	3.4	1.7	1.8	1.1			1.4			1.8		2.4	1.9		1.3	2.1	0.3	1.1	1.4	0.8	1.3	0.7		2.4
137	2-8	0.2	1.1													0.4					0.3				0.2
138	4-8			0.3											0.2		0.4								
139	4-6								0.8				2.2				0.4	0.5							
140	5-9	2.3	0.4							0.5	1.3			1.1											
141	4-10	1.9	2.8								1.9		1.4	0.8	1.6	2.9	0.7		0.4		1.7	0.8	0.9		
142	4-5							0.4																	
143	5-8		1.3																						
144	3-6							0.4											0.6	1.9			1.2		
145	4-7											0.3						0.5							
146	5-6							0.5																	
147	4-5																								
148	3-8	1.4													1.2								1.1		
149	4-5																						0.3		
150	3-7	1.6	1.3											2.1						2.1				0.2	
151	2-4			0.1																					
152	2-5			0.1												0.1									
153	2-5																						0.1		0.1
154	2-4		0.4																						

SPP	DI	UK25	UK26	UK27	UK28	UK29	UK30	UK31	UK32	UK33	UK34	UK35	UK36	UK37	UK38	NO1	NO2	NO3	NO4	NO5	NO6	NO7	NO8	NO9	NO10
155	3-5								0.4	0.4															
156	3-4	0.5	0.4																						
157	3-6	0.8	2.1	0.4				0.4	0.8	1.3	0.8	0.2	0.9	2.7	0.4			0.4	0.4						
158	4				0.5			0.3					0.2		0.4					1.5	1.8	0.9			
159	2-4														0.2					0.6					
160	2-6			0.2				0.1			0.4				0.2				0.3						
161	4		0.9	0.3						1.1						0.3									
162	4																								
163	3-4																								
164	2-6	1.0																0.4							
165	2-7		0.8		0.4					0.9					1.0		0.4	0.2	0.5	1.5	0.8	0.3		0.2	2.2
166	4-7																					1.4			
167	4-6																					1.4			
168	4-6																								
169	5-6												0.1			0.1									
170	4-5				0.1							0.1													
171	4								0.3				0.1										0.1		
172	3-4			0.2																					
173	4-7			0.3														0.1					0.1		
174	4																						0.1		
175	4-5											0.1								0.1					
176	4-5																			0.1	0.1				
177	4-7									0.1									0.1						
178	4-5								0.1	0.1											0.1				
179	4-6									0.1			0.1	0.1			0.1		0.1						
180	4-5												0.1												
181	4							0.1		0.1															
182	4									0.1															
183	3	0.6		0.3																					
184	19					14.3	10.6												6.8			2.2			
185	4																	0.1							
186	5-7				0.5													2.8							
187	4-8	0.1	0.3	0.2		0.1	0.1						0.1		0.1	0.1	0.1		0.1						
188	4-8	0.4	0.2	0.1	0.1	0.1		0.1	0.1	0.1	0.1			0.1	0.1	0.1	0.1	0.1				0.1		0.1	
189	10																								
190	9-12																								
191	11												0.1												
192	8-9																								
193	4-5								0.1			0.1													
194	4-5																								
195	4							0.1					0.1												
196	4-6				0.1	0.1	0.1	0.1		0.1	0.1	0.1	0.1						0.1						
197	4				0.1									0.1											
198	15					0.3									0.2					0.8					
199	8-9														0.3										
200	10																							8.3	
201	4-17	0.4		0.2									0.4		1.4	0.6	0.8			0.4	2.1	2.2			
202	3-18	0.2	0.3	0.3	0.1	1.8	0.6		2.8	0.8	1.9	3.5	1.1	3.5	1.6	1.3		0.1		0.3		0.2	0.3	0.9	1.1
203	2-6			0.2																					
204	2-4																								
205	3-6																								
206	2-3																								
207	3-5	0.1																							
208	1-4	0.1																							
209	2-3																								
210	3-5																								
211	1-3																								
212	4-10				0.4	0.3	2.9		1.4											1.8					
213	4-15				0.2	0.6	1.2	0.4		0.9		2.6	1.8	0.7	1.4	1.8			2.2	5.7					
214	9-14																		0.9						
215	2-7							1.6								0.4			0.4						
216	2-5					0.3	0.2				0.1		0.1			0.1							0.1			
217	2-9					0.3																	0.3		
218	2-3	0.3	0.4																	0.4					
219	3-4																								
220	4-16							0.2										2.4	2.3						
221	3-7			0.3																					
222	2-19	6.1	4.2	2.6	3.4	2.8	1.9	2.7	4.7	2.2		1.4	2.2	8.3	6.1	4.2	2.7	1.6	2.1	2.6		6.2	4.7	2.3	1.7
223	9-14							0.2																	
224	14							0.1										0.2							
225	4-6															0.8						0.5			
226	3-6						0.4																	0.4	
227	3-5		1.3																	1.3					
228	4-12												0.3										0.4		
229	1-3	0.3																							
230	4-9																								
231	1-7			0.4	1.5		1.0								1.6	1.4	1.8		0.8		2.5	1.9			2.7

SPP	DZ	UK25	UK26	UK27	UK28	UK29	UK30	UK31	UK32	UK33	UK34	UK35	UK36	UK37	UK38	NO1	NO2	NO3	NO4	NO5	NO6	NO7	NO8	NO9	NO10
232	2-8					1.4													1.1						
233	1-17	2.5	2.6	3.1	3.7	1.3	1.4		1.5	1.8		1.4	1.8	2.9	2.5		1.6	3.1	2.8	4.6	2.1		0.8	1.3	2.5
234	4-9																	1.9		0.6					
235	3-8											0.3				2.6									
236	4-6																								
237	1-3	0.1			0.1	0.1	0.1	0.1	0.1			0.1			0.1	0.1	0.1				0.1				
238	1-2																								
239	1-2																								
240	1-2																								
241	1-2		0.4																						
242	1-2		0.2							0.1					0.1								0.1		
243	1-2	0.1						0.1																	
244	1-3														0.1										
245	2																								
246	1-2						0.1																		
247	1-2																								
248	2-17	0.1	0.1		0.1			0.1	0.1		0.1		0.1	0.1			0.1	0.1		0.1	0.1	0.1			0.1
249	4-8	21.4	18.2	6.4					5.8									11.7	1.4			11.5	1.8		16.8
250	4-5																								
251	1-2																								
252	4-6			0.4																					
253	1-2																								
254	1-2			0.1																					
255	5																								
256	7-8																								
257	4-7		0.3																						
258	4-6																								
259	2-5					0.2																			
260	19																								
261	8-12						1.3															0.5			
262	5-8																							0.5	
263	3-7					0.2			0.1				0.1												
264	4-8				0.7			1.3					0.6				0.4	0.3				0.2	0.4	0.4	0.3
265	3-8			0.3	2.1			2.2			0.5	1.1		0.9											
266	4																								
267	5-14	0.2														0.2									
268	9-11																								
269	3-9											0.1	0.1												
270	4-16																								
271	15						0.4			0.5															
272	3-6								0.4												0.6				
273	19					2.5										2.1			0.8						
274	10																								
275	3-9																								
276	4-6			0.4	0.4									0.3											
277	4-5														0.4							0.4			
278	4-7		0.8																						
279	3-7																								
280	5-17	1.7		0.9																				1.6	2.7
281	6-9															0.6									
282	2-5					0.2										0.2									
283	5-7		0.4																						
284	3-6																			0.4					
285	5-16																	0.5							
286	14			0.2						0.9															
287	6-16																	0.3	1.5					1.4	1.7
288	3-6											0.3	0.5			0.7									
289	4-5												1.4	0.5											
290	5-17														0.4							0.8	1.3	0.7	0.6
291	3-19	8.1	14.6	3.4	1.3	2.8	2.2	1.6	3.5	2.1	1.5	4.2	2.8	1.4		2.6	5.2	1.3	3.4	8.9	2.6	5.7	1.6	3.1	2.5
292	2-5										0.7														
293	6-13	0.5	1.3						1.5																
294	3-7																								
295	4-6						0.8										0.5	1.7			1.3				
296	2-5																0.8								
297	3-5																								
298	4-9												0.3							0.2	0.2				
299	4-5				0.1																				
300	4																				0.1				
301	5-7							0.5																	
302	15																								
303	16																								
304	19								0.5																
305	19			0.4																					
306	19						0.8	1.2	1.2																
307	9-19			0.8																					
308	19																								

SPP	DI	UK25	UK26	UK27	UK28	UK29	UK30	UK31	UK32	UK33	UK34	UK35	UK36	UK37	UK38	NO1	NO2	NO3	NO4	NO5	NO6	NO7	NO8	NO9	NO10
309	19										0.1				0.1										
310	19				0.2							0.1	0.1		0.1										
311	19				0.1	0.1						0.1			0.1										
312	19									0.1	0.1		0.1												
313	4-10																								
314	4-14												0.1												
315	19			0.2		0.1																			
316	7-10				0.1									0.1						0.1	0.2				
317	19																	0.1							
318	4-13									0.3															
319	19			0.1	0.1													0.1	0.1	0.1					
320	5-8							0.2																	
321	3-16					0.1		0.1								0.1	0.1	0.1							
322	5-10									0.1			0.1									0.1	0.1	0.1	
323	4-19	0.2		0.1	0.1		0.1	0.1	0.1		0.1	0.1					0.1		0.1	0.1		0.1	0.1	0.1	0.1
324	19																								
325	19															0.1		0.1							
326	19			0.1													0.1	0.1			0.1				
327	19															0.1	0.1	0.1	0.1						
328	6-19																0.1	0.1	0.1						
329	19															0.1	0.1	0.1							
330	9-19																								
331	19												0.1	0.1	0.1										
332	19																								
333	19											0.1													
334	15					0.1																			
335	9-16			0.5		2.5	0.8				1.9	2.1													
336	19																			0.1					
337	10																								
338	19										0.1		0.1												
339	19																							0.1	0.1
340	3-19		0.1		0.1	0.1				0.1		0.1			0.1									0.1	0.1
341	8-9					0.1				0.1		0.1													
342	8-9																								
343	4-8																								
344	6-7				0.1																				
345	19																			0.1		0.1	0.1	0.1	0.1
346	19																			0.1		0.1	0.1	0.1	0.1
347	9-16																								
348	3-10	0.1	0.1																						
349	4-13	0.1		0.1																					
350	6-9												0.1												
351	19												0.1												
352	19																0.1								
353	3-10			0.1	0.1	0.1																	0.2	0.1	
354	19															0.1									
355	6				0.1																				
356	19																								
357	19																								
358	17				0.6	1.4						0.8													
359	19					0.9																2.7			2.9
360	19			0.5	1.3	1.5													2.6						
361	19				1.2					1.8			2.7												
362	19																			2.4					
363	19											0.8		2.1					1.8				3.1		
364	19					0.3		0.8					1.3							0.9			2.5		
365	19																								
366	19																								
367	19	0.8						1.5																	
368	17				1.3	2.7						1.2													
369	6-9																								
370	4-16			0.8																				1.9	
371	4-15			0.6																			1.1		
372	4-15											0.6													
373	3-8	7.3	1.5													2.6	9.3								
374	4-8	6.8	10.2	6.1			1.2												4.4						
375	4-7		2.3	0.6																					
376	3-17		0.8		1.3						1.0	1.5	1.6	2.0	1.4	1.8	2.7				7.8		3.7		
377	12										0.5														
378	4-7										0.8														
379	8-13			1.4																	3.6				
380	6-7																								
381	8-15														2.4										
382	5-9																							1.4	
383	4-17												0.3												

A. Species List

1. *Blidingia marina*
2. *Bryopsis plumosa*
3. *Capsosiphon aurealus*
4. *Chaetomorpha linum*
5. *Chaetomorpha melagonium*
6. *Chaetomorpha spp. (Un.)*
7. *Chaetomorpha tortuosa*
8. *Cladophora arcta*
9. *Cladophora flexuosa*
10. *Cladophora fracta*
11. *Cladophora rectangularis*
12. *Cladophora rupestris*
13. *Cladophora utriculosa*
14. *Codium adherens*
15. *Codium bursa*
16. *Codium fragilis*
17. *Codium tomentosus*
18. *Derbesia marina*
19. *Enteromorpha compressa*
20. *Enteromorpha intestinalis*
21. *Enteromorpha linza*
22. *Enteromorpha prolifera*
23. *Monostroma fuscum*
24. *Percusaria percusa*
25. *Prasiola stipitata*
26. *Rhizoclonium riparum*
27. *Spongomorpha arcta*
28. *Ulothrix flacca*
29. *Ulothrix implexa*
30. *Ulothrix speciosa*
31. *Ulva lactuca*
32. *Urospora bangioides*
33. *Urospora isogona*
34. *Urospora spp. (Un).*
35. *Alaria esculentra*
36. *Ascophyllum nodosum*
37. *Asperococcus bullosus*
38. *Chaeptopteris plumosa*
39. *Chorda filum*
40. *Chordaria divaricata*
41. *Chordaria flagelliformis*
42. *Colpomenia peregrina*
43. *Cutleria multifida*
44. *Cystoseria nodicaulis*
45. *Desmarestia aculeata*
46. *Desmotrichium undulatum*
47. *Dictyota dichotoma*
48. *Dictyota membranaceum*
49. *Ectocarpus confervoides*
50. *Ectocarpus fasciculatus*
51. *Ectocarpus minimus*
52. *Ectocarpus siliculosus*

Species List (continued)

53. *Himanthalia lorea*
54. *Giffordia granulosa*
55. *Laminaria digitata*
56. *Laminaraia hyperborea*
57. *Laminaria saccharina*
58. *Leathesia difformis*
59. *Litosiphon laminariae*
60. *Petaloria fascia*
61. *Phaeosaccion collinsii*
62. *Phyllitis fascia*
63. *Phyllitis zosterofilia*
64. *Punctaria lattifolium*
65. *Punctaria tennuisima*
66. *Scytosiphon lomentarius*
67. *Stilophora rhizodes*
68. *Striaria attenuata*
69. *Antithamnion cruciatum*
70. *Antithamnion floccosum*
71. *Audouinella efflorescens*
72. *Audouinella seiriolana*
73. *Audouinella thuretti*
74. *Bangia atro-pupurea*
75. *Brongniartella byssoides*
76. *Calliblepharis ciliata*
77. *Callosiphoria lacinata*
78. *Callosiphoria verrucosa*
79. *Callithamnion arbuscula*
80. *Callithamnion corymbosum*
81. *Callithamnion hookeri*
82. *Callithamnion spp. (Un)*
83. *Catanella caespitosa*
84. *Ceramium arborescens*
85. *Ceramium fustigiata*
86. *Ceramium rubrum*
87. *Ceramium strictum*
88. *Chondrus crispus*
89. *Corallina officinalis*
90. *Dasya hutchinsae*
91. *Delesseria sanguinea*
92. *Dilsea carnosa*
93. *Dudresnaya verticillata*
94. *Dumontia incrassata*
95. *Erythrotrichia carnea*
96. *Furcellaria fastigiata*
97. *Gelidium lattifolium*
98. *Gelidium pussilla*
99. *Gracilaria verrucosa*
100. *Griffithsia floculosa*
101. *Gymnogongrus norvegica*
102. *Gymnogongrus patens*
103. *Halopteris filicina*
104. *Halarachnion ligulatum*
105. *Heterosiphoria plumosa*
106. *Hildenbrandia prototypus*

Species List (continued)

107. *Hypoglossum woodwardii*
108. *Laurencia obtusa*
109. *Laurencia pinnatifida*
110. *Lithophyllum crovani*
111. *Lithophyllum incrustans*
112. *Lithophyllum orbiculatum*
113. *Lithothamnion calcareum*
114. *Lithothamnion lenormandii*
115. *Lithothamnion membranacea*
116. *Lomentaria clavellosa*
117. *Microcladia glandulosa*
118. *Myriogramme Bonnemaissia*
119. *Myriogramme spp. (Un)*
120. *Nemalion helminthoides*
121. *Odonthalia dentata*
122. *Petrocelis cruenta*
123. *Petrocelis Hennedyi*
124. *Phycodrys rubens*
125. *Phyllophora Brodiaei*
126. *Platoma marginifera*
127. *Platoma spp. (Un)*
128. *Plocamium cartilagineum*
129. *Plocamium coccinea*
130. *Plumaria elegans*
131. *Polysiphonia Brodiaei*
132. *Polysiphonia elongata*
133. *Polysiphonia fastigiata*
134. *Polysiphonia fibrata*
135. *Polysiphonia furcellata*
136. *Polysiphonia nigrescens*
137. *Polysiphonia spp. (Un)*
138. *Polysiphonia urceolata*
139. *Polysiphonia violacea*
140. *Porphyra linearis*
141. *Porphyra umbilicalis*
142. *Pterosiphonia thuyoides*
143. *Pterosiphonia spp. (Un)*
144. *Ptilota plumosa*
145. *Rhododermis elegans*
146. *Rhodomela subfusca*
147. *Rhodophyllis bifida*
148. *Rhodymenia palmata*
149. *Sphaeroccocus coronopfolius*
150. *Grantia compressa*
151. *Halichondria cinerea*
152. *Halichondria panicea*
153. *Halichondria sanguinea*
154. *Halichondria spp. (Un)*
155. *Halisarca dujohni*
156. *Hymeniacidon sanguinea*
157. *Leucosolenia complicata*
158. *Leucosolenia coriacea*
159. *Leucosolenia fragilis*
160. *Myxilla incrustans*

Species List (continued)

161. *Ophlitaspongia seiriolana*
162. *Oscarella lobularis*
163. *Pachymatisma johnstoni*
164. *Sycon ciliatum*
165. *Sycon coronatum*
166. *Actinia equina*
167. *Adamsia pallata*
168. *Anemonia sulcata*
169. *Bolocero spp. (Un)*
170. *Bougainvillia ramosa*
171. *Campanularia flexuosa*
172. *Campanularia hincksii*
173. *Campanularia neglecta*
174. *Campanularia volubilis*
175. *Clava cornea*
176. *Clava squamata*
177. *Coryne muscoides*
178. *Coryne pusilla*
179. *Coryne vermicularis*
180. *Coryne verticillata*
181. *Eudendrium rameum*
182. *Eudendrium ramosa*
183. *Haliclystys auricula*
184. *Hormathia digitata*
185. *Hydrallmania falcata*
186. *Metridium senile*
187. *Obelia dichotoma*
188. *Obelia geniculata*
189. *Plumularia halecoides*
190. *Plumularia setacea*
191. *Sagartia sphyrodeta*
192. *Sagartia troglyoides*
193. *Sertularella polyzonias*
194. *Sertularia operculata*
195. *Sertularia spp. (Un)*
196. *Syncoryne eximia*
197. *Syncoryne gravata*
198. *Telia felini*
199. *Telia lofotensis*
200. *Telia tuberculata*
201. *Tubularia indivisa*
202. *Tubularia larynx*
203. *Amphiporus lactifloreus*
204. *Apomatus similis*
205. *Autolytus pictus*
206. *Eunermertes neesii*
207. *Lineus ruber*
208. *Lineus spp. (Un)*
209. *Prosorhonchmus claparedi*
210. *Syllis prolifera*
211. *Tetrastema melanocephalum*
212. *Filograna implexa*
213. *Hydroides elegans*
214. *Lanice conchilega*

Species List (continued)

215. *Lepidonotus squamata*
216. *Nereis pelagica*
217. *Nereis virens*
218. *Nereis spp. (Un)*
219. *Perinereis cultrifera*
220. *Placostegas tridentatus*
221. *Polydora ciliata*
222. *Pomatoceros triqueter*
223. *Sabellaria spinulosa*
224. *Sabellaria spp. (Un)*
225. *Serpula vermicularis*
226. *Spirorbis echinatus*
227. *Spirorbis spirorbis*
228. *Spirorbis vitreus*
229. *Amphithoe rubricata*
230. *Anthura gracilis*
231. *Balanus balanoides*
232. *Balanus balanus*
233. *Balanus crenatus*
234. *Balanus hamerii*
235. *Balanus improvisus*
236. *Cancer pagurus*
237. *Caprella linearis*
238. *Corophium volutator*
239. *Galathea strigosa*
240. *Gammarus locusta*
241. *Harmothoe imbricata*
242. *Idothea baltica*
243. *Idothea granulosa*
244. *Idothea pelagica*
245. *Jaera marina*
246. *Janira maculosa*
247. *Janiropsis breviremis*
248. *Jassa falcata*
249. *Lepas anatifera*
250. *Lepas hilli*
251. *Ligia oceanica*
252. *Macropodia rostrata*
253. *Nymphon gracile*
254. *Phthisica marina*
255. *Pinnotheres pisum*
256. *Porcellana longicornis*
257. *Porcellana platycheles*
258. *Portunus depurator*
259. *Pycnogonum littorale*
260. *Acanthochitona crinatus*
261. *Acmaea tessulata*
262. *Acmaea virginea*
263. *Aeolidia papillosa*
264. *Anomia cepa*
265. *Anomia epphibbium*
266. *Anomia spp. (Un)*
267. *Arca transversa*
268. *Archidoris cinerea*

Species List (continued)

269. *Archidoris pseudoargus*
270. *Buccinum undatum*
271. *Buccinum spp. (Un)*
272. *Cingula alderi*
273. *Colos gracilis*
274. *Crepidula fornicata*
275. *Dendronotus frondosus*
276. *Doto coronatum*
277. *Doto fragilis*
278. *Facelina auriculata*
279. *Goniodoris nodosum*
280. *Hiatella arctica*
281. *Jorunna tomentosa*
282. *Limacia clavigera*
283. *Lacuna pallidula*
284. *Lacuna vincta*
285. *Modiolus barbatus*
286. *Modiolus modiolus*
287. *Modiolus phaseolinus*
288. *Margarites spp. (Un)*
289. *Monia patelliformis*
290. *Musculus discors*
291. *Mytilus edulis*
292. *Onchidoris fusca*
293. *Ostrea edulis*
294. *Patina pallucida*
295. *Pecten maximus*
296. *Punctarella spp. (Un)*
297. *Spisula elliptica*
298. *Trinchesia viridis*
299. *Aetea scabra*
300. *Aetea truncata*
301. *Alcyonidium spp. (Un)*
302. *Amphiblestrum flemingii*
303. *Bicellaria alderi*
304. *Bugula avicularia*
305. *Bugula flabellata*
306. *Bugula fulva*
307. *Bugula neritina*
308. *Bugula turbinata*
309. *Callopora craticulata*
310. *Callopora dumerilli*
311. *Callopora lineata*
312. *Carbasea carbasea*
313. *Cellaria salicornioides*
314. *Cellaria sinuosa*
315. *Celleporella hyalina*
316. *Chartella barleeii*
317. *Chartella papyracea*
318. *Crisia eburnea*
319. *Cryptosula ramosa*
320. *Dendrobeania murrayana*
321. *Electra crustulentra*
322. *Electra monostachys*

Species List (continued)

323. *Electra pilosa*
324. *Epistomia spp. (Un)*
325. *Eschara securifrons*
326. *Esherella abyssicola*
327. *Esherella immersa*
328. *Esherella klugei*
329. *Esherella lacqueata*
330. *Esharina hyndmanni*
331. *Escharina johnstoni*
332. *Escharina vulgaris*
333. *Escharoides coccinea*
334. *Eucratea loricata*
335. *Flustra foliacea*
336. *Haplopoma planum*
337. *Hippodinella lata*
338. *Hippothoa divaricata*
339. *Lagenipora pygmaea*
340. *Membranipora membranaceum*
341. *Membraniporella nitida*
342. *Micropora coriacea*
343. *Phylactella collaris*
344. *Pyripora spp. (Un)*
345. *Porelloides laevis*
346. *Porelloides struma*
347. *Schizobrachiella sanguinea*
348. *Scuparia ambigua*
349. *Scuparia chelata*
350. *Scrupocellaria spp. (Un)*
351. *Sertella spp. (Un)*
352. *Stomachetosella sinuosa*
353. *Tegella unicornis*
354. *Tegella spp. (Un)*
355. *Tubulipora spp. (Un)*
356. *Umbonela verrucosa*
357. *Watersipora complanta*
358. *Amphipholis squamata*
359. *Amphiura spp. (Un)*
360. *Asterias rubens*
361. *Astropecten irregularis*
362. *Ophiopholis aculeata*
363. *Ophiothrix fragilis*
364. *Ophiura albida*
365. *Ophiura texturata*
366. *Paracentrotus lividus*
367. *Psammechinus miliaris*
368. *Strongylocentrotus drobrachiensis*
369. *Archidistema aggregatum*
370. *Ascidia mentula*
371. *Ascidiella aspersa*
372. *Ascidiella scabra*
373. *Botryllus leachi*
374. *Botryllus nigrum*

Species List (continued)

375. Botryllus schlosseri

376. Ciona intestinalis

377. Didemnum gelatinosum

378. Diplosoma listerianum

379. Molgula manhattensis

380. Molgula occulta

381. Polycarpa rustica

382. Polyclinum aurantium

383. Styela clava

B. Sites List

UK1 Culdrose 1980

UK2 Culdrose 1981

UK3 Fowey 1978

UK4 Fowey 1980

UK5 Portland 1978

UK6 Portland 1979

UK7 Portland 1980

UK8 Portland 1982

UK9 Solent 1978

UK10 Solent 1979

UK11 Solent 1980a

UK12 Solent 1980b

UK13 Solent 1981

UK14 Cardigan Bay 1979

UK15 Cardigan Bay 1980

UK16 I.O.M. 1979

UK17 I.O.M. 1980

UK18 Loch Ewe 1980

UK19 Loch Ewe 1981

UK20 Raasay 1978

UK21 Raasay 1980

UK22 S.O.H. 1978

UK23 S.O.H. 1979

UK25 S.O.H. 1981

UK25 St. Kilda 1978

UK26 St. Kilda 1979

UK27 St. Kilda 1981

UK28 North Sea 1979

UK29 North Sea 1980

UK30 May Island 1979

UK31 May Island 1980

UK32 Moray Firth 1983

UK33 Tees 1980

UK34 Tyne 1980

UK35 Flamborough 1978

UK36 Flamborough 1979

UK37 Flamborough 1980

UK38 Flamborough 1982

NO1 Sandfjord

NO2 Farsund

NO3 Kristiansand

NO4 Stavanger

NO5 Bergen 1

NO6 Bergen 2

NO7 Vigra

NO8 Vikna

NO9 Leka

NO10 Vega

C. Depth Zones (DZ)

1.	+ 0.3m	11.	12 - 14m
2.	0 - 0.5m	12.	14 - 16m
3.	0.5 - 1.2m	13.	16 - 18m
4.	1.2m	14.	18 - 20m
5.	1.2 - 1.6m	15.	20 - 25m
6.	1.6 - 3.5m	16.	25 - 30m
7.	3.5 - 5m	17.	30 - 40m
8.	5 - 7.5m	18.	40 - 50m
9.	7.5 - 10m	19.	50 - 60m
10.	10 - 12m		

Appendix 4

'Actinula growth' in *Tubularia larynx*.

Mean stolonic growth (mm), and the effect of temperature and salinity. Values are means of 10 observations per temperature x salinity combination.

Temperature (°C)	Salinity (°/oo)	TIME (days) 2	4	6	8	10	12	14
18	34	1.2	2.1	4.1	6.2	10.1	17.0	26.2
	30	1.2	1.9	3.0	5.8	8.6	13.5	21.5
	28	1.1	1.9	2.4	3.8	5.0	7.0	10.1
12	34	1.4	2.5	3.8	5.7	9.4	15.1	22.4
	30	1.4	2.3	3.7	5.1	7.4	13.3	21.1
	28	1.4	1.8	2.5	3.3	4.4	6.7	8.8
6	34	1.1	1.6	2.2	3.2	4.9	7.1	10.3
	30	1.0	1.4	1.8	2.5	3.4	4.0	5.1
	28	1.1	1.3	1.5	1.9	2.5	3.1	3.8

Appendix 5

'Colony growth' in *Tubularia larynx*.

Mean rate of hydranth production, and the effect of temperature and salinity. Values are means of 12 observations per temperature x salinity combination.

18°C

Time (days)	34°/oo $\bar{x}$	34°/oo SD	30°/oo $\bar{x}$	30°/oo SD	28°/oo $\bar{x}$	28°/oo SD
3	1.8	0.750	1.5	0.522	-	-
6	2.0	0.852	2.3	0.646	-	-
9	3.0	0.951	3.0	1.000	1.6	0.650
12	3.7	0.965	3.8	1.250	1.9	0.660
15	4.0	1.040	4.8	1.078	2.6	0.791
18	5.6	1.431	5.3	1.206	3.0	0.510
21	6.3	1.281	6.2	1.420	3.25	0.450
24	6.4	1.240	7.5	1.368	4.0	0.857
27	8.8	1.262	8.0	1.341	6.0	1.160
30	9.8	1.640	9.4	1.361	6.25	1.050
33	12.7	1.655	10.5	1.368	6.75	1.280
36	13.4	1.439	11.1	1.375	6.5	1.620
12°C						
3	1.4	0.522	-	-	-	-
6	1.6	0.650	1.8	0.410	-	-
9	2.8	0.712	2.0	0.000	-	-
12	2.9	0.530	3.6	1.156	1.7	0.450
15	3.9	0.900	3.8	1.190	1.9	0.280
18	4.8	0.750	4.6	0.924	2.5	0.610
21	7.3	0.674	6.7	0.646	2.7	0.750
24	8.9	0.831	6.8	0.750	4.0	0.774
27	11.2	1.078	7.9	0.539	3.7	0.961
30	13.7	1.555	9.5	1.121	5.0	1.200
33	14.3	1.432	10.5	1.123	5.2	1.280
36	15.3	1.120	12.6	1.348	6.0	0.952

6°C

Time (days)	34°/oo		30°/oo		28°/oo	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
3	1.6	0.491	-	-	-	-
6	2.1	0.300	-	-	-	-
9	2.8	0.615	-	-	-	-
12	3.5	0.662	1.4	0.522	-	-
15	4.2	1.103	1.7	0.646	1.8	0.603
18	5.2	1.192	2.6	0.539	1.8	0.603
21	5.9	1.155	2.7	0.467	3.1	0.700
24	6.6	1.562	3.3	0.447	4.9	1.100
27	7.5	1.213	4.2	0.982	2.8	1.126
30	8.0	1.305	4.2	0.539	3.3	0.741
33	8.6	2.010	4.2	0.750	3.4	0.699
36	9.4	1.400	4.9	0.942	4.1	1.013

Appendix 6

'Colony growth' in *Tubularia larynx*.

Mean rate of hydranth production and the effect of ration. Values are the means of 12 observations per experimental group, in developing colonies fed 3 times per day with one *Artemia* nauplius per hydranth.

18°C

Time (days)	34°/oo $\bar{x}$	34°/oo SD	30°/oo $\bar{x}$	30°/oo SD	28°/oo $\bar{x}$	28°/oo SD
3	1.6	0.051	1.6	0.530	1.1	0.028
6	3.5	0.900	3.2	0.836	1.4	0.798
9	5.3	0.980	4.2	0.942	2.1	0.688
12	6.6	1.232	5.2	0.871	2.8	0.389
15	7.3	1.556	6.1	0.835	3.2	0.621
18	7.9	1.724	7.5	1.032	3.6	0.492
21	9.1	1.563	8.5	1.206	4.1	0.834
24	9.9	1.620	9.6	1.201	4.6	0.778
27	10.7	1.464	10.4	1.280	5.3	1.206
30	11.9	1.242	11.4	1.124	5.7	0.948
33	12.8	1.405	12.3	1.413	6.2	0.918
36	13.9	1.628	13.2	1.506	7.3	1.059
12°C						
3	1.9	0.700	1.9	0.310	-	-
6	3.0	0.744	2.3	0.674	-	-
9	4.9	1.136	3.3	0.904	1.5	0.522
12	5.8	1.078	4.5	0.934	2.1	0.539
15	6.9	1.300	5.9	1.300	3.1	0.700
18	8.4	0.934	6.7	1.190	3.9	0.710
21	10.8	1.470	8.5	0.934	4.9	0.813
24	12.0	1.341	9.4	1.368	5.8	0.750
27	14.3	1.026	11.1	1.221	6.4	0.924
30	14.5	1.210	12.6	1.361	6.9	0.831
33	16.1	1.320	13.1	1.640	7.2	0.786
36	16.5	1.035	13.6	1.501	7.5	0.934

Appendix 7

'Colony growth' in *Tubularia larynx*.

Mean rate of hydranth production, and the effect of light. Values are means of 12 observations per light condition.

A = 12 hr. daylength period (4×10^{-3} lux)

B = 'shaded' conditions (4×10^{-3} lux)

C = continuous light (4×10^{-3} lux)

D = continuous dark.

Time (days)	A		B	
	$\bar{x}$	SD	$\bar{x}$	SD
3	1.8	0.638	1.7	0.731
6	1.9	0.724	2.4	0.935
9	2.3	0.891	2.8	0.962
12	3.4	0.872	3.6	1.204
15	3.9	1.051	5.2	1.278
18	4.6	1.207	6.1	1.465
21	5.8	1.229	7.9	1.392
24	8.2	1.265	8.5	1.624
27	10.1	1.491	9.9	1.581
30	11.8	1.283	12.2	1.422
33	12.6	1.392	12.8	1.429
36	13.2	1.056	14.1	1.407
	C		D	
3	1.8	0.552	2.0	0.622
6	2.2	0.642	2.2	0.714
9	2.8	0.688	3.1	0.865
12	3.0	0.951	3.6	0.928
15	3.8	0.965	4.2	1.019
18	4.7	1.012	4.7	1.183
21	5.9	1.225	6.2	1.207
24	8.2	1.416	7.3	1.192
27	10.3	1.465	9.4	1.166
30	11.4	1.203	11.1	1.305
33	12.0	1.211	12.5	1.227
36	13.6	1.103	13.6	1.128

Appendix 8

Larval development in *Pomatoceros triqueter*. The mean rate of increase in larval length (um) and the effect of temperature and salinity. Values are means of 10 observations per experimental condition.

Day / Salinity	1	4	8	12	16
1. 20°C					
34‰	84	180	237	285	318
30‰	85	194	248	292	325
26‰	88	170	231	275	302
22‰	88	171	205	246	263
18‰	86	181	298	250	266
2. 15°C					
34‰	89	175	243	276	314
30‰	84	174	235	268	295
26‰	85	182	240	275	299
22‰	92	171	200	245	278
18‰	91	164	294	236	260
3. 10°C					
34‰	88	184	234	265	301
30‰	86	188	231	272	305
26‰	78	186	222	253	284
22‰	82	169	196	241	260
18‰	85	155	182	215	245
4. 6°C					
34‰	75	128	204	229	242
30‰	82	113	179	213	236
26‰	83	125	178	208	227
22‰	86	114	155	181	189
18‰	80	118	164	190	213

APPENDIX 9

Laboratory "tubegrowth" in *Pomatoceros triqueter*. The mean rate of increase in fresh weight, including tube, (g), and the effect of temperature, salinity and calcium concentration. Values are means of 10 observations per experimental condition.

20°C

	34°/oo		26°/oo		18°/oo		10°/oo	
Wks	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
0	0.009	0.005	0.008	0.003	0.007	0.003	0.008	0.003
1	0.015	0.006	0.017	0.005	0.022	0.007	0.005	0.002
2	0.019	0.011	0.024	0.005	0.024	0.009	0.004	0.002
3	0.017	0.008	0.027	0.005	0.021	0.006	0.003	0.002
4	0.019	0.006	0.026	0.007	0.016	0.007	0.003	0.002
5	0.019	0.009	0.040	0.051	0.008	0.003	0.004	0.002
6	0.018	0.005	0.022	0.005	0.005	0.004	0.003	0.002
7	0.018	0.005	0.019	0.006	0.006	0.004	0.002	0.001
8	0.021	0.007	0.014	0.004	0.005	0.003	0.002	0.001
9	0.026	0.010	0.012	0.005	0.005	0.002	-	-
10	0.019	0.008	0.010	0.004	0.003	0.002	-	-
11	0.011	0.006	0.008	0.005	0.002	0.001	-	-
12	0.009	0.004	0.009	0.004	0.003	0.001	-	-
13	0.009	0.005	0.007	0.004	0.002	0.001	-	-
14	0.005	0.003	0.007	0.003	0.002	0.001	-	-
15	0.006	0.003	0.005	0.003	0.002	0.001	-	-
$+Ca^{++}$								
0	0.008	0.004	0.007	0.002	0.008	0.002	0.007	0.003
1	0.017	0.005	0.020	0.004	0.023	0.005	0.011	0.003
2	0.024	0.005	0.033	0.007	0.023	0.006	0.008	0.004
3	0.027	0.005	0.046	0.011	0.021	0.003	0.007	0.002
4	0.026	0.007	0.046	0.014	0.022	0.005	0.007	0.004
5	0.040	0.051	0.018	0.005	0.019	0.005	0.006	0.003
6	0.022	0.005	0.017	0.004	0.014	0.005	0.006	0.003
7	0.019	0.006	0.013	0.003	0.012	0.004	0.005	0.003
8	0.014	0.004	0.013	0.003	0.011	0.005	0.004	0.002
9	0.012	0.005	0.013	0.003	0.010	0.005	-	-
10	0.010	0.004	0.014	0.003	0.009	0.005	-	-
11	0.008	0.005	0.010	0.004	0.008	0.004	-	-
12	0.009	0.004	0.010	0.005	0.005	0.003	-	-
13	0.007	0.004	0.004	0.001	0.004	0.002	-	-
14	0.007	0.003	0.004	0.002	0.003	0.002	-	-
15	0.005	0.003	0.003	0.001	0.003	0.002	-	-

15°C

	34°/oo		26°/oo		18°/oo		10°/oo	
Wks	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
0	0.008	0.003	0.006	0.003	0.007	0.004	0.006	0.002
1	0.022	0.012	0.017	0.005	0.011	0.005	0.006	0.002
2	0.19	0.006	0.022	0.008	0.014	0.005	0.005	0.002
3	0.021	0.006	0.015	0.004	0.013	0.005	0.003	0.002
4	0.021	0.008	0.017	0.008	0.013	0.006	0.003	0.001
5	0.019	0.009	0.017	0.004	0.012	0.004	0.002	0.001
6	0.016	0.006	0.014	0.005	0.009	0.003	0.002	-
7	0.013	0.004	0.012	0.003	0.009	0.004	-	-
8	0.014	0.006	0.012	0.004	0.007	0.003	-	-
9	0.010	0.004	0.010	0.005	0.008	0.003	-	-
10	0.012	0.004	0.008	0.004	0.006	0.002	-	-
11	0.009	0.004	0.010	0.005	0.006	0.003	-	-
12	0.008	0.003	0.007	0.004	0.004	0.002	-	-
13	0.007	0.004	0.006	0.003	0.003	0.001	-	-
14	0.009	0.010	0.005	0.003	0.002	0.001	-	-
15	0.005	0.004	0.005	0.003	0.003	0.001	-	-
$+Ca^{++}$								
0	0.007	0.002	0.009	0.003	0.007	0.003	0.008	0.003
1	0.017	0.004	0.018	0.003	0.016	0.003	0.011	0.002
2	0.020	0.005	0.022	0.004	0.019	0.005	0.007	0.003
3	0.021	0.004	0.023	0.004	0.019	0.007	0.007	0.002
4	0.019	0.005	0.020	0.006	0.023	0.012	0.004	0.002
5	0.018	0.003	0.018	0.003	0.016	0.004	0.004	0.002
6	0.015	0.004	0.019	0.005	0.015	0.003	0.003	0.002
7	0.012	0.004	0.021	0.005	0.011	0.003	0.003	0.002
8	0.012	0.005	0.016	0.004	0.012	0.004	0.002	0.001
9	0.011	0.004	0.014	0.003	0.012	0.005	0.002	0.001
10	0.010	0.004	0.015	0.003	0.011	0.004	-	-
11	0.010	0.004	0.012	0.003	0.011	0.004	-	-
12	0.010	0.004	0.013	0.003	0.010	0.003	-	-
13	0.008	0.002	0.011	0.003	0.007	0.003	-	-
14	0.007	0.004	0.008	0.003	0.007	0.003	-	-
15	0.008	0.003	0.006	0.004	0.006	0.002	-	-

10°C

	34°/oo		26°/oo		18°/oo		10°/oo	
Wks	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
0	0.009	0.004	0.008	0.004	0.007	0.003	0.008	0.004
1	0.019	0.007	0.013	0.006	0.011	0.004	0.004	0.001
2	0.016	0.008	0.014	0.005	0.010	0.005	0.003	0.002
3	0.016	0.003	0.015	0.004	0.008	0.004	0.003	0.001
4	0.017	0.006	0.013	0.006	0.009	0.005	0.002	0.001
5	0.013	0.004	0.011	0.003	0.008	0.003	0.002	0.001
6	0.012	0.005	0.012	0.004	0.007	0.003	0.002	0.001
7	0.013	0.003	0.012	0.004	0.006	0.003	-	-
8	0.009	0.004	0.012	0.003	0.006	0.003	-	-
9	0.008	0.003	0.008	0.004	0.007	0.004	-	-
10	0.008	0.005	0.008	0.004	0.005	0.002	-	-
11	0.008	0.004	0.008	0.004	0.005	0.002	-	-
12	0.005	0.003	0.007	0.004	0.003	0.001	-	-
13	0.006	0.003	0.007	0.003	0.003	0.001	-	-
14	0.004	0.003	0.005	0.002	0.002	0.001	-	-
15	0.006	0.002	0.003	0.001	0.002	0.001	-	-
$+Ca^{++}$								
0	0.008	0.003	0.008	0.004	0.007	0.002	0.007	0.002
1	0.010	0.003	0.016	0.005	0.012	0.003	0.008	0.003
2	0.018	0.005	0.016	0.005	0.019	0.006	0.005	0.003
3	0.017	0.004	0.025	0.007	0.013	0.006	0.003	0.001
4	0.017	0.006	0.016	0.003	0.017	0.005	0.002	0.001
5	0.019	0.006	0.016	0.003	0.016	0.005	0.002	0.001
6	0.016	0.005	0.012	0.004	0.014	0.005	0.002	0.001
7	0.014	0.003	0.011	0.003	0.011	0.003	-	-
8	0.014	0.005	0.014	0.005	0.010	0.003	-	-
9	0.009	0.004	0.014	0.003	0.009	0.004	-	-
10	0.008	0.001	0.010	0.003	0.010	0.003	-	-
11	0.008	0.003	0.011	0.004	0.008	0.004	-	-
12	0.009	0.004	0.010	0.003	0.008	0.003	-	-
13	0.006	0.003	0.007	0.003	0.006	0.003	-	-
14	0.004	0.003	0.006	0.003	0.006	0.003	-	-
15	0.005	0.002	0.006	0.002	0.005	0.002	-	-

6°C

	34°/oo		26°/oo		18°/oo		10°/oo	
Wks	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
0	0.008	0.003	0.007	0.003	0.007	0.002	-	-
1	0.004	0.002	0.005	0.002	0.004	0.002	-	-
2	0.004	0.002	0.004	0.001	0.002	0.001	-	-
3	0.005	0.002	0.003	0.001	0.002	0.001	-	-
4	0.003	0.002	0.002	0.001	0.002	0.001	-	-
5	0.003	0.002	0.002	0.001	0.002	0.001	-	-
6	0.003	0.001	-	-	-	-	-	-
7	0.003	0.001	-	-	-	-	-	-
8	0.002	0.001	-	-	-	-	-	-
9	0.002	0.001	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-
$+Ca^{++}$								
0	0.008	0.002	0.008	0.002	0.007	0.001	-	-
1	0.004	0.002	0.005	0.002	0.006	0.002	-	-
2	0.004	0.002	0.003	0.001	0.003	0.001	-	-
3	0.004	0.001	0.003	0.001	0.003	0.001	-	-
4	0.003	0.001	0.002	0.001	0.003	0.001	-	-
5	0.002	0.001	0.002	0.001	0.002	0.001	-	-
6	0.002	0.001	-	-	-	-	-	-
7	0.002	0.001	-	-	-	-	-	-
8	0.002	0.001	-	-	-	-	-	-
9	0.002	0.001	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-

Appendix 10

Laboratory 'tubegrowth' in *Pomatoceros triqueter*. The mean rate of increase in fresh weight including tube, (g), and the effect of daylength period (light intensity, 4×10^{-3} lux). Values are means of 10 observations per experimental condition.

	12 hrs		8 hrs		4 hrs		0 hrs	
Wks	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
0	0.007	0.003	0.007	0.003	0.006	0.003	0.008	0.004
1	0.023	0.010	0.020	0.006	0.019	0.008	0.019	0.008
2	0.014	0.005	0.020	0.008	0.022	0.010	0.020	0.006
3	0.017	0.008	0.019	0.007	0.016	0.007	0.016	0.005
4	0.020	0.008	0.018	0.007	0.013	0.006	0.014	0.006
5	0.012	0.005	0.015	0.007	0.013	0.006	0.013	0.004
6	0.012	0.004	0.012	0.004	0.016	0.003	0.009	0.003
7	0.009	0.005	0.012	0.005	0.017	0.003	0.016	0.003
8	0.012	0.008	0.010	0.004	0.008	0.003	0.009	0.006

Appendix 11

Field 'tubegrowth' in *Pomatoceros triqueter*. The mean rate of increase in fresh weight including tube, (g), at two buoy sites (F buoy and N1 buoy) near Plymouth, and the effect of depth. Values are means of 20 observations per depth.

F. Buoy (i) IM

TIME (months)	1	2	3	4	5	6	7	8	9	10
0 (starting weight)	0.0028	0.0067	0.0142	0.0034	0.0050	0.0094	0.0073	0.0086	0.0060	0.0021
A	0.0472	0.0794	0.0426	0.0487	0.0492	0.0483	0.0515	0.0572	0.0403	0.0386
S	0.0517	0.0419	0.0312	0.0466	0.0308	0.0365	0.0674	0.0269	0.0618	0.0351
O	0.0648	0.0144	0.0592	0.0201	0.0406	0.0282	0.0522	0.0320	0.0094	0.0155
N	0.0120	0.0251	0.0071	0.0170	0.0138	0.0180	0.0149	0.0389	0.0064	0.0110
D	0.0084	0.0119	0.0052	0.0287	0.0141	0.0043	0.0103	0.0061	0.0126	0.0118
J	0.0032	0.0137	0.0039	0.0228	0.0100	0.0045	0.0112	0.0015	-	0.0041
F	0.0021	0.0116	0.0023	0.0008	0.0019	-	0.0210	0.0032	-	0.0022
M	0.0170	0.0024	0.0247	0.0017	0.0051	-	0.0114	0.0070	-	0.0283
A	0.0128	0.0236	0.0062	0.0103	0.0433	-	0.0252	0.0216	-	0.0196
M	0.0130	0.0314	0.0268	0.0363	0.0275	-	0.0104	0.0318	-	0.0236

11	12	13	14	15	16	17	18	19	20	Mean	Standard Deviation
0.0042	0.0027	0.0083	0.0071	0.0021	0.0103	0.0049	0.0036	0.0019	0.0064	0.0058	0.00323
0.0597	0.0381	0.0504	0.0529	0.0348	0.0736	0.0295	0.0341	0.0316	0.0425	0.0477	0.0132
0.0371	0.0247	0.0316	0.0419	-	0.0472	0.0384	0.0570	0.0422	0.0422	0.0416	0.0118
0.0294	0.0272	0.0366	0.0280	-	0.0310	0.0492	0.0400	0.0212	0.0245	0.0312	0.0155
0.0126	0.0170	0.0114	0.0202	-	0.0164	0.0123	0.0244	0.0139	0.0386	0.0172	0.0089
0.0093	0.0248	0.0136	0.0460	-	0.0185	0.0371	0.0121	0.0260	0.0053	0.0161	0.0114
0.0046	0.0181	0.0110	0.0122	-	0.0041	0.0035	0.0272	0.0091	0.0073	0.0095	0.0071
0.0023	0.0073	0.0020	0.0031	-	0.0045	0.0029	0.0016	0.0081	0.0040	0.0038	0.0052
0.0101	0.0258	0.0042	0.0026	-	0.0133	0.0151	0.0037	0.0085	0.0261	0.0128	0.0105
0.0251	0.0050	0.0113	0.0175	-	0.0184	0.0064	0.0139	0.0314	0.0146	0.0180	0.0099
0.0472	0.0139	0.0068	0.0203	-	0.0252	0.0312	0.0117	0.0562	0.0227	0.0258	0.0131

F. Buoy (ii) 5M

TIME (months)	1	2	3	4	5	6	7	8	9	10
0 (starting weight)	0.0052	0.0206	0.0114	0.0029	0.0034	0.0016	0.0047	0.0060	0.0010	0.0046
A	0.0433	0.0962	0.0216	0.0414	0.0384	0.0557	0.0614	0.0475	0.0307	0.0284
S	0.0619	0.0206	0.0379	0.0474	0.0360	0.0318	0.0136	0.0504	0.0452	0.0118
O	0.0282	0.0276	0.0232	0.0358	0.0301	0.0164	0.0464	0.0170	0.0340	0.0154
N	0.0195	0.0216	0.0142	0.0130	0.0226	0.0350	0.0493	0.0180	0.0091	0.0121
D	0.0130	0.0081	0.0073	0.0146	0.0117	0.0124	0.0078	0.0159	0.0043	0.0032
J	0.0052	0.0091	0.0047	0.0082	0.0027	0.0055	0.0101	0.0133	0.0064	0.0040
F	0.0030	0.0042	0.0026	0.0056	0.0134	0.0012	0.0008	0.0086	0.0021	0.0011
M	0.0244	0.0128	0.0135	0.0063	0.0134	0.0081	0.0332	0.0091	0.0095	0.0042
A	0.0072	0.0193	0.0286	0.0123	0.0144	-	0.0426	0.0314	0.0135	0.0118
M	0.0537	0.0283	0.0273	0.0098	0.0637	-	0.0515	0.0304	0.0427	0.0113

11	12	13	14	15	16	17	18	19	20	Mean	Standard Deviation
0.0131	0.0027	0.0036	0.0057	0.0107	0.0093	0.0068	0.0075	0.0115	0.0124	0.0072	0.0048
0.0514	0.0460	0.0437	0.0668	0.0704	0.0339	0.0572	0.0476	0.0316	0.0249	0.0469	0.0178
0.0560	0.0632	0.0265	0.0333	0.0369	0.0225	0.0124	0.0235	0.0456	0.0340	0.0355	0.0156
0.0321	0.0400	0.0148	0.0571	0.0154	0.0203	0.0183	0.0364	0.0440	0.0520	0.0302	0.0129
0.0105	0.0227	0.0119	0.0368	0.0314	0.0231	0.0198	0.0245	0.0293	0.0141	0.0219	0.0100
0.0132	0.0102	0.0084	0.0113	0.0210	0.0158	0.0063	0.0059	0.0124	0.0170	0.0109	0.0046
0.0046	0.0092	0.0051	0.0021	0.0037	0.0106	0.0023	0.0014	0.0090	0.0010	0.0059	0.0034
0.0020	0.0061	0.0031	0.0022	0.0017	0.0006	0.0048	0.0051	0.0012	0.0064	0.0037	0.0031
0.0276	0.0139	0.0040	0.0088	0.0162	-	0.0273	0.0147	0.0083	0.0464	0.0159	0.0117
0.0170	0.0155	0.0273	0.0142	0.0130	-	0.0132	0.0450	0.0108	-	0.0198	0.0111
0.0462	0.0334	0.0227	0.0149	0.0226	-	0.0317	0.0128	0.0255	-	0.0310	0.0160

F. Buoy (iii) IOM

TIME (months)	1	2	3	4	5	6	7	8	9	10
0 (starting weight)	0.0026	0.0056	0.0072	0.0034	0.0047	0.0021	0.0017	0.0048	0.0097	0.0040
A	0.0342	0.0248	0.0370	0.0160	0.0283	0.0492	0.0516	0.0412	0.0423	0.0568
S	0.0394	0.0564	0.0537	0.0216	0.0147	0.0376	0.0282	0.0255	0.0261	0.0346
O	0.0225	0.0256	0.0276	0.0360	0.0221	0.0161	0.0133	0.0272	0.0301	0.0215
N	0.0162	0.0138	0.0218	0.0235	0.0176	0.0125	0.0393	0.0281	0.0190	0.0244
D	0.0157	0.0102	0.0091	0.0130	0.0082	0.0068	0.0113	0.0151	0.0122	0.0076
J	0.0143	0.0051	0.0030	0.0076	0.0021	0.0014	0.0004	0.0060	0.0011	0.0024
F	0.0162	0.0021	0.0064	0.0113	0.0032	0.0241	0.0210	0.0025	0.0076	0.0035
M	0.0151	0.0129	0.0163	0.0120	0.0041	0.0189	0.0117	0.0142	0.0083	0.0079
A	0.0085	0.0126	0.0023	0.0184	-	-	0.0136	0.0212	0.0093	0.0130
M	0.0292	0.0357	0.0103	0.0160	-	-	0.0173	0.0455	0.0206	0.0227

11	12	13	14	15	16	17	18	19	20	Mean	Standard Deviation
0.0018	0.0025	0.0104	0.0047	0.0053	0.0026	0.0057	0.0064	0.0033	0.0086	0.0048	0.0025
0.0382	0.0356	0.0413	0.0201	0.0480	0.0593	0.0294	0.0306	0.0122	0.0311	0.0363	0.0128
0.0416	0.0287	0.0320	0.0092	0.0315	0.0212	0.0472	0.0434	0.0340	0.0270	0.0326	0.0120
0.0223	0.0316	0.0185	0.0152	0.0226	0.0191	0.0241	0.0062	0.0314	0.0320	0.0232	0.0073
0.0201	0.0135	0.0101	0.0212	0.0197	0.0082	0.0175	0.0136	0.0194	0.0091	0.0184	0.0072
0.0062	0.0046	0.0027	0.0163	0.0154	0.0075	0.0122	0.0096	0.0126	0.0114	0.0103	0.0038
0.0071	0.0055	0.0100	0.0024	0.0016	0.0032	0.0073	0.0012	0.0042	0.0039	0.0044	0.0034
0.0221	0.0087	0.0046	0.0180	0.0110	0.0098	0.012	-	0.0033	0.0226	0.0110	0.0075
0.0124	0.0103	-	0.0135	0.0091	0.0129	0.0142	-	0.0164	0.0180	0.0127	0.0037
0.0073	0.0084	-	0.0115	0.0175	0.0062	0.0256	-	0.0183	0.0191	0.0131	0.0068
0.0244	0.0310	-	0.0217	0.0298	0.0323	0.0470	-	0.0104	0.0117	0.0253	0.0113

NI. Buoy (i) IM

TIME (months)	1	2	3	4	5	6	7	8	9	10
0 (starting weight)	0.0135	0.0027	0.0045	0.0086	0.0031	0.0112	0.0041	0.0022	0.0073	0.0052
A	0.0861	0.0584	0.0312	0.0347	0.0693	0.0534	0.0460	0.0312	0.0440	0.0287
S	0.0310	0.0292	0.0430	0.0400	0.0412	0.0871	0.0356	0.0403	0.0382	0.0562
O	0.0241	0.0415	0.0406	0.0424	0.0686	0.0945	0.0719	0.0425	0.0517	0.0692
N	0.0091	0.0110	0.0078	0.0156	0.0204	0.0218	0.0220	0.0312	0.0270	0.0141
D	0.0073	0.0052	0.0102	0.0140	0.0110	0.0186	0.0085	0.0120	0.0210	0.0077
J	0.0124	0.0047	0.0063	0.0122	0.0120	0.0251	0.0103	0.0087	0.0111	0.0036
F	0.0041	0.0079	0.0110	0.0165	0.0095	0.0173	0.0125	0.0015	0.0021	0.0044
M	0.0252	0.0311	0.0158	0.0116	0.0123	0.0217	0.0136	0.0240	0.0189	0.0213
A	0.0183	0.0031	0.0049	0.0072	0.0089	0.0122	0.0144	0.0313	0.0096	0.0102
M	0.0218	0.0243	0.0473	0.0519	0.0302	0.0173	0.0376	0.0534	0.0146	0.0276

11	12	13	14	15	16	17	18	19	20	Mean	Standard Deviation
0.0087	0.0022	0.0041	0.0120	0.0058	0.0049	0.0063	0.0148	0.0024	0.0090	0.0067	0.0039
0.0572	0.0380	0.0453	0.0306	0.0214	0.0309	0.0294	0.0617	0.0472	0.0337	0.0449	0.0162
0.0356	0.0417	0.0240	0.0996	0.0426	0.0521	0.0315	0.0301	0.0391	0.0232	0.0409	0.0135
0.0410	0.0526	0.0414	0.0532	0.0351	0.0480	0.0367	0.0510	0.0346	0.0358	0.0488	0.0164
0.0314	0.0178	0.0096	0.0213	0.0300	0.0112	0.0155	0.0326	0.0254	0.0173	0.0196	0.0080
0.0231	0.0194	0.0104	0.0225	0.0127	0.0084	0.0180	0.0142	0.0036	0.0042	0.0125	0.0061
0.0071	0.0064	0.0050	0.0112	0.0218	0.0047	0.0033	0.0121	0.0089	0.0045	0.0095	0.0057
0.0024	0.0085	0.0072	0.0115	0.0127	0.0031	0.0062	0.0068	0.0111	0.0207	0.0089	0.0056
0.0040	0.0078	0.0122	0.0135	0.0210	0.0152	0.0328	0.0214	0.0138	0.0045	0.0170	0.0078
0.0233	0.0130	0.0063	0.0114	0.0051	0.0246	0.0161	0.0090	0.0140	0.0122	0.0127	0.0071
0.0468	0.0235	0.0376	0.0455	0.0306	0.0617	0.0253	0.0136	0.0204	0.0230	0.0326	0.0141

NI. Buoy (ii) 5M

TIME (months)	1	2	3	4	5	6	7	8	9	10
0 (starting weight)	0.0140	0.0072	0.0031	0.0048	0.0050	0.0024	0.0017	0.0039	0.0056	0.0026
A	0.0424	0.0318	0.0270	0.0409	0.0331	0.0263	0.0314	0.0534	0.0510	0.0282
S	0.0281	0.0375	0.0316	0.0491	0.0226	0.0522	0.0361	0.0428	0.0473	0.0437
O	0.0516	0.0808	0.0574	0.0820	0.0334	0.0453	0.0688	0.0343	0.0478	0.0320
N	0.0089	0.0131	0.0122	0.0095	0.0112	0.0244	0.0128	0.0184	0.0061	0.0130
D	0.0062	0.0071	0.0183	0.0087	0.0069	0.0100	0.0042	0.0029	0.0053	0.0127
J	0.0041	0.0128	0.0217	0.0074	0.0064	0.0121	0.0060	0.0032	0.0106	0.0124
F	0.0123	0.0116	0.0043	0.0070	0.0020	0.0058	0.0140	0.0126	0.0031	0.0047
M	0.0131	0.0222	0.0109	0.0208	0.0114	0.0124	0.0357	0.0218	-	0.0210
A	0.0284	0.0119	0.0043	0.0082	0.0132	0.0160	0.0224	0.0093	-	0.0064
M	0.0567	0.0302	0.0189	0.0533	0.0286	0.0244	0.0407	0.0381	-	0.0459

11	12	13	14	15	16	17	18	19	20	Mean	Standard Deviation
0.0035	0.0027	0.0051	0.0064	0.0042	0.0076	0.0013	0.0024	0.0037	0.0018	0.0044	0.0028
0.0226	0.0341	0.0322	0.0265	0.0518	0.0484	0.0347	0.0353	0.0517	0.0580	0.0369	0.0099
0.0292	0.0306	0.0363	0.0277	0.0566	0.0404	0.0582	0.0364	0.0473	0.0560	0.0396	0.0102
0.0737	0.0290	0.0394	0.0536	0.0418	0.0829	0.0375	0.0420	0.0316	0.0533	0.0507	0.0183
0.0081	0.0109	0.0216	0.0230	0.0161	0.0197	0.0300	0.0224	0.0118	0.0132	0.0153	0.0063
0.0061	0.0086	0.0092	0.0133	0.0026	0.0144	0.0162	0.0094	0.0121	0.0160	0.0095	0.0045
0.0089	0.0069	0.0073	0.0042	0.0123	0.0227	0.0025	0.0061	0.0082	0.0014	0.0088	0.0056
0.0219	0.0084	0.0130	0.0104	0.0083	0.0096	0.0122	0.0134	0.0022	0.0121	0.0093	0.0049
0.0124	0.0314	0.0228	0.0146	0.0135	0.0078	0.0119	0.0232	0.0245	0.0128	0.0180	0.0075
0.0086	0.0197	0.0163	0.0298	0.0126	0.0034	0.0071	0.0202	0.0066	0.0307	0.0144	0.0086
0.0317	0.0542	0.0128	0.0336	0.0194	0.0372	0.0324	0.0491	0.0352	0.0212	0.0349	0.0127

NI. Buoy (iii) IOM

TIME (months)	1	2	3	4	5	6	7	8	9	10
0 (starting weight)	0.0082	0.0041	0.0029	0.0146	0.0122	0.0018	0.0026	0.0048	0.0062	0.0030
A	0.0291	0.0534	0.0366	0.0473	0.0321	0.0852	0.0310	0.0290	0.0692	0.0410
S	0.0414	0.0717	0.0580	0.0426	0.0484	0.0430	0.0371	0.0296	0.0318	0.0239
O	0.0327	0.0263	0.0251	0.0302	0.0291	0.0492	0.0358	0.0510	0.0578	0.0316
N	0.0400	0.0245	0.0337	0.0342	0.0190	0.0106	0.0122	0.0131	0.0168	0.0250
D	0.0225	0.0124	0.0062	0.0175	0.0223	0.0246	0.0182	0.0017	0.0264	0.0193
J	0.0231	0.0028	0.0049	0.0151	0.0084	0.0056	0.0047	0.0118	0.0131	0.0020
F	0.0121	0.0096	0.0112	0.0084	-	0.0038	0.0220	0.0140	0.0106	0.0011
M	0.0213	0.0126	0.0824	0.0331	-	0.0142	0.0116	0.0037	0.0148	-
A	0.0139	0.0055	0.0124	0.0173	-	0.0023	0.0067	0.0103	0.0060	-
M	0.0156	0.0203	0.0417	0.0305	-	0.0282	0.0246	0.0341	0.0108	-

11	12	13	14	15	16	17	18	19	20	Mean	Standard Deviation
0.0080	0.0024	0.0071	0.0068	0.0032	0.0021	0.0045	0.0086	0.0101	0.0051	0.0060	0.0035
0.0421	0.0596	0.0347	0.0515	0.0230	0.0220	0.0343	0.0317	0.0494	0.0582	0.0420	0.0162
0.0386	0.0403	0.0460	0.0324	0.0384	0.0256	0.0303	0.0341	0.0272	0.0290	0.0389	0.0114
0.0426	0.0319	0.0437	0.0725	0.0593	0.0424	0.0513	0.0265	0.0284	0.0230	0.0403	0.0135
0.0119	0.0241	0.0162	0.0310	0.0231	0.0403	0.0098	0.0160	0.0258	0.0082	0.0219	0.0104
0.0066	0.0175	0.0043	0.0151	0.0212	0.0062	0.0148	0.0125	0.0191	0.0085	0.0148	0.0072
0.0212	0.0140	0.0032	0.0127	0.0051	0.0078	0.0013	0.0154	0.0022	0.0036	0.0091	0.0064
0.0017	0.0066	0.0120	0.0041	0.0063	-	-	0.0028	0.0075	0.0096	0.0086	0.0058
0.0129	0.0083	0.0117	0.0213	0.0224	-	-	0.0431	0.0240	0.0321	0.0194	0.0105
0.0114	0.0216	0.0081	0.0136	0.0142	-	-	0.0236	0.0159	0.0186	0.0125	0.0068
0.0226	0.0145	0.0173	0.0245	0.0068	-	-	0.0186	0.0472	0.0232	0.0237	0.0107

NI. Buoy (iv) 20M

TIME (months)	1	2	3	4	5	6	7	8	9	10
0 (starting weight)	0.0018	0.0032	0.0047	0.0126	0.0057	0.0022	0.0025	0.0019	0.0066	0.0028
A	0.0218	0.0307	0.0326	0.0417	0.0204	0.0226	0.0397	0.0483	0.0455	0.0429
S	0.0302	0.0432	0.0443	0.0356	0.0318	0.0272	0.0416	0.0520	0.0293	0.0288
O	0.0135	0.0247	0.0286	0.0522	0.0320	0.0414	0.0145	0.0196	0.0313	0.0182
N	0.0099	0.0026	0.0250	0.0107	0.0112	0.0083	0.0041	0.0167	0.0121	0.0218
D	0.0072	-	0.0183	0.0061	0.0055	0.0015	0.0042	0.0033	0.0086	0.0091
J	0.0041	-	0.0120	0.0116	0.0077	0.0044	0.0052	0.0026	0.0050	0.0131
F	0.0048	-	0.0092	0.0105	0.0211	0.0061	0.0034	0.0056	0.0074	0.0022
M	0.0113	-	0.0081	0.0037	0.0046	0.0026	0.0036	0.0134	0.0028	0.0056
A	0.0120	-	0.0224	0.0135	-	0.0061	0.0098	0.0144	0.0138	0.0267
M	-	-	-	-	-	-	-	-	-	-

11	12	13	14	15	16	17	18	19	20	Mean	Standard Deviation
0.0071	0.0063	0.0022	0.0110	0.0037	0.0024	0.0042	0.0058	0.0023	0.0044	0.0046	0.0029
0.0218	0.0346	0.0283	0.0330	0.0263	0.0172	0.0292	0.0316	0.0201	0.0387	0.0313	0.0099
0.0292	0.0221	0.0216	0.0442	0.0185	0.0355	0.0268	0.0452	0.0360	0.0402	0.0338	0.0092
0.0241	0.0262	0.0300	0.0173	0.0452	0.0229	0.0264	0.0394	0.0413	0.0126	0.0288	0.0106
0.0180	0.0153	0.0164	0.0197	0.0072	0.0090	0.0061	0.0102	0.0058	0.0231	0.0131	0.0062
0.0074	0.0069	0.0028	0.0114	0.0037	0.0180	0.0050	0.0043	0.0130	0.0014	0.0113	0.0187
0.0010	0.0064	0.0017	0.0027	0.0112	0.0059	0.0087	0.0028	0.0106	0.0130	0.0068	0.0040
0.0043	0.0096	0.0039	0.0110	0.0023	0.0072	0.0028	0.0064	0.0136	0.0014	0.0072	0.0048
0.0041	0.0128	0.0012	0.0073	0.0036	0.0068	0.0122	0.0037	0.0058	0.0124	0.0065	0.0038
0.0026	0.0162	0.0056	0.0153	0.0049	0.0106	0.0133	0.0022	-	-	0.0118	0.0067