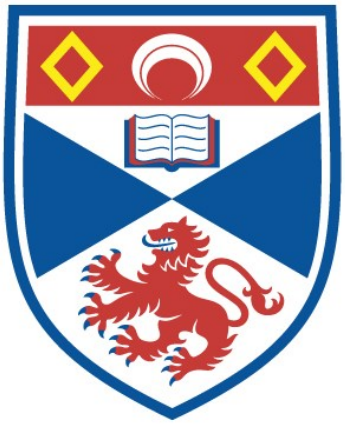
RELATIONSHIPS BETWEEN THE NUDIBRANCH 'ADALARIA PROXIMA' AND ITS PREY, THE BRYOZOAN 'ELECTRA PILOSA'

Helen White

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1993

Relationships between the nudibranch *Adalaria proxima* and its prey, the bryozoan *Electra pilosa*

by Helen White

Submitted for the Degree of Doctor of Philosophy

in the University of St. Andrews.

Department of Biology and Pre-clinical Medicine

September 1992

Th
B350

For my parents and Louise

"Ruder heads stand amazed at those prodigious pieces of Nature, Whales, Elephants, Dromidaries and Camels; these, I confess, are the colossus and majestic piece of her hand: but in these narrow Engines there is more curious mathematicks; and the civility of these little Citizens more neatly sets forth the Wisdom of their maker."

Sir Thomas Browne: *Religio medici*

Declaration

a) I, Helen Judith White, hereby certify that this thesis has been composed by myself, that it is a record of my work, and that it has not been accepted in partial or complete fulfilment of any other degree or professional qualification.

Signed Date 29-9-92

b) I was admitted to the Faculty of Science of the University of St. Andrews under Ordinance General No. 12 on 1st. October, 1988 and as a candidate for the degree of Ph. D. on 29th. September, 1989.

Signed Date 29-9-92

c) I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate to the degree of Ph. D.

Signature of Supervisor Date 29.9.92

d) In submitting this thesis to the University of St. Andrews I understand that I am giving permission for it to be made available for use in accordance with the regulations of the University Library for the time being in force, subject to any copyright vested in the work not being affected thereby. I also understand that the title and abstract will be published, and that a copy of the work may be made and supplied to any *bona fide* library or research worker.

Acknowledgements

First and foremost, a big thank you to my supervisor, Dr. C.D. Todd, for all his help and who had to tolerate me for much longer than he dared imagine.

I am especially grateful to everybody at the Gatty, staff and students alike, for all the encouragement and friendship. Cheers everyone! However, it would be rude not to thank; Roland Jack for lugging kerbstones in the pouring rain; Pete Baxter for real technical assistance; Margaret Robertson for her "quality" newspapers; Dr. W. Lambert for valuable discussions in times of crisis and for introducing me to Tetris; Dr. M.G. Bentley for his ready "wit" and loads of help; Jane Williamson for her office; Toni Beddow for the Met. data, Micha Bayer for his references and Mary Auckland, Mary Tierney, Jeni Boyle, Isabel Lafuente, Dave Matthews, and Demetris Maroulis being there. Thanks must also go to June "the pedant" Chisholm and all the others who helped in the last minute crisis; Claire Peddie, Karen Bell, but especially Helen Jones and Richard Smullen for the cheese roll, tippex and vote of confidence.

I would also like to thank Dr. R. Ingram for the use of the Delta-T Area Meter, Dr. R. Gibson and John Joyce of Dunstaffnage Marine Laboratory, Oban for the use of facilities and to the residents of the hostel for introducing me to the Mantrap and Oban nightlife!

Finally to Susan Howard and Scott Peake, Slange!

This work was carried out under the tenure of an NERC studentship awarded to the Department of Biology and Pre-clinical Medicine, and I am grateful for this support.

Abstract

The cosmopolitan distribution of the anascan bryozoan *Electra pilosa* manifests itself in the phenotypic plasticity of growth morphology expressed. Long-term field observations of the bryozoan on the essentially ephemeral substrata of the marine macroalgae, *Fucus serratus* and *Laminaria digitata*, at two contrasting sites in Scotland, confirm this.

Despite its potential for exponential indeterminate growth, *Electra pilosa* rarely realises this in the field. Observations show that any pattern of growth is complicated and distorted by the influences of physical (colony abrasion and the dynamics of the substratum) and biotic disturbance (predation by the nudibranch *Adalaria proxima*).

Rather than effect an visible induced defensive response after sustaining injury from mechanical damage or *Adalaria proxima*, *Electra pilosa* was demonstrated to maintain a high specific growth rate. It is suggested that this would preclude the settlement of potential competitors and ensure the fitness of this competitively weak, opportunistic species. Adoption of a stellate colony morphology was not concomittant with higher predation susceptibility but rather represented a plastic adaptive response to an ephemeral, dynamic environment

The lack of a metamorphic response to anything other than the presence of live *Electra pilosa* clearly demonstrated that metamorphosis of *Adalaria proxima* is by some component of the live, intact bryozoan. This component is neither water-soluble or extractable, and the inductive property is eliminated by homogenisation and freezing. Although hypotheses for this are evaluated, the mechanism of induction and the reasons for this relationship remain unclear.

Adaptations to this close nudibranch-bryozoan association are therefore evident in the life history strategies of both species.

Table of Contents

Chapter 1

Introduction to *Electra pilosa* and *Adalaria proxima*

1.1. Introduction

Historically, the Bryozoa has been a phylum of confused terminologies since the initial observations of sixteenth century naturalists (Hyman, 1959; Ryland, 1967, 1970, 1976). Perhaps such "overburdening of large and fantastic terminology" (Hyman, 1959) and the microscopic nature of the phylum had, until the latter half of this century, deprived the Bryozoa of attentions deserved from all but the most definitive studies. This despite their comprising five thousand extant species with perhaps four times that number in the fossil records. (Ryland, 1970), and being among the "principle constituents" of fouling communities (Ryland, 1967).

The most destructive predators of the Bryozoa are the nudibranchs (Ryland, 1976). The diverse morphologies seen within this polyphyletic molluscan order are primarily attributed to their respective prey associations (Todd, 1981). The intimate relationship between the bryozoan *Electra pilosa* and its nudibranch predator *Adalaria proxima* is such that their life histories are largely concomitant. The nature of this close association and the consequences for the two species has attracted limited consideration in the literature. Whilst this relationship is acknowledged (Thompson, 1958; Todd, 1981; Todd & Havenhand, 1989), it has not been studied to any considerable degree.

This thesis investigates this relationship between the nudibranch *Adalaria proxima* and its bryozoan prey *Electra pilosa*; a relationship which ultimately influences the growth dynamics of the bryozoan and the metamorphic event in the nudibranch.

It is the aim of this first chapter to introduce the two species and summarise present understanding of their life histories in the context of their respective phyla.

1.2. Animals Studied

1.2.1. *Electra pilosa*

The Bryozoa are abundant and diverse, and constitute one of the most successful colonial phyla. Their physiology and ecology has been thoroughly reviewed by Ryland (1970, 1976). The basic unit of the colony is the zooid, an asexually replicated, morphological unit which independently performs physiological or structural roles such as feeding, reproduction or support but which is in organic continuity and may be closely integrated with the rest of the colony (Boardman & Cheetham, 1973; Ryland, 1976).

The basic zooid is an autozooid (feeding zooid) comprising a circular or crescentic lophophore bearing slender, ciliated, post-oral tentacles. The anterior part of the body forms an introvert within which the lophophore and tentacles can be withdrawn. The alimentary canal is closely looped so that the anus opens near the mouth, but just outside the lophophore. Excretory organs are absent. There is no respiratory or circulatory system but a colonial nervous system is present in some species. Colonies, but not all zooids, are hermaphrodite. Each zooid secretes a rigid or gelatinous wall, so providing support for the colony. The lophophore, gut and their associated musculature are collectively referred to as the polypide. Whilst colony longevity in the Bryozoa varies greatly, the life span of individual polypides is a matter of only a few weeks (Gordon, 1976). The polypide breaks down and is replaced several times in the lifespan of each autozooid. The substances resulting from the breakdown are partly resorbed whilst unresorbed residues often remain as brown bodies which may be ejected via the gut of the new polypide or remain thereafter in the body cavity (Ryland, 1976). The body wall of the autozooid, including cellular and skeletal components, and the space it encloses, constitutes the cystid. In all Bryozoa a network of mesenchymatous tissue, the funiculus, closely associated with the gut,

occurs within the body cavity of each zooid and provides organic communication with the rest of the colony.

However, morphological variation of zooids continues transitionally via the processes of astogeny (colony growth) and ontogeny (zooidal growth) to forms in which a markedly different function is performed. In the extremes of polymorphism of zooids, most notably in the Cheilostomata, all organs associated with the functions of may be lacking (Boardman & Cheetham, 1973).

The phylum comprises three classes; Phylactolaemata, Stenolaemata and Gymnolaemata. The first of these is exclusively freshwater and, although widely distributed, comprises only 40 to 50 species. The Stenolaemata are characterised by elongate zooids that are cone- or tube-shaped and have terminal apertures. Zooids are budded in unpartitioned, multizooidal budded zones and increase in length during most or all of their ontogenetic development. The sole extant stenolaemate order, the Cyclostomata are marine. Gymnolaemate Bryozoa, found in both freshwater and marine environments, comprise two orders; the soft bodied Ctenostomata and the calcified, far more diversified Cheilostomata. Zooids are generally box- or sac-shaped, with dimensions of the principal body cavity determined early in ontogenetic development. Polymorphism of zooids characterises nearly all gymnolaemates. The cheilostomes may be further divided into two suborders, the Anasca and Ascophora. Anascans possess frontal surface membranes which are directly involved in the eversion of the lophophore. The flexibility of these membranes allows for the compensation of hydrostatic changes upon protrusion and retraction of the lophophore. The Ascophora are distinguished by the presence of a distensible ascus (inner compensation sac) which performs the role of the anascan frontal membrane (Hayward & Ryland, 1979).

The anascan cheilostome bryozoan *Electra pilosa* , that occurs circumglobally, is ubiquitous on a wide variety of substrata. Zooids are typically oval, although shape

and dimensions may vary (Okamura, 1987). The lightly calcified gymnocyst occupies approximately half of the frontal surface and is closely perforated by large round pores. The oval frontal membrane is surrounded by 4 to 12 spines which are simple evaginations of the gymnocyst, together with a sometimes enlarged proximal spine (Bobin, 1968; Ryland & Hayward, 1977).

Electra pilosa is a non-brooding cheilostome and fertilisation occurs in the intertentacular organ as the ova are discharged. A fertile zooid produces 10 to 20 ovoid eggs ≈ 60μm in length. These undergo maturation in the coelom prior to their discharge through the intertentacular organ which lies between the two dorsomedial tentacles (Ryland & Stebbing, 1971). The spermatozoa are retained within the intertentacular organ and are released through the terminal pores of the two dorsomedial tentacles upon the release of ova (Silén, 1966, 1972). Cross fertilisation may be mediated by epidemic discharge of spermatozoa (Silén, 1966). Post-fertilisation, development proceeds and the egg hatches into a bivalve-shelled, planktotrophic cyphonautes larva (Atkins, 1955a, 1955b; Ryland & Stebbing, 1971). Further development ensues until the larva attains competence, whereupon settlement and metamorphosis into the sessile ancestrula occurs (described in detail by Ryland & Stebbing, 1971). At metamorphosis the larval organisation breaks down and a reversal of internal polarity is observed. The rheotropic behavioural orientation of the larva at settlement thus importantly influences the initial direction of colony growth (Ryland & Stebbing, 1971). From the ancestrula disto-lateral budding initiates astogeny (postlarval colony development) and the characteristic quincuncial series zooid arrangement (Marcus, 1926; Silén, 1987). Growth of the colony is influenced by temperature (Menon, 1972), nutritional regime (Jebram, 1980a, 1980b), seasonality (Okamura, 1987), flow-regime (Okamura, 1988) and inter- and intra-specific competition (Okamura 1988, 1992; Rubin, 1985, 1987). Ultimately, however, expressed growth is modified by the effects of substratum loss, colony damage and predation.

1.2.2. *Adalaria proxima*

It is certainly paradoxical that within the gastropod molluscs one finds the successful but primitive prosobranchs with a strongly developed shell-operculum system and anterior mantle cavity, indicative of torsion and spiral coiling of the visceral mass, and the opisthobranchs which have achieved success through the gradual abolition of these characteristics (Thompson, 1976). Evolutionary progression is manifested in the largest order of the opisthobranchs, the Nudibranchia (Thompson, 1976). Members of this polyphyletic order of true "sea slugs" have undergone complete detorsion of the visceral mass and are devoid of the shell (in the adult), operculum, mantle cavity, osphradium and internal gills (Barnes, 1980; Todd, 1981). Relieved of the cumbersome yet protective shell, the nudibranchs have exploited the benthic, interstitial and pelagic oceanic habitats often denied to other gastropod orders, whilst adopting "active dynamic biological and chemical defensive adaptations" (Thompson, 1976).

The order comprises four suborders: Dendronotoidea, Doridoidea, Arminoidea and Aeolidoidea (Thompson & Brown, 1976; Todd, 1981), which are essentially trophic groupings; a fact reflected in the diverse morphology of the order. Literature pertaining to the ecology and systematics of the Nudibranchia have been reviewed by Harris (1973) and Todd (1981, 1983).

The small dorid *Adalaria proxima* was the subject of a lengthy and comprehensive study by Thompson (1958). Since then, it has been studied with respect to growth, reproduction, metamorphosis and larval behaviour in a series of papers by Todd and co-workers (Todd, 1979a, 1987, 1990; Todd & Doyle, 1981; Havenhand *et al.*, 1986; Todd & Havenhand, 1985, 1988; Havenhand & Todd, 1988a, 1988b, 1989; Kempf & Todd, 1989; Todd *et al.*, 1989, 1991). Such interest has been due, largely, to its sympatric coexistence with the planktotrophic dorid *Onchidoris muricata*.

Adalaria proxima attains a maximum size of 18 to 20mm in length (≈ 40mg dry weight) (Havenhand & Todd, 1988a) and is a boreo-arctic species which inhabits the lower littoral zone of rocky shores in association with its bryozoan prey *Electra pilosa* (Thompson, 1958; Thompson & Brown, 1976). The nudibranch is strictly annual, simultaneously hermaphrodite and semelparous, although more than one spawn mass may be produced (Havenhand & Todd, 1988a). Increase in body mass is linear and the growth rate is constant such that the daily growth increment represents a decreasing proportion of the increasing body size. Weight specific growth rate thus decreases gradually with time to virtually zero on the commencement of spawning (Havenhand & Todd, 1988a). Respiration rates vary, largely independent of seasonal temperature changes, although fluctuations due to short-term changes were observed, until the onset of spawning in March, after which respiration rates are dictated by environmental temperature (Havenhand & Todd, 1988a).

The commencement of spawning is accompanied by the onset of rapid degrowth (-0.78 to -4.24 $J.day^{-1}$) (Havenhand & Todd, 1988b). Accordingly, the spawning period is short (March to May) with rapid degrowth leading to a correspondingly rapid onset of senescence and death. Such degrowth is attributed to the fact that feeding rates observed in *Adalaria proxima* are not sufficient to offset reproductive costs. Rapid degrowth contributing 19% to the daily energy flux to reproduction (Todd & Havenhand, 1988) thus leads to a correspondingly rapid onset of senescence and death (Todd & Havenhand, 1988). Spawn production is not closely related to body size (Havenhand & Todd, 1988b).

Fertilisation is internal and usually reciprocal (Todd & Havenhand, 1988), following which spawning results in the characteristic spawn mass. Each ovum, ≈ 165 µm diameter (Todd & Havenhand, 1988), is encapsulated and surrounded by the mucous matrix of the spawn mass (Thompson, 1958). Development within the egg requires 36 to 39 days at 9 to 10° C (Thompson, 1958). The processes of cleavage,

gastrulation and assumption of the veliger form was described in detail by Thompson (1958). The eggs hatch into fully developed, short-term pelagic lecithotrophic larvae. The evolution of a lecithotrophic strategy by *Adalaria proxima* is presumed to be due to an "unpredictability" in its allocation of assimilated energy during spawning. The enhanced survival to metamorphosis characteristic of a lecithotrophic strategy thus serves to counteract the effects of the "unpredictability" of reproductive output (Todd, 1979). Feeding in the otherwise functionally lecithotrophic larvae of *A. proxima* has been described by Thompson (1958, 1962) and Kempf and Todd (1989). Ingested unicellular algal cells phagocytosed by digestive cells, identical to those described histologically and ultrastructurally in the planktotrophic larvae of various nudibranch species, were observed in the left digestive diverticulum of a complete digestive system (Thompson, 1959). Facultative planktotrophy in *A. proxima* is, however, insufficient to supplement the extra-embryonic yolk reserves, for both fed and starved larvae showed a reduction in tissue mass from hatching. The somewhat extended longevity of fed larvae would suggest a certain degree of nutrient gain acquired from ingested unicellular algal cells, and this assimilation of food is indeed substantiated by biochemical analysis, though feeding is never obligatory for metamorphic success (Kempf & Todd, 1989). The retention of a functional larval gut in *A. proxima* is thus presumed a requirement for the period of post-larval detrital feeding prior to handling the adult prey (Todd, 1990).

Upon attaining competence after the short obligate planktonic phase (1 to 2 days) the larvae metamorphose on contact with *Electra pilosa*, delaying the metamorphic event indefinitely in its absence (Thompson, 1958). Nonetheless, artificial induction of metamorphosis by elevated K^+ and choline chloride has been demonstrated (Todd *et al.*, 1991). Metamorphosis involves drastic changes, but no change in orientation. Numerous structural alterations proceed contemporaneously in the conversion of the veliger, adapted to a planktonic life, to the dorid form adapted to a browsing, benthic life. On completion of metamorphogenesis the juveniles begin to

feed on the bryozoan (Thompson, 1958), although Todd (1990) observed an intermediate detrital feeding stage, the juveniles becoming active only after depletion of the immediate food source.

1.3. Aims of this thesis.

1) Accounts of long-term field observations of epiphytic bryozoans are limited (Bushnell, 1966; Cancino, 1983). The influence of biotic and abiotic factors upon the ecology of *Electra pilosa* within the context of community organisations of marine macroalgae have received considerable attention in the literature. However, an understanding of the life-history of the cheilostome bryozoan in the field is limited. Through continued observations of *E. pilosa* a better understanding of the actual life-history of one of Britain's more common intertidal Bryozoa may be achieved.

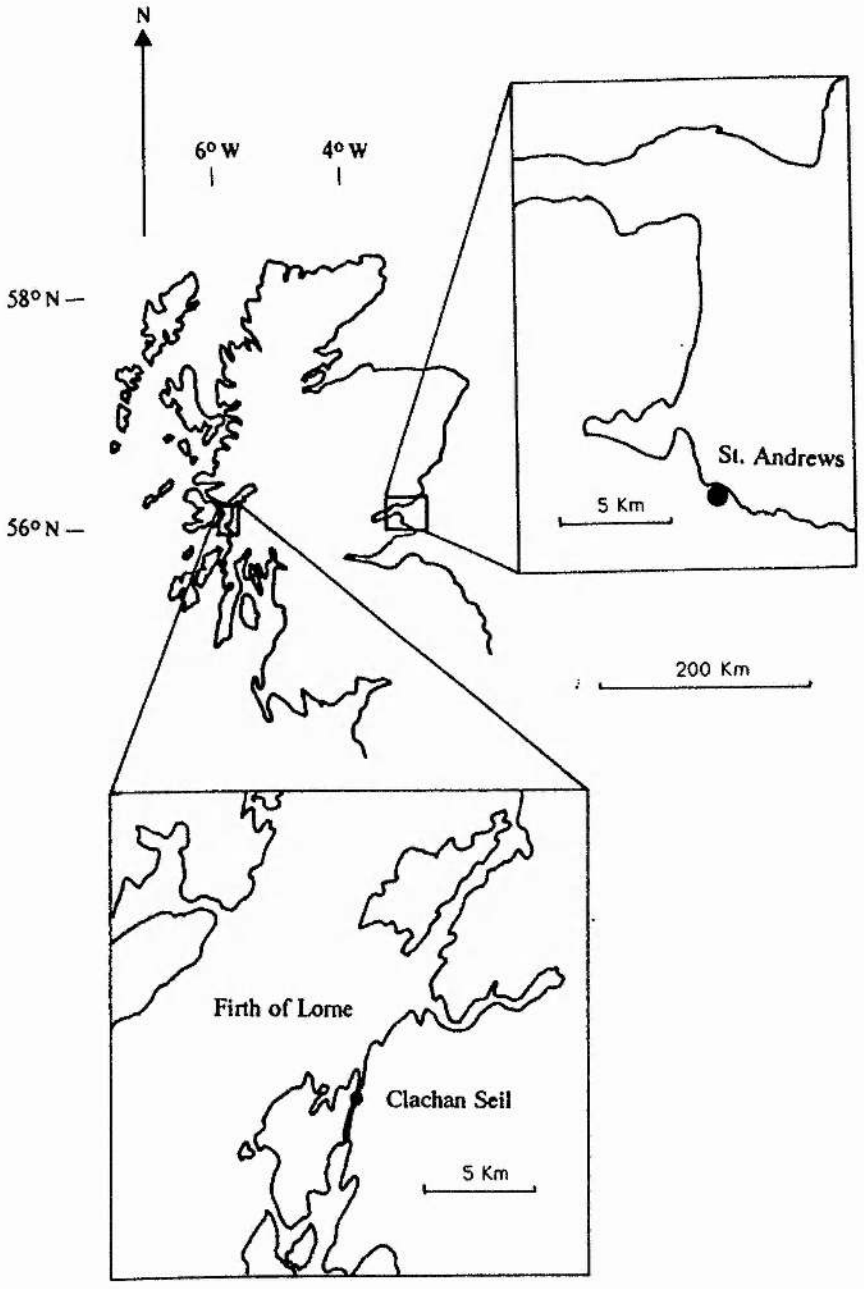
2) The phenotypic plasticity demonstrated in the induced response of the bryozoan *Membranipora membranacea* to nudibranch predation may be similarly provoked in *Electra pilosa*. The presence of enlarged spines in zooids of *E. pilosa* has been reported in response to overgrowth (Stebbing, 1973a, 1973b) although the process of enlargement has not been observed. Observation of the growth of the bryozoan after mechanical and predation damage might demonstrate a possibly inducible response in *E. pilosa*.

3) Since the observations of Thompson (1958) on the ability of *Electra pilosa* to induce metamorphosis in *Adalaria proxima*, little has been done to investigate the manner and nature of the inductive properties of the bryozoan. In the light of the comprehensive study of artificial metamorphic induction by choline and elevated potassium of *A. proxima* (Todd *et al.*, 1991) and the extraordinary length of time since Thompson's original work (1958), further investigation of this event was initiated.

Chapter 2

Field sites studies

Figure 2.1. The geographical location of the two field sites, St. Andrews, Fife and Clachan Seil, Argyll.


N
6° W
4° W
58° N
56° N
St. Andrews
5 Km
200 Km
Firth of Lorne
Clachan Seil
5 Km

2.1. Introduction

Two contrasting field sites provided the experimental animals and areas of study in this investigation; St. Andrews, Fife (east coast, Scotland) and Clachan Seil, Argyll (west coast, Scotland) (fig. 2.1). Both sites have been the subject of study on a number of previous occasions (Clachan Seil: Lewis, 1964; Todd & Lewis, 1984; Todd & Turner, 1986 and 1988; Turner, 1988. St. Andrews: Laverack & Blackler, 1974; Conolly, 1982; Conolly and Drew, 1985; Turner, 1988) due in part to their proximity to marine laboratory facilities: Gatty Marine Laboratory, St. Andrews and Dunstaffnage Marine Laboratory, Oban, Argyll.

2.2. St. Andrews

The East Rocks, St. Andrews (56° 20'N, 2° 47'W), hereafter referred to as St. Andrews, is an "exposed shore", calculated to be 12 on the Grenager and Baardseth exposure index (Conolly & Drew, 1985). Winds are predominantly south-easterly, though the summer months are characterised by westerly winds (I. Johnston pers. comm.). The site (fig. 2.2), east of the Gatty Marine Laboratory and at the foot of the Kinkell Braes, comprises calciferous sandstone and igneous rocks thrown into a series of anticlinal and synclinal folds, intersected by faults (Laverack & Blackler, 1974). The water is heavily polluted by the continuous release of domestic sewage (primary sedimentation treatment only) from the St. Andrews sewer to the west of the field site at a rate of ≈ 264,000 litres.day^{-1}, with flow dependent on rainfall (Conolly & Drew, 1985). Suspended material is very much in evidence after heavy ground swell.

Salinity in the bay (34.45—34.5‰) is presumed to be influenced by the influx of freshwater from the River Tay (Scotland's largest river) off Dundee Bar and to a lesser extent the River Eden 2km north west of St. Andrews. More specific to the site, however, is the proximity of the Kinnessburn which flows into the harbour, west of

Figure 2.2. The St. Andrews field site.

Figure 2.3. Surface seawater temperatures for a) St. Andrews Bay and b) the Firth of Lorne, between January 1990 and December 1991, obtained from the Meteorological Office. Open circles denote 1990 temperatures; closed circles are for 1991.

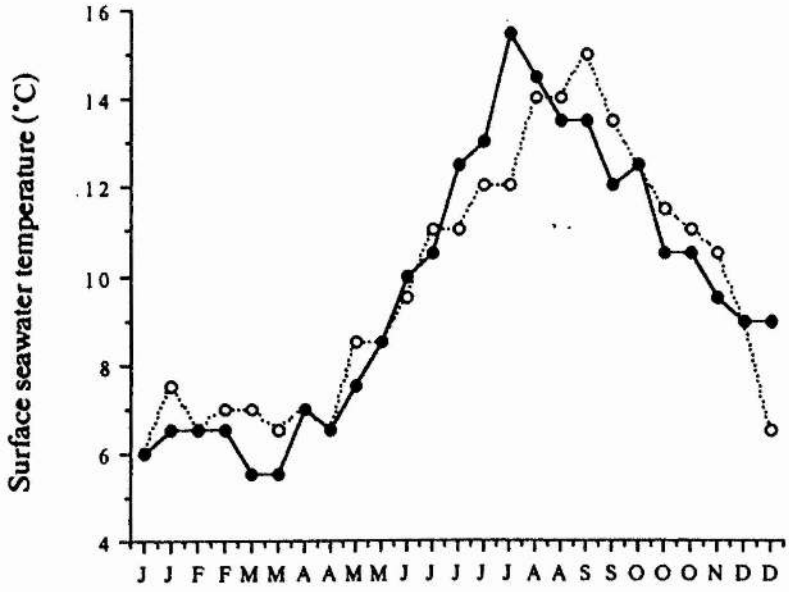


Figure 2.3a.

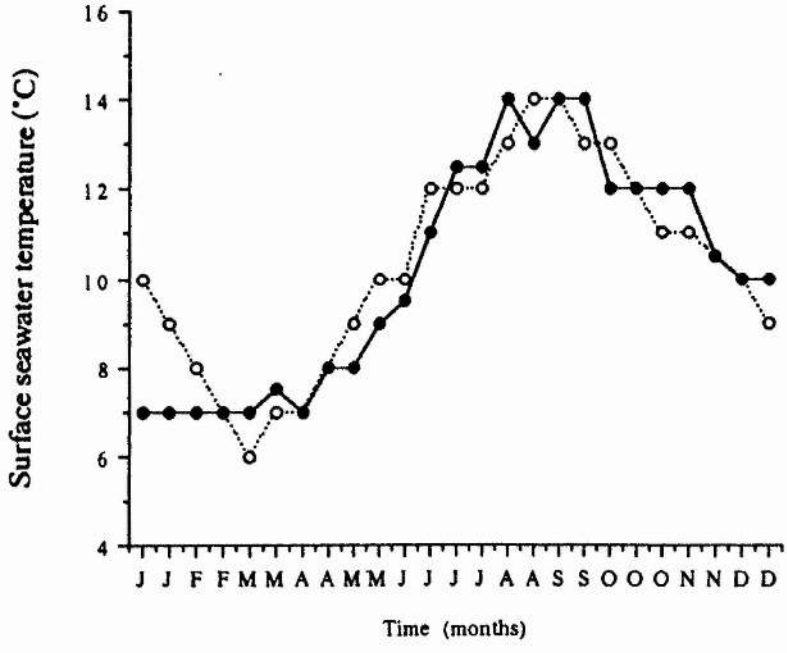


Figure 2.3b.

the East Rocks (Laverack & Blackler, 1974). Mean fortnightly sea water temperatures obtained from the Meteorological Office for January 1990—December 1991 (fig. 2.3a) showed a range of 15.5° C—3.5° C and a similar pattern for the two years. The data are not distinct from previous quoted temperatures of Laverack and Blackler, 1974 and Conolly, 1982.

The accessibility of the site was highly dependent on the prevailing tidal and weather conditions. The height of the tide, and the distance to which it receded were markedly affected by wind strength and duration, and unusually high or low barometric pressure. The mean tidal amplitude ranges from 2.4m (neaps) to 4.8m (springs). At St. Andrews, 2.9m below Ordnance Datum (Newlyn) (Admiralty Tide Tables), there is a marked seasonal variation in the actual low water level of the spring tides such that for tides of the same predicted low water height, the ebb is often to a lower level in summer than winter (Laverack & Blackler, 1974). The actual area studied was a gully comprising bedrock and boulders, running perpendicular to the shore, from the *Laminaria digitata* zone to the mid *Fucus serratus* zone. The flora and fauna of St. Andrews are sparse, algae are small and the associated epifaunal assemblages are simple, with a low percent cover (pers. obs.). *Adalaria proxima* is not common but is found in the bases of *F. serratus* and in moderate densities under stones in close association with the bryozoan *Electra pilosa.*

2.3. Clachan Seil

Clachan Seil (56°17' N, 5°37' W) by contrast, is a "sheltered" shore (Lewis, 1964); a tidal narrow, 1km long by 30m wide (Todd and Turner, 1986), that separates the island of Seil from the Argyll mainland. At high water the narrowness of the sound and the closeness of the terrestrial vegetation belie the fact that this is a marine environment (fig. 2.4a). At low tide, however, the kelp beds and dense fucoid vegetation are conspicuous and a rocky shoreline of boulders, smaller rocks and gravel

Figure 2.4. The Clachan Seil site at high water and low water.

is visible (fig. 2.4b). The large *Fucus serratus* plants and dense beds of *Laminaria digitata* support a complex, species rich epifauna. Populations of *Adalaria proxima* coexisting with the planktotrophic dorid, *Onchidoris muricata*, are seasonally abundant on fronds of *F. serratus*, although sparse on the blades of *L. digitata* despite the presence of, often quite large, colonies of *Electra pilosa.*

The mean tidal amplitude at Oban ranges from 1.1m (neaps) to 3.3m (springs) (Admiralty Tide Tables). At Clachan Seil, however, there are tidal sills at both extremities of the narrows such that the spring tides ebb to the same level between the sills, irrespective of open coast fluctuations. "Drainage over the sills all but ceases at low water springs and thus the enclosed narrows are never emersed" (Todd & Turner, 1986). The tidal flow velocity is rapid, 0.025 $m.s^{-1}$ at low springs, and gives the impression of a fast flowing stream.

Mean fortnightly sea water temperature data for the Firth of Lorne, Argyll (fig. 2.3b) showed very similar seasonal variations for both 1990 and 1991 with a range of 6—14°C, and salinity is 33—34‰ (Todd & Turner, 1986). Due to the complexity of the local mainland and island topography, all factors are subject to local variation. At Clachan Seil, on spring ebbs the surface sea water temperature may rise during summer whilst surface salinity may be influenced by fluctuations in freshwater run-off, especially in the winter months.

Chapter 3

Settlement and growth of *Electra pilosa*

3.1. Introduction

3.1.1. Life history strategies of the Bryozoa

The view that the life history parameters of any species are the result of evolutionary pressures exerted by its physical and biotic environment lead Kaufmann (1970, 1973) to propose a tripartite classification of colony morphology for Bryozoa, and later other colonial animals (1973). The classification of "vine-like, encrusting and upright bushy forms each expressed by linear, quadratic and exponential functions of time respectively" (Kaufmann, 1973) was additionally related to r- and K-selection strategies. This somewhat ingenuous approach has been modified considerably since its inception, most specifically with regard to encrusting, unilamellar Bryozoa.

Experimental data provided by various authors do not agree with this compartmentalisation: Menon (1972) provided evidence of exponential growth in a series of laboratory and field experiments with *Membranipora membranacea*, *Electra pilosa* and *Conopeum reticulum*, verified by Winston's (1976) laboratory based investigation of the growth of *Conopeum tenuissimum*. Wass & Vail (1978), however, in their studies of the Australian Bryozoan, *Valdemunitella valdemunita*, proposed a linear model of growth and cited Kaufmann's (1976) experiments as confirming their findings. Unfortunately, Kaufmann (1976) gave no details of his experimental proceedure or results. Thorpe (1979), dismissed Kaufmann's (1973; 1976) conclusions and presented a theoretical model which proposed that growth would be exponential for colonies un-restricted by either food supply or space.

Such discrepancies in the apparent growth patterns of encrusting Bryozoa led to Buss (1979a) and Jackson (1979a), independently outlining further subdivisions for descriptive growth of encrusting colonial animals. Jackson's (1979a) model of morphological strategy attributed the adoption of a particular growth form to

variations in mortality processes (selective forces), such as competition, predation and physical disturbance. The response to these selective forces is manifest in the growth form displayed by a colonial organism and lies within the two extremes of the sheet-runner continuum (Bishop, 1989). Sheet colonies (multiserial forms) possess contiguous, coherent rows of individuals whose growth reflects a confrontational strategy displayed in the morphological and behavioural responses directed towards maintenance of colony integrity (Buss, 1979a; Jackson, 1979a; Silén, 1987, McKinney & Jackson, 1991). This, in contrast to competitively inferior runners (uniserial growth) whose pattern of diffuse growth, a consequence of restricted budding, demonstrate a fugitive strategy of refuge location and withstand frequent partial mortality (Buss, 1979a; Jackson, 1979a; Silén, 1987, McKinney & Jackson, 1991). Buss (1979a), however, presented a graphical model which outlined the relationship between growth morphology and life-history characteristics. In this model morphological and behavioural responses to competition and substratum-space utilization provided the incentives for the adoption of a particular growth strategy. More recently, the question of the ecological and evolutionary significance of this variation in growth form lead McKinney & Jackson (1992) to present a new growth form model. This model considered the risks of mortality associated with particular growth forms in terms of differences in their modes and directions of growth. The predicted zooidal characteristics (morphology, spatial arrangement and polymorphism) and distribution, with varying degrees of disturbance, of multiserial and uniserial growth forms were confirmed by both recent and fossil Bryozoa in the field. Thus, the risk model was able to relate the adaptive significance of different growth forms as strategies to reduce risk of mortality due to known ecological processes.

3.1.2. Life hisory of *Electra pilosa*

The anascan cheilostome bryozoan *Electra pilosa*, perhaps the most common bryozoan to be found around the shores of the British Isles (Ryland & Hayward 1977), displays the "utmost catholicity" (Ryland, 1976) in its choice of suitable substratum for settlement (Ryland, 1976; Ryland & Hayward, 1977; Jebram, 1980a, 1980b; Silén, 1987). Such a cosmopolitan distribution manifests itself in the variation of colonial and zooidal forms of the bryozoan, though not to the extent that the species is unrecognisable (Ryland & Hayward, 1977). Colony formation of *E. pilosa*, in common with other encrusting Bryozoa, begins with the settlement of the larva followed by its metamorphosis into the primary zooid (ancestrula). Subsequent astogenic growth, iterative asexual budding of new zooids, commences with the ancestrula and then by successive generations of peripheral zooids (Marcus, 1926, Boardman & Cheetham, 1973; Silén, 1987; Bishop, 1989). Silén (1987), proposed that this pattern of astogeny is dependent on two factors: 1) the potential of each zooid to form four buds orientated at 90^{o} to each other and 2) the realisation of that potential.

The modular form of *Electra pilosa* dispenses with metabolic allometry (Hughes & Hughes, 1986) and one would expect growth to be exponential (Sebens, 1987), in accordance with the findings of Menon (1972) and Jebram (1980a, 1980b). However, the budding potential is not realised. Budding is merely disto-lateral (Silén, 1987) as space, restricted by parental zooids, becomes limited and new zooid formation is confined to the colony margins (Hughes & Hughes, 1986; Rubin, 1987; Silén, 1987; Bishop, 1989). Colony growth, therefore, is restricted and should, in common with other encrusting two-dimensional colonies, proceed as a quadratic function of time (Hughes & Hughes, 1986). The composite multiserial growth of *E. pilosa* producing the characteristic irregular and stellate colony (Marcus, 1926) enables the colony to exceed the quadratic rate (Hughes & Hughes, 1986; Rubin, 1987). Certainly the lobed periphery provides a much increased perimeter with a

subsequent increase in peripheral zooids. Hughes & Hughes (1986) argued that these peripheral zooids, although remaining constant in size, bud faster as the colony grows, perhaps enabled by the translocation of metabolites from non-replicating zooids restrained within the colony itself.

Composite multiserial growth, exhibited by *Electra pilosa*, was seen by Silén (1987) as the intermediate between sheet (multiserial) and runner-like (uniserial) forms, in the manner of Jackson's (1979a) sheet-runner continuum model. Silén (1987) suggested that this composite multiserial growth is more closely allied to the primitive obligate uniserial pattern than to obligate multiserial growth, and thus questioned our present understanding of cheilostome phylogeny. Certainly a review of current bryozoan taxonomy is suggested by the work of McKinney & Jackson (1992).

The morphological plasticity exhibited by *Electra pilosa*, whilst fundamentally associated with the achievement and maintenance of a high specific growth rate (Rubin, 1987), enables the cheilostome to respond to inimical ambient conditions (Rubin, 1987) or to poor nutritional regimes (Jebram, 1980a, 1980b; see Winston, 1976). This response was viewed by Buss (1979a) to be an adaptation to refuge location in such situations, but Rubin (1987) argued that rapid growth leading to increased colony size is more important in refuge location than is colony shape. Okamura (1992), however, considered that the expressed growth morphologies of *E. pilosa* were a consequence of the mechanisms of astogeny. In favourable conditions, the major axes radiating from the ancestrula grow rapidly across the substratum, at rate greater than concurrent disto-lateral budding, to produce the resultant stellate morphology. In more inimical environments, a circular, or more regular growth form occurs in consequence of the comparable rates of budding at all astogenetic levels of the colony. Bishop (1989) considered these previous models of encrusting cheilostome growth and offered an alternative based on his own arguments that uniserial growth is not indicative of competitively inferior species, but as a "positive

adaptation to particular configurations of substrate space". He also incorporated spot/dot colonies into his own model (encrusting colonies which achieve only a very small maximum size) and presented a quite different vision of encrusting cheilostome growth patterns.

The inherent problems associated with partial mortality and colony fission were recognised by Jackson & Winston (1981) and McKinney & Jackson (1992), and only through long-term observations can one actually assess and account for life-history patterns. Surprisingly then, there have been few instances of long-term observations of bryozoan growth in the field (Bushnell, 1966; Hayward & Ryland, 1975; Wass & Vail, 1978; Jackson & Winston, 1981; Wood, 1983; Cancino, 1983, 1986), despite numerous laboratory based investigations (Menon, 1972; Winston, 1976; Jebram, 1980a, 1980b; Silén, 1987, *etc.*). Certainly there have been no previous instances of long-term monitoring of *Electra pilosa* colony growth in the field before. However, an understanding of the growth patterns of *E. pilosa* alone cannot account for the life-history of *E. pilosa* in the field: knowledge of the substratum is of fundamental importance.

3.1.3. The macroalgal substrata

Dominating the littoral and shallow sublittoral of rocky shores, the Phaeophyta provide a substratum resource for colonisation by a variety of sessile epifauna. Studies have been conducted primarily on the littoral fucoid, *Fucus serratus* (Hagerman, 1966; the series of papers by Boaden, Seed & O'Connor reviewed by Seed & O'Connor, 1981; Wood & Seed, 1980; Oswald *et al.*, 1984) and to a lesser extent the kelps, *Laminaria digitata, L. hyperborea, L. saccharina* and *Macrocystis* sp. (Sloane *et al.*, 1957; de Burgh & Fankboner, 1978; Bernstein & Jung, 1979; Seed & Harris, 1980; Fletcher & Day, 1983) and *Ecklonia radiata* (Cancino, 1983, 1986; Schultze *et al.*, 1990).

3.1.3a. *Fucus serratus*

Fucus serratus is the dominant low shore fucoid on European shores (Schonbeck & Norton, 1980) whose limits of distribution are set by physiology (upper) and competition (lower) (Schonbeck & Norton, 1978, 1980). The alga bears characteristic strap-like serrated fronds that arise from a disc holdfast, with pronounced midrib and wings. In autumn and winter the apices bear flattened and pointed fruiting bodies, whilst non-fertile regions may possess soft silken hairs (Barrett & Yonge, 1985).

Growth is a biphasic process commencing with the formation of a primary thallus within 0.5cm of the apex. Secondary growth, characterised by basal defoliation, proceeds distally and an internode may lose up to 10 percent of its length as the wings disintegrate and the midrib thickens to form a stipe, whilst fruiting denudes the frond apically (Knight & Parke, 1950; Boney, 1966; Wood, 1983). Repeated dichotomies, resulting from vertical divisions of the apical cells, accompany thallus length (Boney, 1966) and have been shown to vary in response to local environmental conditions (Knight & Parke, 1950).

3.1.3b. *Laminaria digitata*

The adult sporophytes of the genus *Laminaria* are among the largest and most prominent of the marine algae (reviewed by Kain, 1979). *L. digitata*, the primary intertidal laminarian species of the north-eastern Atlantic (Kain, 1979) is confined to the lower littoral zone and then extends to the shallow sublittoral. Its upper limit is constrained by the effects of desiccation, the alga being unable to tolerate more than minimal tidal emersion, whilst the lower distribution is restricted by the more competitive *L. hyperborea* (Kain, 1979). Lüning (1979), however, argued that the higher growth rate of *L. digitata*, which restricts the storage of reserve products,

confines the alga to regions where photosynthesis is not reduced by light restriction either through depth or shading effects.

Growth in the Laminariales, which may be followed by Parke's (1948) punched hole method, is effected by basal meristematic tissue growth. The result is that the "old lamina becomes a worn appendage on the end of the developing one" (Boney, 1966). Thus, the fronds of laminarians behave like "moving belts of tissue" (Mann, 1972); frond tissue being generated at the base and eroded at the frond tip (Parke, 1948; Kain, 1979), accompanied by variation in chemical composition (Black, 1954; Conolly, 1982; Conolly & Drew, 1985). Two distinct phases of growth are observed (Parke, 1948); a period of fast growth (January to June [Parke, 1948]) during which time there is extensive elongation due to cell enlargement with vacuolation (Boney, 1966) and a period of slow growth (July onwards [Parke, 1948]) involving an accumulation of storage carbohydrates (Black, 1954) fated to become the food reserve for winter or for sporogenesis (Kain, 1979).

3.1.4. Sessile epiphytic communities of marine macroalgae

The sessile assemblages epiphytic on marine macroalgae have been the subject of a number of publications (reviews by Hayward, 1980; Seed & O'Connor, 1981; Seed, 1985; Wahl, 1989; Williams & Seed, 1992). However, despite their widespread distribution and suitability for experimental investigation these communities have still not received the attention they warrant, especially when one considers the extensive literature available for communities on marine hard substrata (e.g. Connell, 1966, 1972; Dayton, 1971; Paine, 1974; Menge & Sutherland, 1976; Osman, 1977; Jackson, 1977; Sousa, 1984). Initial interest in macroalgal communities catalogued representative taxa and species (Colman, 1940; Hagerman, 1966; Sloane, *et al.*, 1957). Only recently has the emphasis shifted towards an understanding of community organisation in these assemblages (Stebbing, 1973a,

1973b; Hayward & Harvey, 1974; Seed & O'Connor, 1981; Seed, 1985; Williams & Seed, 1992). Inevitably, comparisons have been made between macroalgal epifaunas and those of marine hard non-renewable substrata with respect to community organisation (Seed & O'Connor, 1981). Marine hard substrata are fundamentally space-limited communities (Connell, 1961; Dayton, 1971, Paine, 1974), the structuring forces of which are both physical and biological (predation and competition). Seed & O'Connor (1981), however, proposed that the two major factors structuring algal epifaunal communities are: 1) larval selectivity and 2) seasonal generation of new substrata available for colonisation (but see Fletcher & Day, 1983). The potential host's growth is important in as much as it represents the rate of substratum renewal; conversely, mortality, whether complete or partial (i.e. frond abrasion) dictates the availability of the substratum for colonisation (Wood, 1983). In this investigation the term epiphyte is used to refer to any macrofauna inhabiting the host plants in the manner of a number of other publications, but see also Wahl's definitions of marine epibiosis (1989).

It is argued that the non-random distribution of sessile marine invertebrates on hard and algal substrata is caused by both larval selectivity and site-specific post settlement mortality (competition, predation and substratum loss) (Keough & Downes, 1982; Keough, 1986; Hurlbut, 1991; Walters & Wethey, 1991). Both Keough (1986) and Hurlbut (1991) maintained that the location of settlement is critical to the post settlement survival of the individual due to the influence of site-specific environmental parameters (but see Durante & Chia, 1991). As a consequence of the non-uniformity of mortality processes across a substrate there will be spatial positions where the fitness of an individual will be greater than for individuals of the same species occupying a different region of substratum (Buss, 1979a).

The entire length of the attached alga represents a microenvironmental gradient (Seed, 1985). Larval selective settlement is exhibited by epiphytic Bryozoa in response to differing cues such as aggregation, topographic features of the

substrata, algal substratum and surface microflora (reviewed by Meadows & Campbell, 1972; Crisp, 1974; Scheltema, 1986; Ryland, 1976, 1979; Buss, 1979a; Seed & O'Connor, 1981; Seed, 1985; Williams & Seed, 1992). A number of settlement responses are apparent in the settlement and immediate growth of *Electra pilosa*. On *Fucus serratus*, the Bryozoan is predominantly distal (Seed & O' Connor, 1981) and favours the concave surface of the partially folded fronds (Boaden *et al.*, 1975, 1976; Wood & Seed, 1980). Furthermore, *E. pilosa* exhibits orientated growth towards the younger, distal regions of the plant (Ryland & Stebbing, 1971). Such responses are attributable to the pursuit of spatially predictable refuges (Buss, 1979a), in avoidance of potential competitors and a maximisation of survival time on a plant which grows from apical meristems (Ryland & Stebbing, 1971; Boaden *et al.*, 1975; Wood & Seed, 1980; Oswald *et al.*, 1984). The precise mechanisms by which larvae detect the age-gradient is not understood. It may be in response to bacterial films and surface microflora (Wisely, 1958; Crisp & Ryland, 1960; Ryland, 1974; Mihm *et al.*, 1981; Brancato & Woollacott, 1982), physiological gradients (Knight & Parke, 1950; King & Schramm, 1976; Kain, 1979; Oswald *et al.*, 1984; Maki *et al.*, 1989; Wahl, 1989) or the production of antimicrobial compounds (Conover & Sieberth, 1966; Sieberth & Conover, 1965; Hornsey & Hide,1974, 1976; Al-Ogily & Knight-Jones, 1977). Algal epifauna are thus subjected to a "biologically dynamic" substratum (Seed & O' Connor, 1981) which is constantly changing with respect to both physical dimensions and chemical composition.

3.1.5. Aims of this chapter

Accounts of long-term field observations of epiphytic Bryozoa are limited (Bushnell, 1966; Hayward & Ryland, 1975; Cancino, 1983). Although the role of *Electra pilosa* in the community organisations of marine macroalgae, specifically those of *Fucus serratus*, and the influence of plant and environment have received

considerable attention in the literature (reviews by Hayward, 1980; Seed & O' Connor, 1981; Seed, 1985; Williams & Seed, 1992), and understanding of the life-history of the cheilostome bryozoan in the field is limited.

Two contrasting sites, Clachan Seil, Argyll (west coast of Scotland: sheltered from wave action) and St. Andrews, Fife (east coast of Scotland: exposed to wave action) promote differing growth forms in *Electra pilosa.* East coast colonies appear small and regular, whereas colonies on the west coast are large and stellate. Equally, the greater surface area offered by *Laminaria digitata* than *Fucus serratus* should provide a greater scope for settlement and growth of the bryozoan. Through continued observations of *E. pilosa* at these two sites, a better understanding of the actual life-history of one of Britain's more common bryozoans can be achieved.

3.2. Materials and methods

3.2.1. Settlement of *Electra pilosa*

Between February 1990 and September 1991 settlement of *Electra pilosa* was recorded with respect to algal frond area and position on the frond, for mature plants of both algal species at the two sites and observations were made monthly where possible.

Fucus serratus plants can be divided into segments, each consisting of a Y level or dichotomy (Boaden *et al.*, 1975; Wood & Seed, 1980). These segments were numbered Y1, Y2,...Yn commencing at the base of the plant. Fucoids grow by means of apical meristems, such that the frond represents an age-gradient with the basal dichotomy being the oldest region of the plant. In addition, a further ecological criterion to assess settlement, that of the recognisable convex and concave surfaces of the partially folded frond (Hayward & Harvey, 1974; Wood & Seed, 1980), was employed.

Laminaria digitata possesses meristematic tissue near the frond base such that the frond itself represents an age-gradient (Black, 1954; Cancino, 1986). Settlement positions of *Electra pilosa* were recorded with respect to their distance from the transition point (where stipe and frond converge).

Plants were removed from the low intertidal zone (0.4 to 0.6m above chart datum), cleared of any *Electra pilosa* present using the blunt side of a scalpel blade (Bushnell, 1966) and deployed in the field with reattachment by means of cable ties to *in situ* host plants. Evidence of settlement in the ensuing month could then be ascertained. It should be noted that at Clachan Seil attachment of plants to host plants resulted in high plant loss. Thus, four ropes were placed from the middle of the tidal narrow to the low littoral, within the *Fucus serratus* zone. All plants were attached to these ropes using cable ties.

At Clachan Seil only, settlement of the bryozoan was also observed, for a limited time period, May 1991 to September 1991, on slate panels. These were machine cut to a plane finish, of dimensions 15cm by 15cm and 12mm thick, with the central area (10cm by 10cm) stained blue using a non-toxic marker pen (Todd & Turner, 1986). After allowing microfloral filming of the panels a week before deployment, they were then placed in the field and attached to the ropes with cable ties. Upon retrieval, panels were removed to St. Andrews and examined under a Wild M8 stereomicroscope.

The available surface area for settlement and growth of *Electra pilosa* was determined with the aid of a Delta-T Area Meter (Delta-T Devices Ltd.) and the length of all plant fronds was recorded. From these data (number of individuals.cm^{-2}.cm^{-1}), settlement densities were plotted against time. By incorporating plant length into the experimental parameters the units of measurement in this investigation were number of settled individuals.cm^{-1}. In addition, a one-way analysis of variance (ANOVA) (Sokal & Rohlf, 1981) was performed on the settlement data collated from the concurrently run panel experiment at Clachan Seil and comparable data for settlement on *Fucus serratus* and *Laminaria digitata* . This statistical analysis tests the assumption that the differences, if any, among the mean settlement densities on each substratum during this time period, are due to the different substrata examined.

To determine the spatial dispersion of settlement the null hypothesis tested was that settlement is directly related to the available substratum area. Using the mean area of 10 percent increments of the total plant length for six plants of both species from both east and west coast sites, the expected distribution was calculated. The observed and expected numbers were compared with a χ^2 goodness-of-fit test (Sokal & Rohlf, 1981). Rejection of the null hypothesis would imply that settlement follows a non-uniform distribution not related to available substratum area. However, it should be noted that the substrata were not sampled in proportion to their relative abundance and availability to the larvae in the field (Hurlbut, 1991).

3.2.2. Growth of mature host plants

Growth rates for mature plants of the two algal species were calculated. At Clachan Seil, fifty-one *Fucus serratus* plants were tagged over a period of 453 days, commencing in June 1990, in four regions adjacent to the ropes (table 3.1). Twenty-three plants on four rocks were followed at St. Andrews over a period of 445 days from June 1990. Plants were numbered and coloured twine marked their position in the field. Growth was followed by measuring frond elongation (length of longest frond) to the nearest 0.5cm (Knight & Parke, 1950) (table 3.2). Although elongation alone is no full measure of growth, dichotomy of the fronds and development of the lateral frondage proceeds simultaneously with linear extension (Knight & Parke, 1950). Frond growth was measured as as $cm.day^{-1}.cm^{-1}$ and thus the data are plotted as day^{-1} against time.

Fifty-six tagged *Laminaria digitata* plants at Clachan Seil and sixty-four plants at St. Andrews were studied over a period of 440 days (Clachan Seil) and 456 days (St. Andrews), commencing in June 1990 (tables 3.3 to 3.4). Plants were monitored in areas adjacent to experimental plants in the same manner as for *Fucus serratus*. Frond growth was measured by following the displacement of a 5cm diameter hole, punched 10cm from the transition point. A new reference hole was punched at each observation date (Parke, 1948; Sundene, 1964; Mann, 1972; Kain, 1979; Seed & Harris, 1980; Conolly, 1982; Conolly & Drew, 1985). Measured growth, therefore, was the sum both of primary and secondary elongation (Parke, 1948). The length of the longest lamina and the hole displacement were recorded on each occasion, to the nearest 0.5cm. Actual frond growth was recorded as day^{-1} against time in the same manner as for *Fucus serratus* for the reasons explained above. Frond elongation data acquired from the length of the longest lamina were plotted as day^{-1} against time to provide a further assessement of growth.

Multiple regression analysis (Sokal & Rohlf, 1981) was performed to assess the influence on the elongation rate of the variables, season and site, and to establish a possible linear functional relationship. For the purposes of analysis the year was divided into four seasons each of three months.

The loss of a plant was regarded as death of that individual and each loss was replaced with a new plant. Mortality rates (percentage number of plants lost per day) were calculated for each month and plotted against time.

3.2.3. Growth of established *Electra pilosa* colonies

At the two sites the growth patterns of individual established colonies were followed over a period of time from spring 1990. Such colonies were monitored on a monthly basis, where possible, by the use of tagged plants (tables 3.5 to 3.8). On each occasion at St. Andrews, plants were removed from the field to the laboratory, where zooids were drawn and counted using a *camera lucida* attached to a Wild M8 stereomicroscope. At Clachan Seil, however, access to such facilities was not available and fieldwork time limited. Accordingly, colonies growing on *Laminaria digitata* were photographed and zooid numbers counted from the negatives at a later date. The convoluted and irregular surface of *Fucus serratus* fronds necessitated the use of acetate traces of individual colonies and colony area was ascertained by digitiser.

At St. Andrews, colony growth was also followed *in situ* both for *Laminaria digitata* and *Fucus serratus* plants. These plants were tagged with coloured twine to aid retrieval in the field. Colonies were photographed *in situ* and their position on the

---

Tables 3.1—3.4 (following pages). Growth of mature *Fucus serratus* and *Laminaria digitata* plants at St. Andrews and Clachan Seil. The tables detail percentage plant recovery for each observation.

Table 3.1. *Fucus serratus* growth plants observed at Clachan Seil.

* plant lost

23.06.90	23.07.90	22.08.90	17.10.90	05.11.90	02.12.90	31.01.91	17.03.91	16.04.91	15.05.91	15.06.91	13.07.91	10.08.91	09.09.91
1	*												
2	*												
3	*												
4	*												
5	->	->	->	->	->	*							
6	->	->	->	->	->	*							
7	->	->	->	->	->	*							
8	->		->	->	*								
9	->	->	*										
10	->	->	*										
11	->	->	->	->	*								
12	->	->	->	->	->	->	->	->	->	->	->	->	->
13	->	->	*										
14	->	*											
15	->	*											
16	->	->	->	->	->	->				->	->		->
17	*												
18	->	->	->	->	->	->	->	->		->	->	->	->
19	->	->	->	->	->	->	->	->		->	->	->	->
20	->	->	->	->	->	->	*						
	21	->	*										
	22	->	->	->	->	->	*						
	23	->		->	*								
	24	->		->	->	->	->	->	->	*			
	25	->		->	->	->	->	->	->	->	*		
			26	->	->	*							
			27	->	->	*							
			28	->	->	*							
			29	->	*								
				30	->	->	->	->	->			->	*
				31	->	->	->	->	->	->		->	->
				32	->	->	->	->	->	->	->	->	->
					33	*							
					34	->	->	->	->	*			
					35	->	->	->	->	*			
						36	->	->	->	->	->	->	->
						37	->	->	->	->	->	->	->
						38	->	->	->	->	->	->	->
						39	->	->	->	->	->	->	->
						40	->	->	->	->	->	->	->
						41	->	->	->	->	->	->	->
						42	->	->	*				
								43	->	->	->	->	->
								44	->	->	-	->	->
								45	->	->	->	->	->
									46	->	->	->	->
									47	->	->	->	->
									48	->	->	->	->
									49	->	->	->	->
									50	->	->	->	*
										51	*		
% plant recovery	75	80	56.25	100	80.95	65	85	100	85	90.91	86.36	94.74	90

Table 3.2. *Fucus serratus* growth plants observed at St. Andrews.

* plant lost

11.06.90	11.07.90	09.08.90	07.09.90	19.10.90	05.12.90	18.02.91	31.03.91	14.05.91	12.06.91	12.07.91	09.08.91	11.09.91
1	->	->	->	->	->	->	*					
2	->	->	->	->	->	->	*					
3	->	->	->	->	->	->	->	->	->	->	->	->
4	->	->	->	->	->	->	->	->	->	->	->	->
5	->	->	->	->	*							
6	->	->	->	->	->	->	->	->	->	->	->	->
7	->	->	->	->	->	->	->	->	->	->	->	->
8	->	->	->	->	->	->	->	->	->	->	->	->
9	->	->	->	->	->	->	->	->	->	->	->	->
10	->	->	->	->	->	->	->	->	->	->	->	->
11	->	->	->	->	->	->	->	->	->	->	->	->
12	->	->	->	->	->	->	->	->	->	->	->	->
13	->	->	->	->	->	->	->	->	->	->	->	->
14	->	->	->	->	->	->	->	->	->	->	->	->
15	->	->	->	->	->	->	->	->	->	->	->	->
16	->	->	->	->	->	->	->	->	->	->	->	->
17	->	->	->	->	->	->	->	->	->	->	->	->
18	->	->	->	->	->	->	->	->	->	->	->	->
19	->	->	->	->	->	->	->	->	->	->	->	->
20	->	->	->	->	->	->	->	->	->	->	->	->
							21	->	->	->	->	->
							22	->	->	->	->	->
							23	->	->	->	->	->
% plant recovery	100	100	100	100	95	100	89.47	100	100	100	100	100

Table 3.3. *Laminaria digitata* growth plants observed at St. Andrews.

* plant lost

12.06.90	12.07.90	06.08.90	06.09.90	04.12.90	18.02.91	31.03.91	14.05.91	12.06.91	12.07.91	09.08.91	11.09.91
1	*										
2	->	*									
3	->	->	->	*							
4	->	->	*								
5	->	->	*								
6	->	->	*								
7		->	->	*							
8	->	->	->	*							
9	*										
10	*										
11		->	->	->	->	->		->	*		
12		->	->	*							
13		->	->	->	->	->	->	->	*		
14		->	->	->		->	->	->	->	->	->
15		->	->	*							
16	->	->	->	->	*						
17	->	*									
18	->	->	->	->	->	->		->	->	->	
19		->	->	*							
20	->	->	->	->	*						
21	->	->	->	->	*						
22	->	->	->	->	->	*					
23	->	->	->	->	->	->	*				
24	->	->	*								
25	->	->	->	->	->	->	->	->	*		
26	->	->	->	->	->	->	->	->	->	->	*
28	->	->	->	*							
29	->	->	->	->	->	->	->	->	->	->	->
30	->	->	->								
				31	*						
				32	*						
				33	*						
				34	*						
				35	*						
				36	*						
				37	*						
				38	*						
				39	*						
				40	*						
				41	->	->	->	->	->	->	->
				42	->	->	->	->	->	->	->
				43	*						
				44	*						
				45		->	->	->	->	->	->
				46	->	->	->	->		->	*
				47	->	->	*				
				48	->	->	->	->	->	->	*
				49	->	->	->	->	->	->	*
				50		->	*				
						51	->	->	->	->	->
						52	->	->	->		->
						53	->	->	->	->	->
						54	->	->	->	*	
						55	->	->	->		->
						56	->	->	->	->	*
						57	->	->	->	->	->
						58	->	->	->		->
						59	->	->	->	->	->
						60	->	->	->	*	
										61	->
										62	->
										63	->
										64	->
% plant recovery	50	90	88	68.18	42.86	100	82.14	95.65	83.33	75	75

Table 3.4. *Laminaria digitata* growth plants observed at Clachan Seil.

* plant lost

26.06.90	23.07.90	22.08.90	17.10.90	05.11.90	02.12.90	31.01.91	17.04.91	15.05.91	15.06.91	14.07.91	11.08.91	09.09.91
1	->	*										
2	->	*										
3	*											
4	->	->	->	->	*							
5	->	->	->	->	*							
6	->	->	->	->	->	->	->	->	->	->	->	->
7	->	->	->	->	->	->	->	->	->	->	->	->
8	*											
9	*											
10	*											
11	*											
12	->	->	->	->	->	->	->	->	->			->
13	->	->	*									
14	->	->	->	*								
15	->	->	->	*								
16	*											
17			->	->	->	->	*					
18			->	->	->	->	*					
19	->	->	->	->	->	->		->	->	->	->	*
20	*											
	21	->	->	->	*							
	22	->	->	->	->	->	*					
	23	->	*									
	24	->	*									
	25	*										
	26	*										
	27	->	->	->	->	->	->	*				
	28	->	->	->	->	->	->		->	->	*	
	29	->	->	->	->	->	->		->	->	*	
		30	->		->	->	->	->	->	->	*	
				31	->	->	->	->	->	->	->	*
				32	->	->	->	->	->	->	->	->
				33	->	->	->	->	->	->	->	->
				34	->	->	->	->	->	->	->	->
				35	->	->	->	->	->	->	->	->
				36	->	->	->	->	->	*		
						37	->	->	->	->	->	*
						38	->	->	->	->	->	->
						39	->	->	*			
							40	->	*			
							41		->		->	
							42		->	*		
							43		->	*		
							44	*				
							45	*				
								46		->	->	->
								47		->	->	->
								48		->	->	->
								49		->	->	->
								50		->	->	->
									51	->	->	->
									52	->	->	->
									53	->	->	->
									54	->	->	->
									55	->	->	->
											56	*
% plant recovery	55	80	85.71	92.86	84.21	94.12	68.42	73.68	73.68	68.18	95	95

plant mapped onto an acetate overlay of the whole frond. To assess whether colony growth was influenced by the retrieval and handling of plants a paired t-test (Sokal & Rohlf, 1981) was performed. This tests the null hypothesis that the true difference between the paired observations is zero, and was applied to the arc-sine transformed data ($\arcsin\sqrt{p}$) obtained from the percentage mortality and retrieval of *in situ* colonies and plants and retrieved colonies and plants. The arc-sine transformation stretches out both tails and compresses the middle of the distribution, and in that way yields normally distributed data which meets the assumption of the t-test (Sokal & Rohlf, 1981).

Growth was expressed as the specific growth rate which is hereafter referred to as the apparent specific growth rate (aSGR). The aSGR represents observations of individual colonies at times t_1 and t_2 which does not take into account the possibilities of predation or colony abrasion in the interim. Calculation of this growth rate employed the geometric mean (the back-transformed mean of the logarithmically transformed data). The geometric mean, when the data are all positive and unequal, is always less than the arithmetic mean (Sokal & Rohlf, 1981). Growth of *Electra pilosa* colonies cannot be described as a straight line between two points of observation, since growth is exponential (Hughes & Hughes, 1986). Thus, the use of the geometric mean is more pertinent to growth of the bryozoan.

Multiple regression analysis (Sokal & Rohlf, 1981) was performed to identify the significant relationships between the dependent variable, aSGR of *Electra pilosa*, and the independent variables, season, site and plant species (for an explanation of this analysis and season see section 3.2.2).

Tables 3.5–3.8 (following pages). Established *Electra pilosa* colonies followed at St. Andrews and Clachan Seil. The tables detail percentage recovery of host plants and all colonies. In addition the percentage of colonies that were not lost was recorded, *i.e.* colony mortality attributable solely to colony abrasion or predation.

Table 3.5

E.pilosa colonies on *F. serratus* at St. Andrews.

D all zooids dead

* plant lost

30.03.90	30.04.90	30.05 90	26.06.90	08.08.90	06.09.90	19.10.90	04.12.90	15.02.91	31.03.91	13.05.91	17.06.91	12.07.91	15.08.91	11.09.91	11.10.91
1	D														
2	->	->	->	D											
3	->	->	->	*											
4	->	->	->	D											
		5	D												
		6	D												
			7	D											
				8	->	->	*								
				9	->	->	D								
							10	*							
							11	*							
							12	*							
							13	*							
								14	*						
								15	*						
								16	*						
								17	D						
								18	->	D					
									19	->	->	D			
									20	->	->	D			
									21	->	->	D			
										22	->	D			
												23	->	D	
													24	*	
														25	->
														26	*
%recovery plants	100	100	100	75	100	100	50	0	20	75	100	100	100	50	50
% recovery all colonies	45	28.6	31.6	0	50	87.5	0	0	10	29.4	42.9	0	54,.5	0	11.8
% recovery colonies on retrieved plants	50	28.6	31.6	0	50	87.5	0	0	33.33	29.4	42.9	0	54.5	0	40

Table 3.6

E. pilosa colonies on *L. digitata* at St. Andrews.

D all zooids dead

* plant lost

29.05.90	25.06.90	08.08.90	06.09.90	19.10.90	04.12.90	15.02.91	31.03.91	13.05.91	17..06.91	17.07.91	15.08.91	11.09.91	11.10.91	10.11.91
1	→	•												
2	→	→	→	•										
3	→	•												
4	→	→	→	→	D									
		5	→	D										
		6	→	D										
		7	→	•										
			8	→	D									
					9	→	•							
					10	→	•							
					11	•								
					12	→	•							
					13	→	•							
						14	→	D						
						15	→	•						
						16	→	•						
						17	→	D						
							18	→	•					
								19	•					
								20	•					
									21	•				
									22	→	•			
										23	→	•		
										24	D			
											25	•		
											26	→	•	
												27	•	
												28	→	•

% recovery plants	100	50	100	66.67	100	80	50	60	0	20	66.67	33.33	33.33	0
% recovery all colonies	60	4.8	52.6	18.2	0	66.67	40	24	0	16.67	10	18.75	12.5	0
% recovery colonies on retrieved plants	60	9.1	52.6	66.67	0	100	40	30	0	30	14.3	42.9	22.22	0

Table 3.7

E. pilosa colonies on *F. serratus* at Clachan Seil

D all colonies dead

* plant lost

23.06.90	23.07.90	22.08.90	17.10.90	05.11.90	02.12.90	31.01.91	17.03.91	16.04.91	15.05.91	15.06.91	13.07.91	10.08.91	19.09.91
1	→	→	•										
2	→	→	D										
3	→	→	D										
4	•												
5	•												
	6	→	→	→	→	→	→	D					
	7	→	D										
			8	→	→	→	→	→	D				
				9	→	D							
				10	→	→	→	→	D				
						11	→	→	D				
						12	→	•					
								13	→	•			
								14	→	D			
									15	→	→	D	
									16	→	→	→	→
									17	→	→	D	
											18	→	→
											19	→	→
												20	→
												21	→
%recovery plants	100	100	40	100	100	100	100	80	60	83.33	100	100	100
%recovery all colonies	69.4	12.3	5.4	72.7	81.25	37.5	78.9	47.1	45	48.9	41.7	82.61	52.6
% recovery colonies on retrieved plants	69.44	12.3	7.69	72.7	81.25	87.5	78.9	80	45	69	41.67	82.6	54.5

Table 3.8

E. pilosa colonies on *L. digitata* at Clachan Seil.

D all colonies dead

* plant lost

	23.04.90	24.05.90	23.06.90	23.07.90	22.08.90	17.10.90	05.11.90	02.12.90	31.01.91	17.03.91	16.04.91	15.05.91	15.06.91	13.07.91	10.08.91	19.09.91
	1	→	*													
		2	→	*												
				3	→	•										
				4	→	•										
				5	D											
				6	→	•										
				7	→	→	D									
						8	→	→	•							
						9	→	→	→	•						
						10	→	→	•							
							11	→	→	→	•					
							12	→	•							
									13	•						
									14	•						
									15	→	•					
									16	→	→	D				
										17	→	D				
										18	→	D				
											19	D				
											20	D				
												21	→	•		
												22	→	•		
												23	→	→	D	
														24	→	•
														25	→	•
% recovery plants		100	0	0	100	25	75	100	40	50	60	100	100	33.33	66.67	0
% recovery all colonies		100	0	0	47.6	18.2	81.8	100	33.3	36.4	40	0	62.1	4	72.7	0
% recovery colonies on retrieved plants		100	0	0	47.6	66.7	81.8	100	100	66.7	44.4	0	62.1	4.8	72.7	0

3.3. Results

3.3.1. Settlement

Over the period February 1990 to September 1991, settlement of *Electra pilosa* at St. Andrews (fig. 3.1) appeared to be cyclical on both algal species, although it is relevant to note that the data set is for eighteen months only. The period of peak settlement was from July 1990 to March 1991 and commenced again in September 1991. Whilst this pattern was observed both on *Fucus serratus* and *Laminaria digitata*, the peak values of settlement were more than double on the kelp: *F. serratus* 0.002 $cm^{-1}.day^{-1}$ (September 1990); *L. digitata* 0.0042 $cm^{-1}.day^{-1}$ (July 1990); *F. serratus* 0.0017 $cm^{-1}.day^{-1}$ and *L. digitata* 0.0078 $cm^{-1}.day^{-1}$ (September 1991).

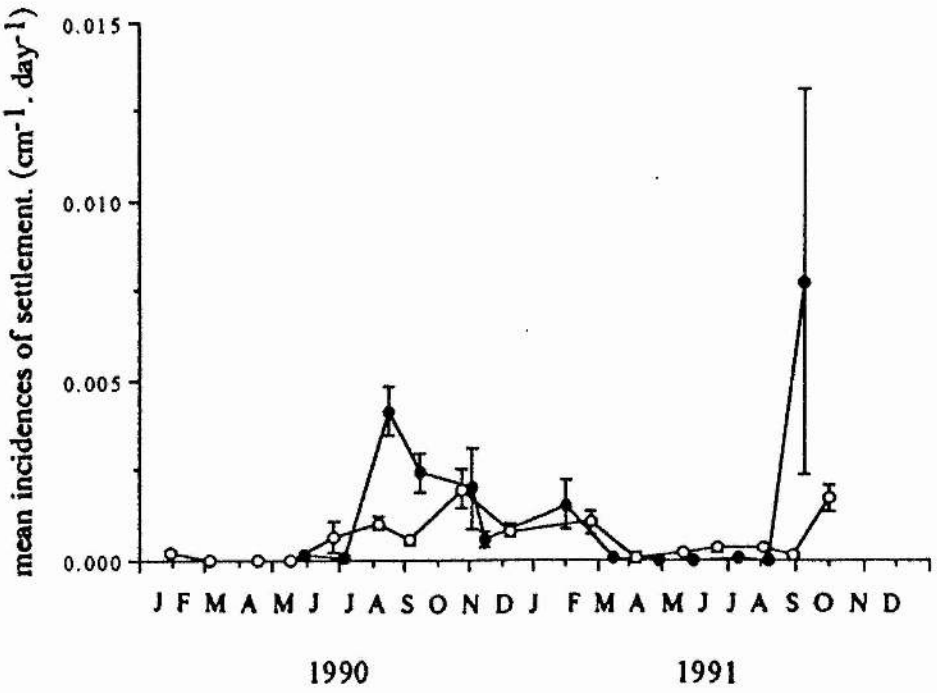
At Clachan Seil, the density of settlement was much reduced (fig. 3.1). Indeed, settlement of the bryozoan on *Laminaria digitata* was almost negligible throughout the entire period of observations. The same cyclical pattern of settlement on *Fucus serratus* seen at St. Andrews was, however, observed, although the same *caveat* applies. Maximum settlement (0.0017 $cm^{-1}.day^{-1}$) was observed on *F. serratus* between July 1990 and February 1991 with minor peaks in June 1990 and July 1991.

One-way ANOVA from the concurrently run panel experiment at Clachan Seil (May 1991 and September 1991) showed that although there was no significant difference between settlement densities on the panels or *Laminaria digitata* during this particular time period (table 3.5), there was a significant difference between settlement densities on *Fucus serratus* and the panels ($F = 787.13$, $p < 0.05$).

The distributions of the recently settled colonies on *Fucus serratus* at both St. Andrews ($\chi^2_{(9)} = 16.99$, $p > 0.05$) and Clachan Seil ($\chi^2_{(9)} = 55.84$, $p > 0.05$) (fig. 3.2a) and on *Laminaria digitata* at Clachan Seil ($\chi^2_{(9)} = 53.1643$, $p < 0.05$) (fig. 3.2b) did not conform to the null hypothesis. The selection of available frond substrata is not related to the area available. Rather, there was a greater density of settlement on

Figure 3.1. Seasonal mean settlement density (mean numbers. cm^{-1}. day^{-1}) ± SE of *Electra pilosa* on *Fucus serratus* and *Laminaria digitata* at St. Andrews and Clachan Seil. Open circles denote settlement of the bryozoan on *F. serratus* plants, closed circles denote settlement on the fronds of *L. digitata*.

St. Andrews



Clachan Seil

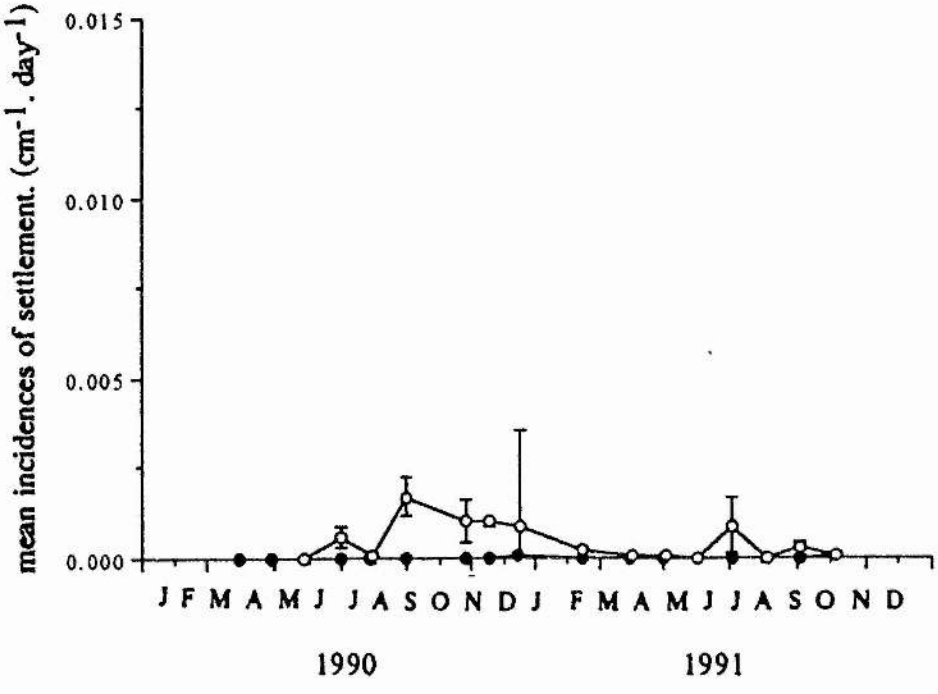


Table 3.9. Mean settlement density of *Electra pilosa* (mean number of settlements.cm^{-1}) at Clachan Seil on three alternative substrata.

Table 3.9

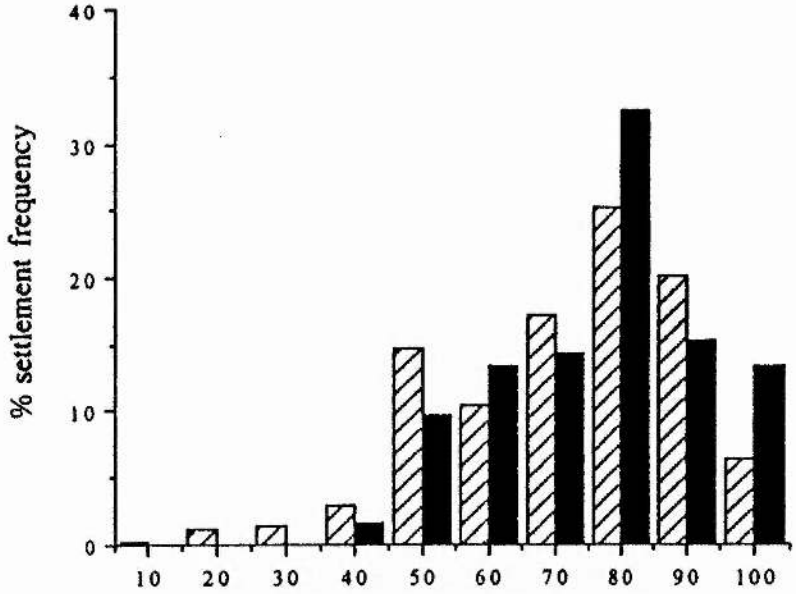
Date	Panel	*Fucus serratus*	*Laminaria digitata*
15.05.91	0.0030	—	—
15.06.91	0.0042	0.0280	—
14.07.91	0.0026	—	—
11.08.91	0.0010	0.0077	0.0002
10.09.91	—	0.0016	0.0032

One-way ANOVA showing F-statistic

	Panel	*Fucus serratus*	*Laminaria digitata*
Panel	—	787.13 *	3.39
Fucus serratus	—	—	0.22
Laminaria digitata	—	—	—

Figure 3.2a. Settlement models for *Electra pilosa* on *Fucus serratus* at St. Andrews and Clachan Seil. Hatched columns denote expected numbers; blocked columns denote observed numbers.

St. Andrews



Clachan Seil

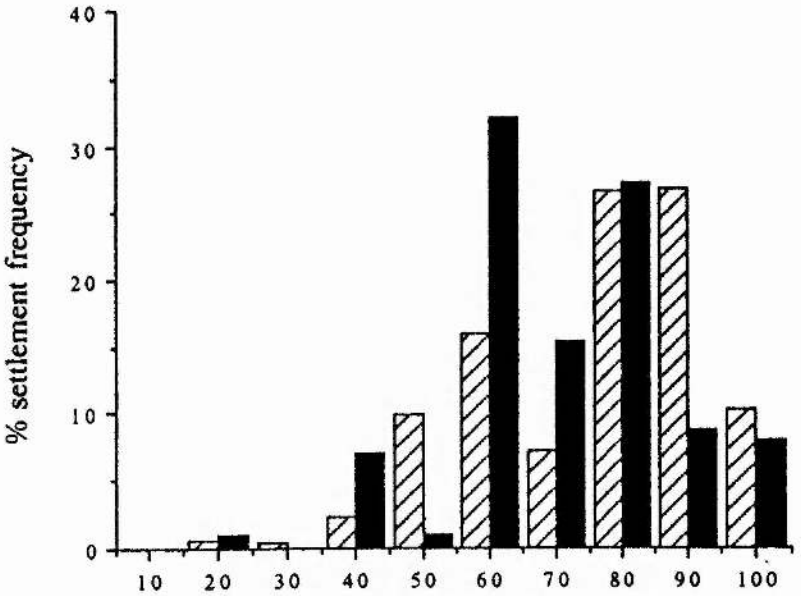
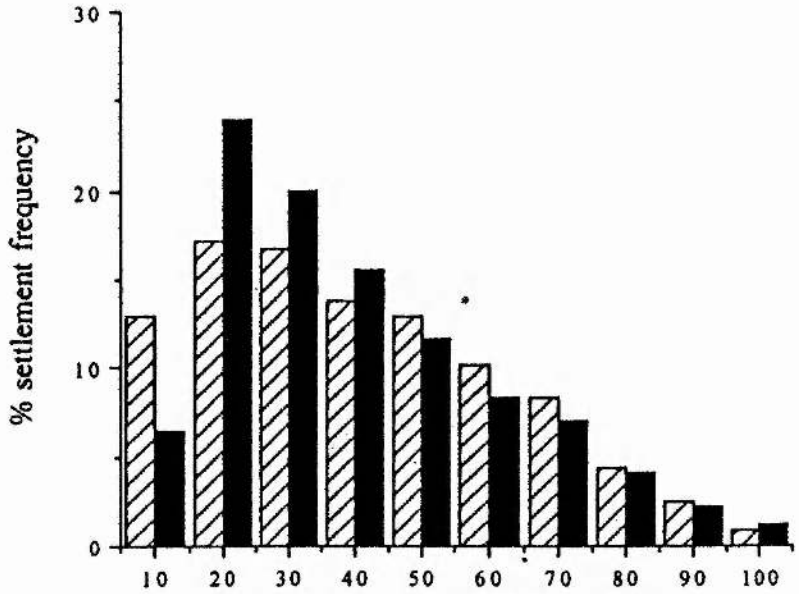
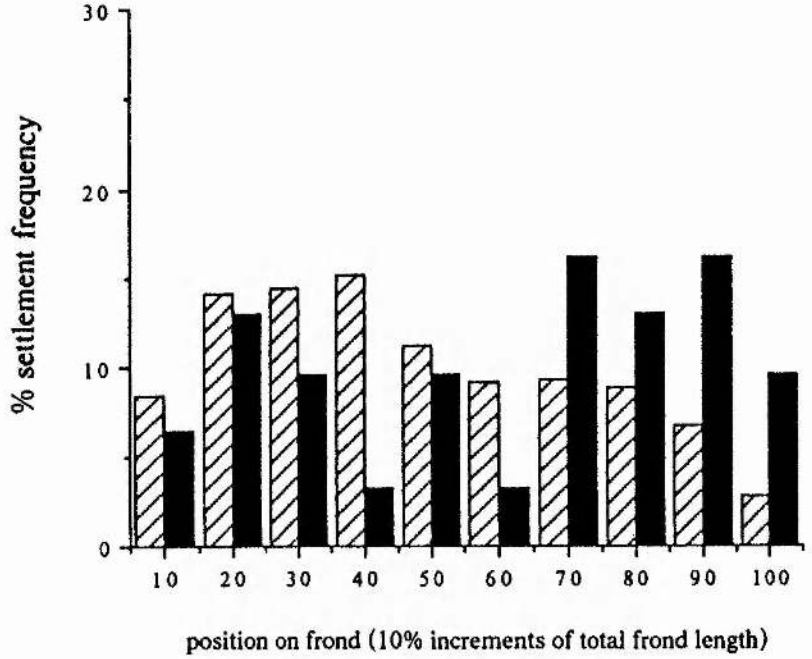


Figure 3.2b. Settlement models for *Electra pilosa* on *Laminaria digitata* at St. Andrews and Clachan Seil. Hatched columns denote expected numbers; blocked columns denote observed numbers.

St. Andrews



Clachan Seil



the more distal regions of the fucoids, specifically 80% of the total plant length (St. Andrews) and 60 to 80% of the total plant length (Clachan Seil). A greater density of distal settlement was also recorded on the laminarian at Clachan Seil.

However, the pattern of settlement of the bryozoan on the fronds of *Laminaria digitata* at St. Andrews (fig. 3.2b) ($\chi^2_{(9)} = 7.45$ p, < 0.05) did accept the null hypothesis. This is nicely illustrated by fig. 3.3b. The frequency of observed and expected settlements clearly show this conformity. It is interesting to note, however, the much lower than expected degree of settlement on the first 10% increment of the laminarian fronds.

3.3.2. Plant growth

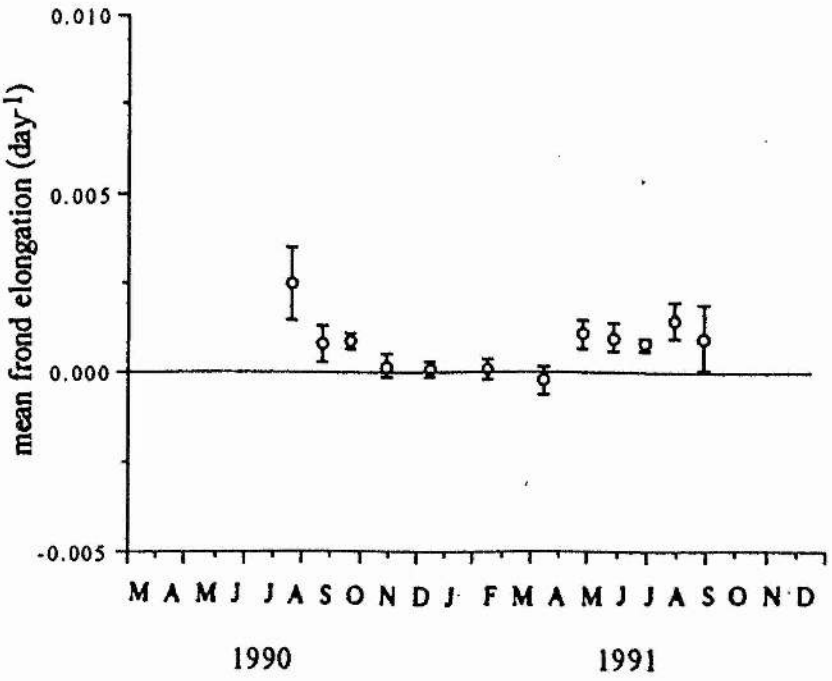
3.3.2a. *Fucus serratus*

The mean elongation rate of mature *Fucus serratus* at both sites (figs. 3.3) was not continuous throughout the year. At St. Andrews, from the high rate attained in June 1990, growth declined to a minimum between September 1990 and April 1991 when growth effectively ceased. The elongation rate was restored to the previous June 1990 value from May 1991 to August 1991 before declining yet again. The growth rates recorded at both sites were much lower than those observed by both Knight & Parke (1950) and Hatton (1932). It is important to note, however, that the results presented have the units day^{-1} rather than the $cm.day^{-1}$ values of the previous authors.

This apparent seasonality of growth is evident from both multiple regression analysis (table 3.10), as is the significance of initial plant length, and the trajectories plotted of change in elongation rate (day^{-1}) for individual plants with three or more observations (fig. 3.4a). The trajectories demonstrate the uniformity of a growth pattern between plants, despite occasional variants. Mortality of plants occurred only in

Figure 3.3. Seasonal change in mean frond elongation (day^{-1}) ± SE of mature *Fucus serratus* plants at a) St. Andrews and b) Clachan Seil.

a.


0.010
0.005
0.000
-0.005
mean frond elongation (day-1)
M A M J J A S O N D J F M A M J J A S O N D
1990
1991

b.

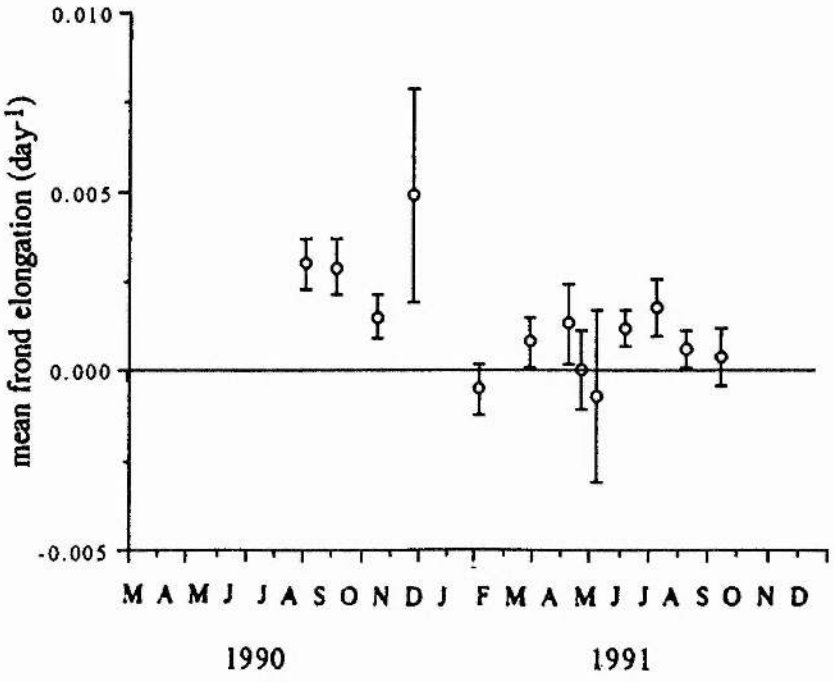
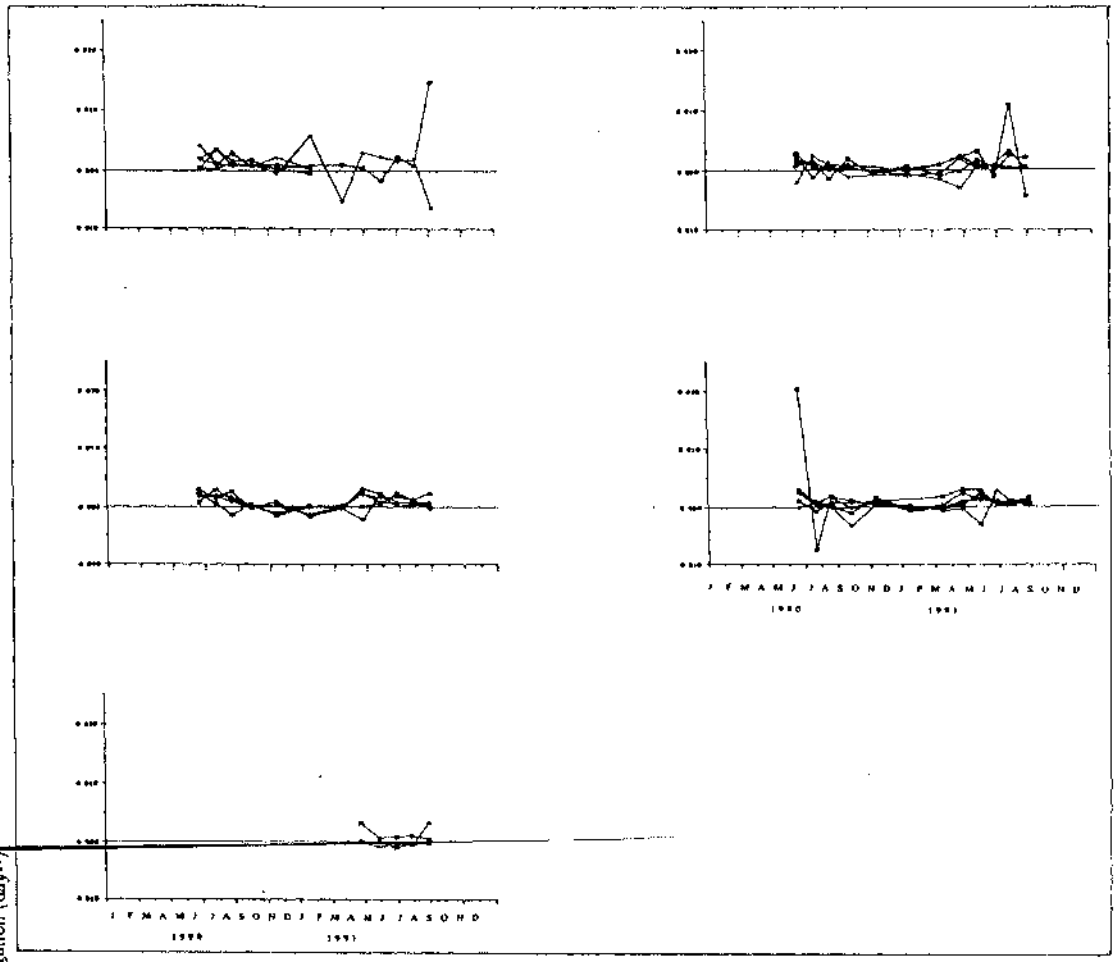

0.010
0.005
0.000
-0.005
mean frond elongation (day-1)
M A M J J A S O N D J F M A M J J A S O N D
1990
1991

Figure 3.4. Growth trajectories for the frond elongation (day^{-1}) of individual mature *Fucus serratus* plants at a) St. Andrews and b) Clachan Seil.

St. Andrews



frond elongation (day-1)

Clachan Seil

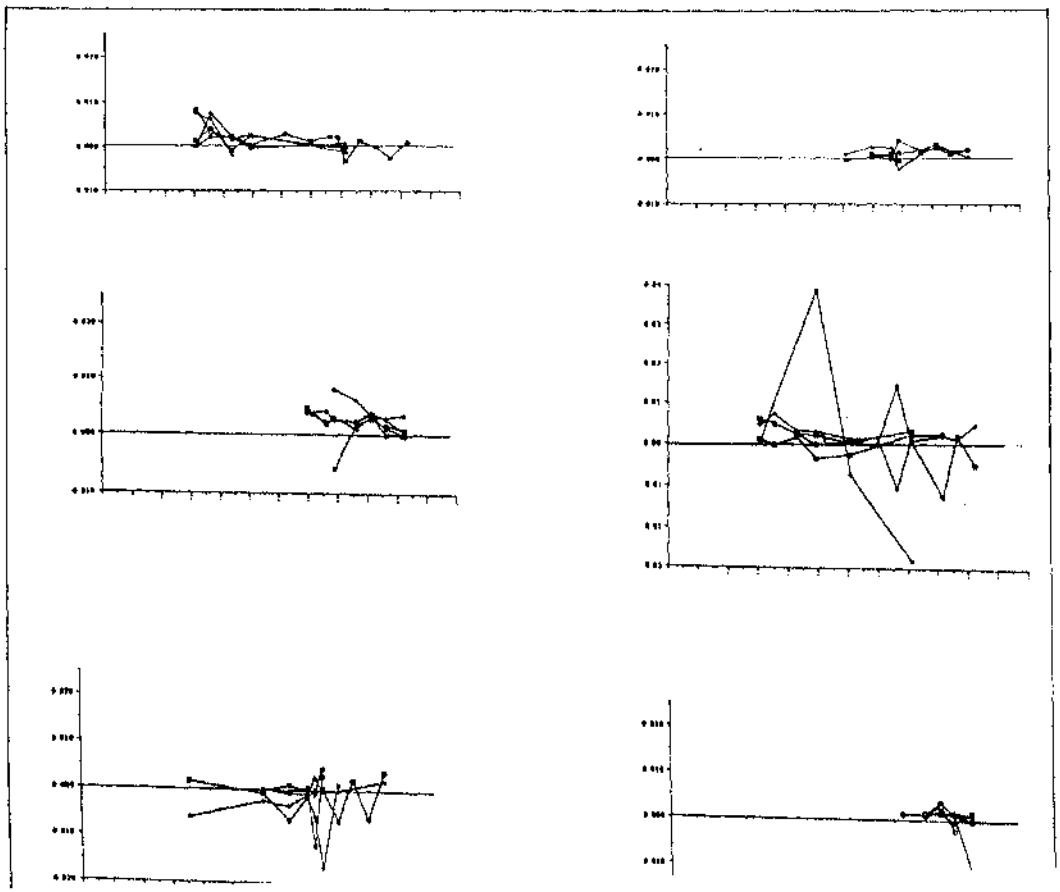


Table 3.10. Multiple regression analysis of the influence of the independent variables, initial plant length and season, on the dependent variable, growth ($cm.day^{-1}$) of *Fucus serratus*.

Table 3.10

St. Andrews

Independent variables	coefficient	SE of coefficient	p
initial length	-0.0027	0.0008	0.001 ***
season	-0.0295	0.0084	0.001 ***

Clachan Seil

Independent variables	coefficient	SE of coefficient	p
initial length	-0.0002	0.0008	0.810
season	-0.0241	0.0215	0.263

December 1990 and March 1991 (table 3.2) and there is little evidence of frond abrasion/loss from the trajectories of individual plants.

Such seasonality was not recorded at Clachan Seil. Mean elongation rates were sporadic (fig. 3.3), and although rates were higher in the summer months (June to September 1990 and July to August 1991) any trend was less apparent. The non-uniformity of plant growth is reiterated in the trajectories of *Fucus serratus* plants at the site (fig. 3.4). Higher mortality (table 3.1) and more negative rates of elongation (frond abrasion or loss) compounded the situation. The high incidence of frond abrasion between May and June 1991 is evident in the recorded mean elongation values (fig. 3.3). This essentially removed a possible trend of an increased growth rate during these months which might otherwise have been expected. However, it is interesting to note that the mean elongation rate of *Laminaria digitata* at Clachan Seil (fig. 3.5b) was also negative at this time which might lead to the supposition that such a heightened level of frond abrasion may be due to some extrinsic environmental factor at the west coast site.

The contrast between growth patterns at the two sites is confirmed by the lack of significance both of season and initial plant length at Clachan Seil (table 3.10). Whether this is an annual feature of growth at the west coast cannot be confirmed by the findings of this study.

3.3.2b. *Laminaria digitata*

Mean actual growth of *Laminaria digitata* was continuous at both field sites (fig. 3.5) with the exception of the observation for October 1990 at St. Andrews These observations were much lower than those of Conolly (1982) who recorded values of approximately 0.4 cm.day^{-1} at St. Andrews, although again it is important to note that the results presented here are day^{-1}. The disparity between maximum and minimum

Figure 3.5. Seasonal change in mean frond elongation and mean actual growth (day^{-1}) ± SE of mature *Laminaria digitata* at a) St. Andrews and b) Clachan Seil. Closed circles denote actual frond growth; open circles represent frond elongation.

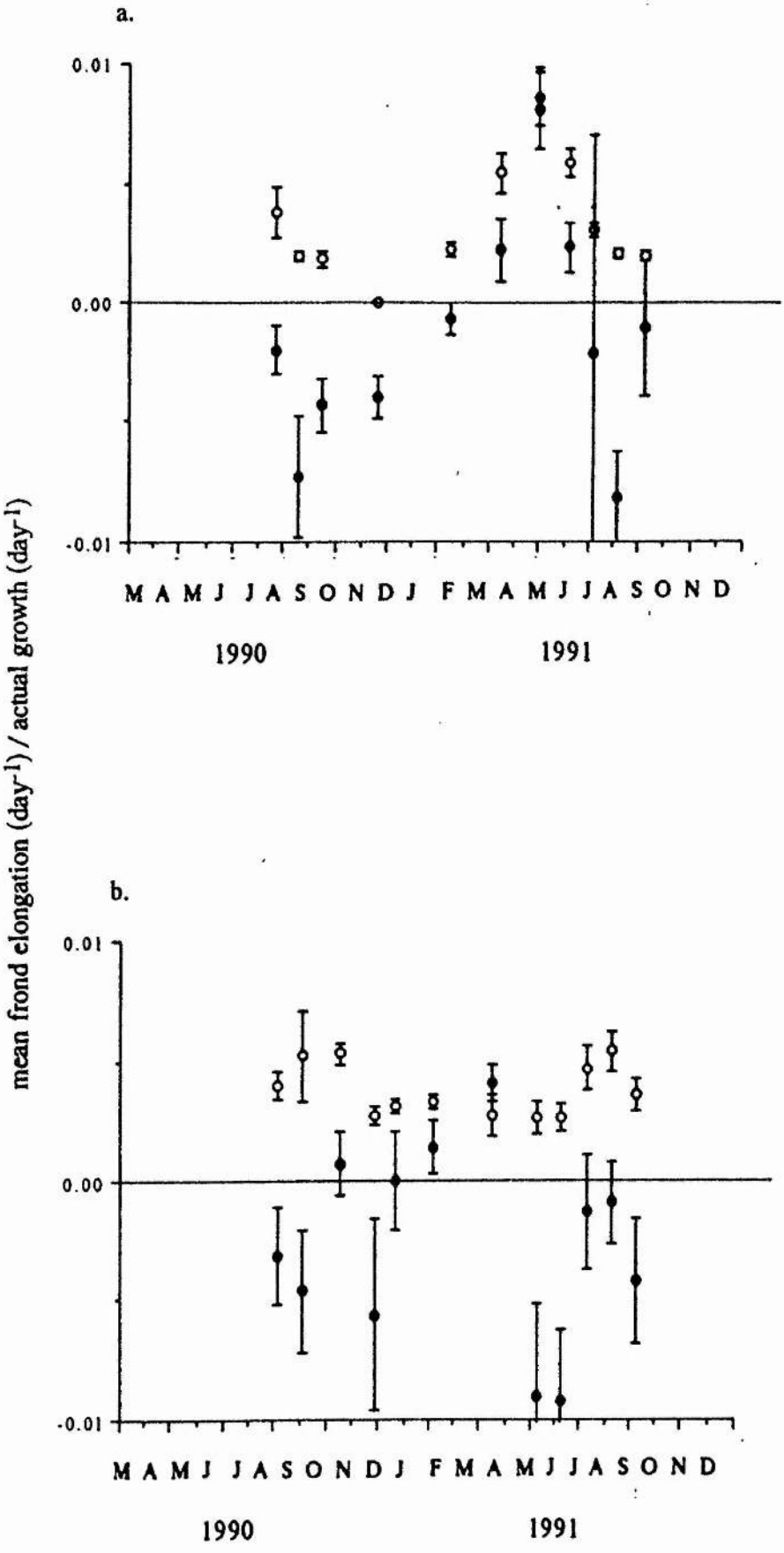
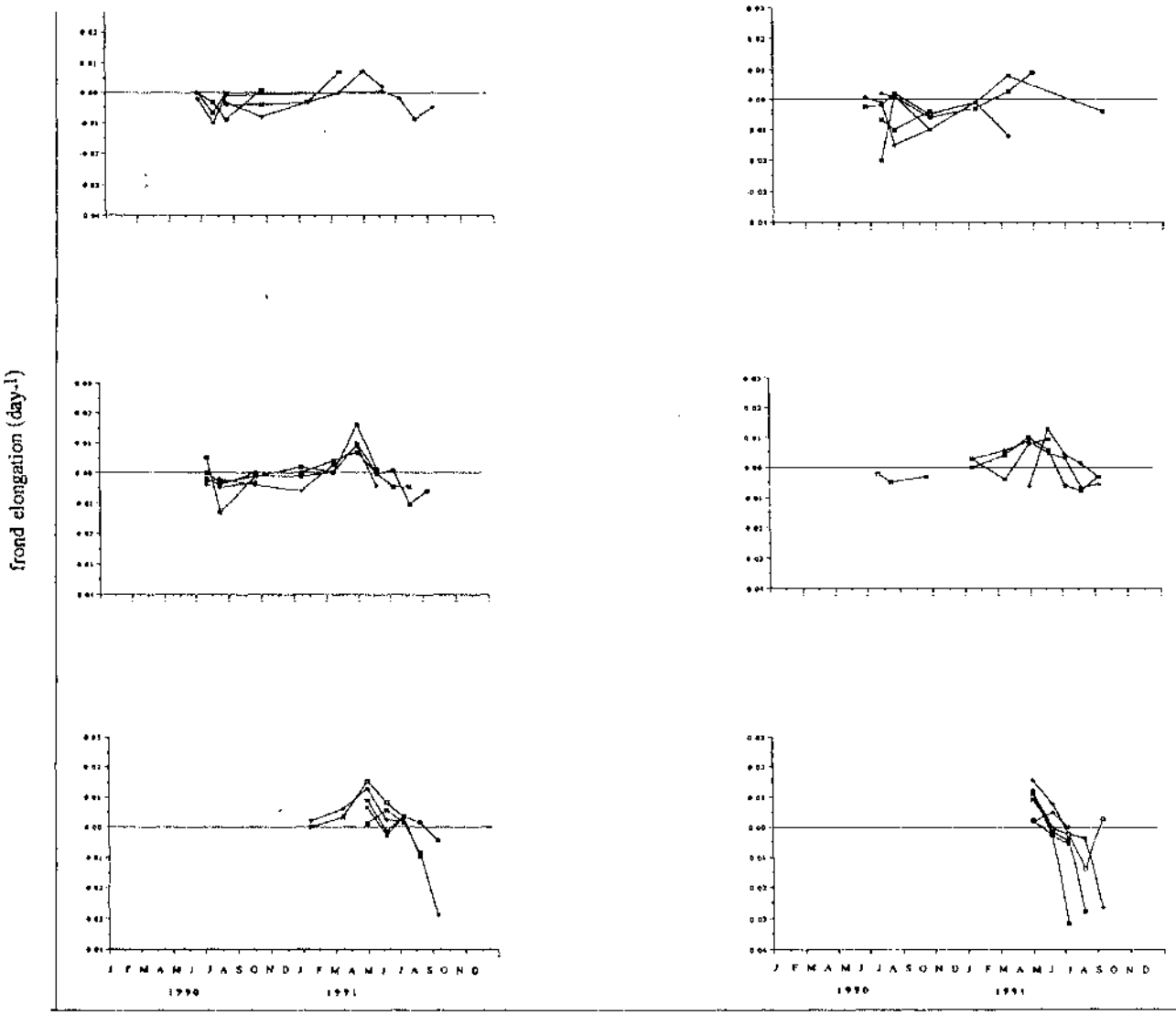

a.
0.01
0.00
-0.01
M A M J J A S O N D J F M A M J J A S O N D
1990
1991
b.
0.01
0.00
-0.01
M A M J J A S O N D J F M A M J J A S O N D
1990
1991
mean frond elongation (day-1) / actual growth (day-1)

Figure 3.6a. Growth trajectories of actual frond growth and elongation (day^{-1}) of individual mature *Laminaria digitata* plants at St. Andrews.


frond elongation (day-1)
J F M A M J J A S O N D J F M A M J J A S O N D
1990
1991

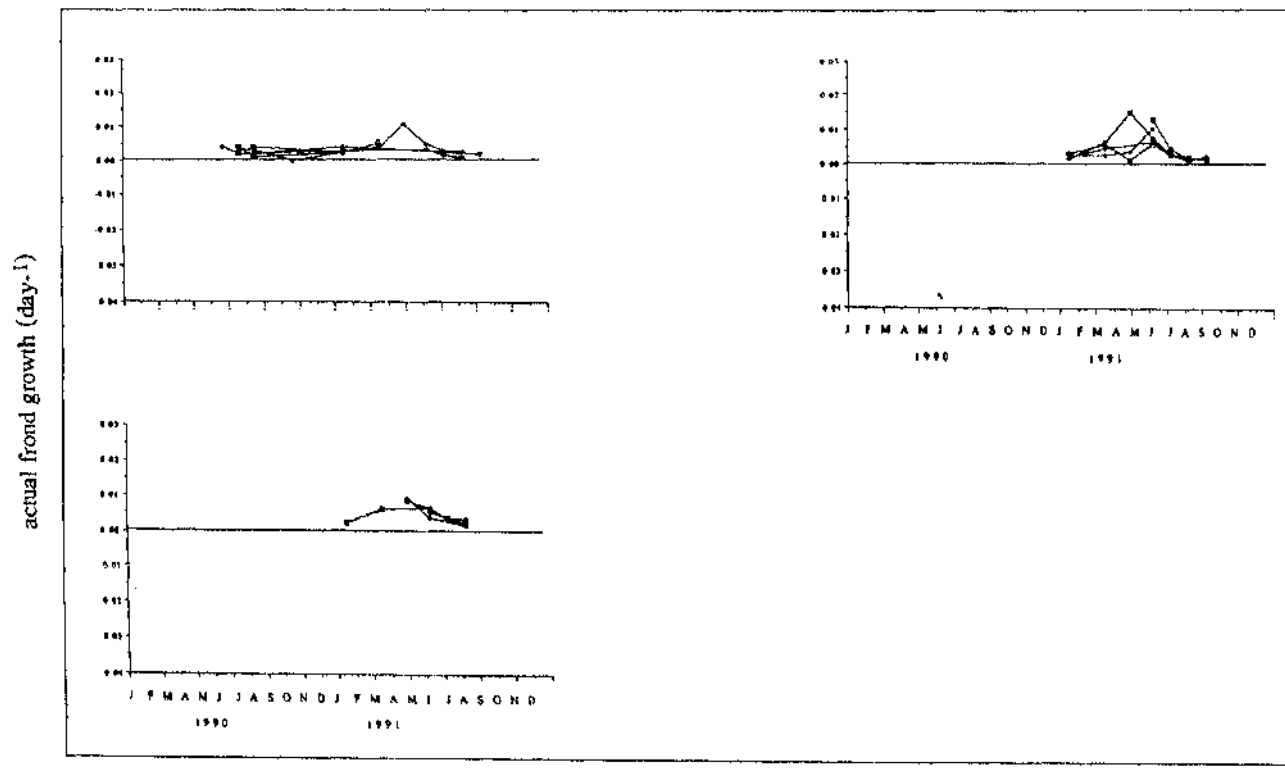
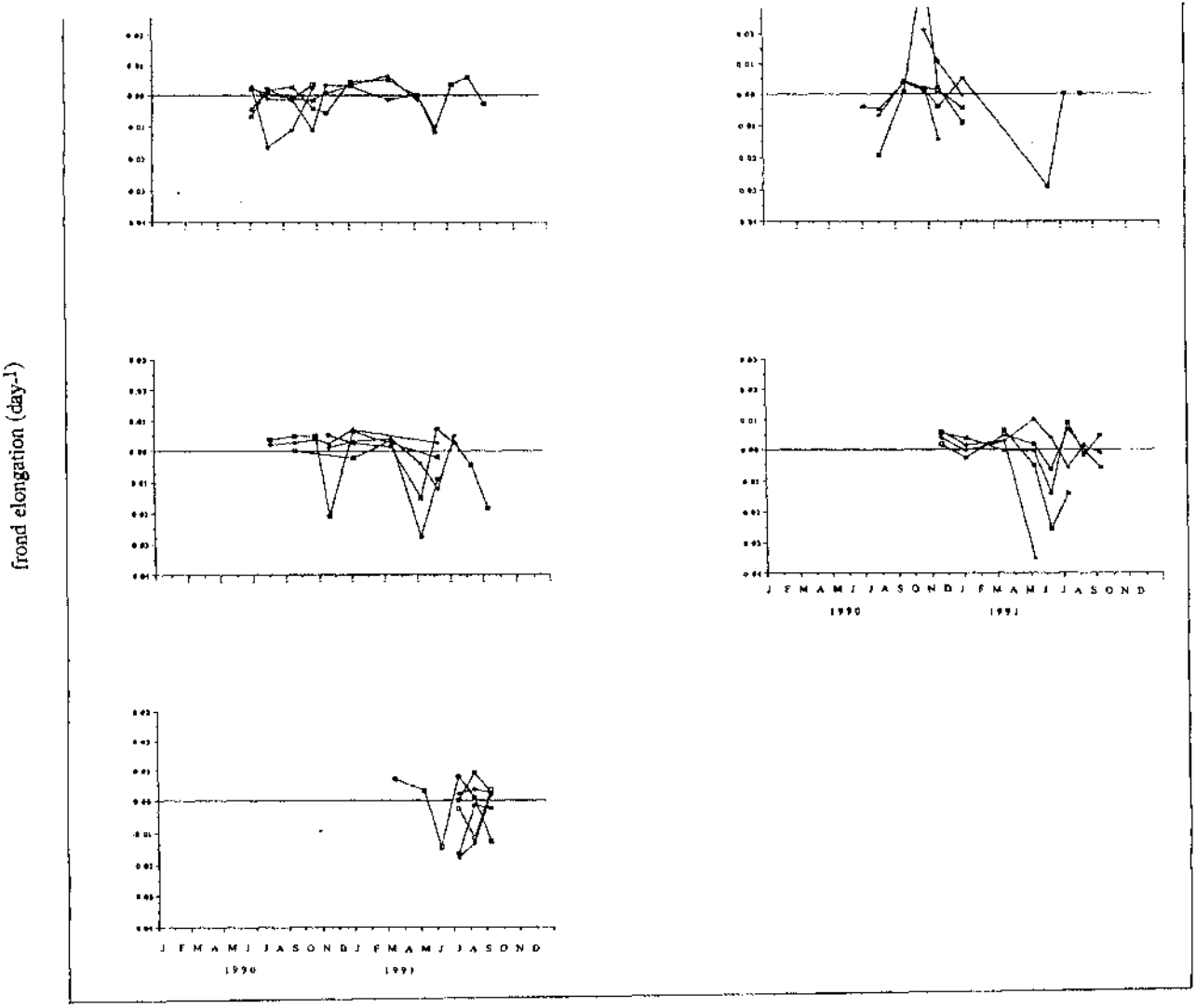

actual frond growth (day-1)
J F M A M J J A S O N D J F M A M J J A S O N D
1990
1991

Figure 3.6b. Growth trajectories of actual frond growth and elongation (day^{-1}) of individual mature *Laminaria digitata* plants at Clachan Seil.


frond elongation (day-1)
J F M A M J J A S O N D J F M A M J J A S O N D
1990
1991

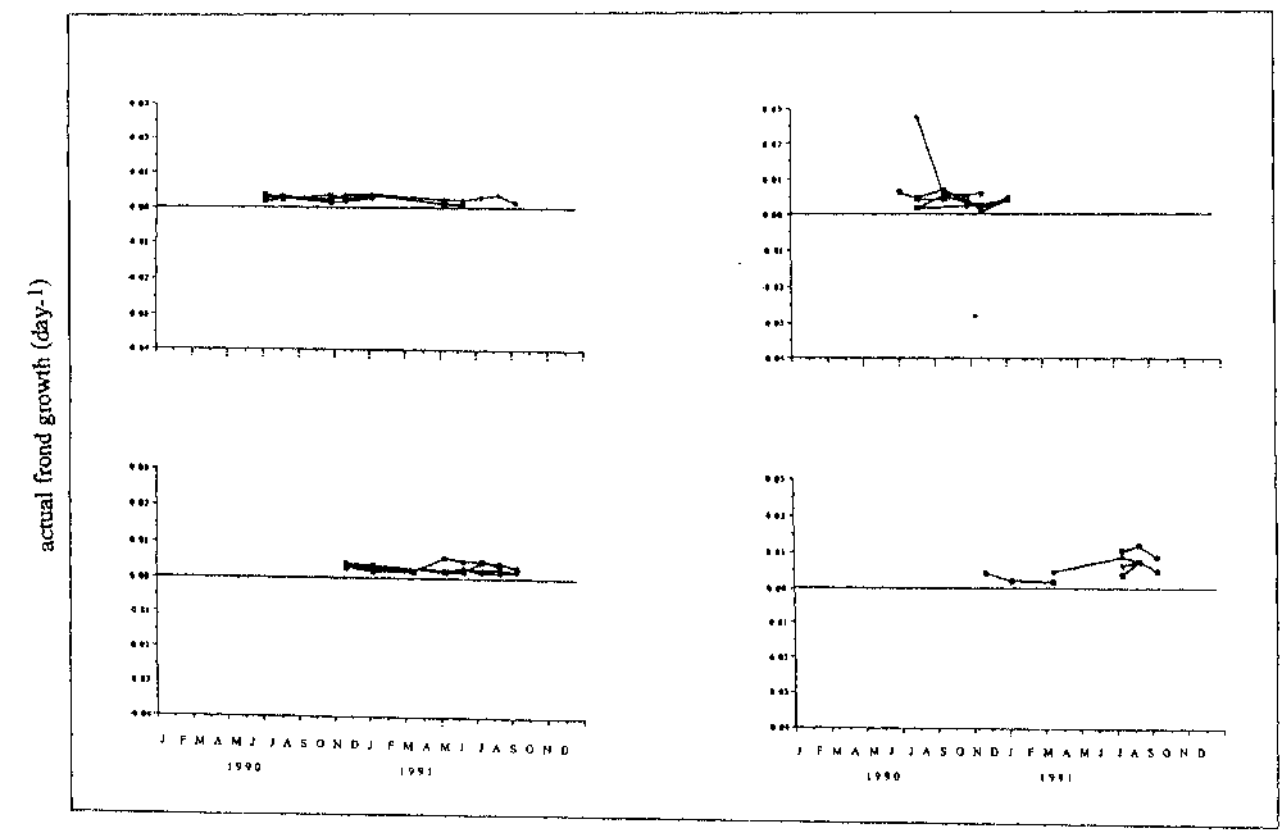

actual frond growth (day-1)
J F M A M J J A S O N D J F M A M J J A S O N D
1990
1991

Table 3.11. Multiple regression analysis of the influence of the independent variables, initial plant length and season, on the dependent variable, growth ($cm.day^{-1}$) of *Laminaria digitata*.

Table 3.11

St. Andrews: frond elongation

variables	coefficient	SE of coefficient	p
initial length	-0.0063	0.0016	0.000 ***
season	-0.1121	0.0465	0.017

St. Andrews: actual frond growth

variables	coefficient	SE of coefficient	p
initial length	0.0006	0.0006	0.337
season	-0.0725	0.0164	0.000 ***

Clachan Seil: frond elongation

variables	coefficient	SE of coefficient	p
initial length	-0.0050	0.0014	0.000 ***
season	0.3730	0.0934	0.000 ***

Clachan Seil: actual frond growth

variables	coefficient	SE of coefficient	p
initial length	0.0010	0.0002	0.000 ***
season	-0.0136	0.0170	0.427

values at St. Andrews was greater than at Clachan Seil and statistical analysis by multiple regression (table 3.11) shows that season is indeed very highly significant at St. Andrews whilst apparently not significant at all at Clachan Seil.

Mean frond elongation (fig. 3.5) (probably better interpreted as mean frond loss because values are generally negative) was cyclical at St. Andrews with peaks in June 1990 and May 1991. Such a pattern was also apparent at the west coast site; minima were recorded in June 1990 and May 1991 and maximum elongation rates in May and November 1990 and April and September 1991. Seasonality is significant at both field sites although to a greater degree at Clachan Seil.

The plant trajectories (figs. 3.6a and 3.6b) demonstrate the uniformity of actual growth and the variability of frond elongation between individuals, although it should be noted that whilst frond elongation can indeed be negative, due to the effects of frond abrasion, actual growth of the plant can never be less than zero. Plant mortality at both locations was high (tables 3.3 to 3.4) with numerous plants being lost immediately following tagging (*i.e.* never recovered) as a consequence of stipe breakage. It is relevant also to note that mean elongation at Clachan Seil fell to an absolute minimum in May 1991 corresponding to the time of maximum mean elongation at St. Andrews.

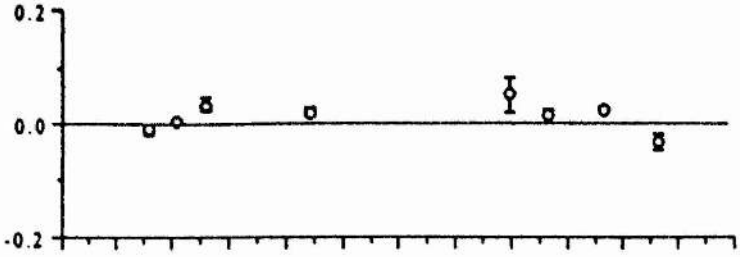
Multiple regression analyses showed that initial plant length was significantly correlated with frond elongation at both sites, and with actual growth at Clachan Seil (table 3.11). Longer fronds had higher rates of elongation and actual growth.

3.3.3. Growth of established colonies of *Electra pilosa*

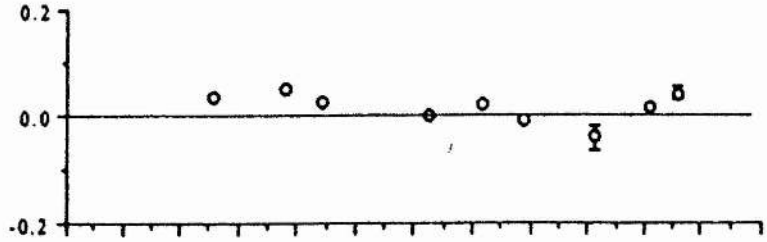
Both east and west coast populations of *Electra pilosa* showed very low values for mean aSGR (figs. 3.7a and 3.7b) in 1990 and 1991, although the growth of established colonies on *Laminaria digitata* at Clachan Seil showed quite marked negative fluctuations. This is reiterated by multiple regression analysis which

Figure 3.7. Seasonal change in mean aSGR ± SE of established colonies of *Electra pilosa* on the fronds of *Fucus serratus* and *Laminaria digitata* at a) St. Andrews and b) Clachan Seil.

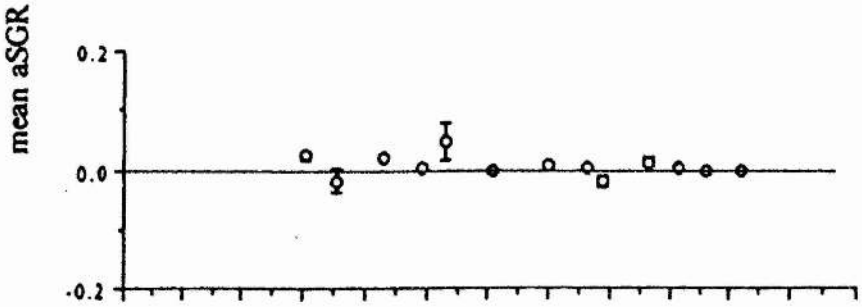
a. *Electra pilosa* on *Fucus serratus*



a. *Electra pilosa* on *Laminaria digitata*



b. *Electra pilosa* on *Fucus serratus*



b. *Electra pilosa* on *Laminaria digitata*

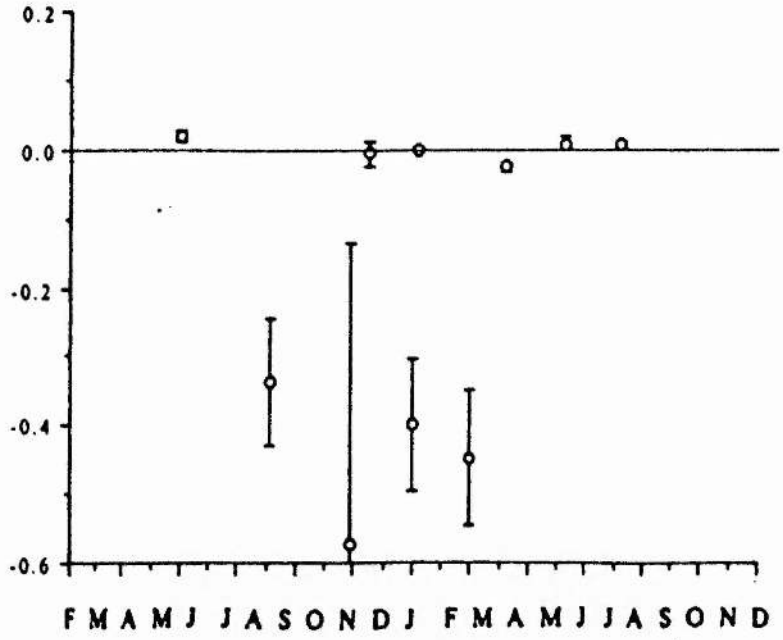


Table 3.12. Multiple regression analyses of the influence of the independent variables, colony size (zooid number) and season, on the aSGR of *Electra pilosa* on both host plant species at St. Andrews and Clachan Seil.

Table 3.12

St. Andrews: *Fucus serratus*

variables	coefficient	SE of coefficient	p
colony size	-0.0000	0.1364	0.073
season	0.0046	1.2777	0.207

St. Andrews: *Laminaria digitata*

variables	coefficient	SE of coefficient	p
colony size	-0.0000	0.0000	0.000 ***
season	-0.0017	0.0022	0.449

Clachan Seil: *Fucus serratus*

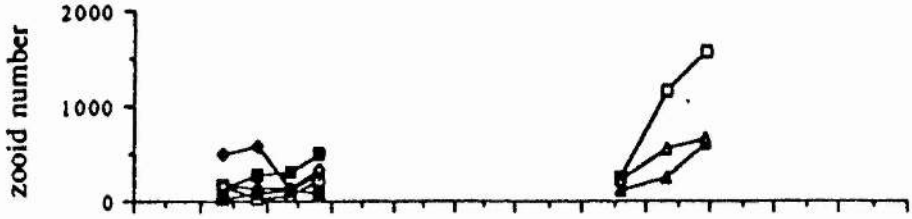
variables	coefficient	SE of coefficient	p
colony size	-0.0000	0.0000	0.000 ***
season	0.3730	0.0934	0.000 ***

Clachan Seil: *Laminaria digitata*

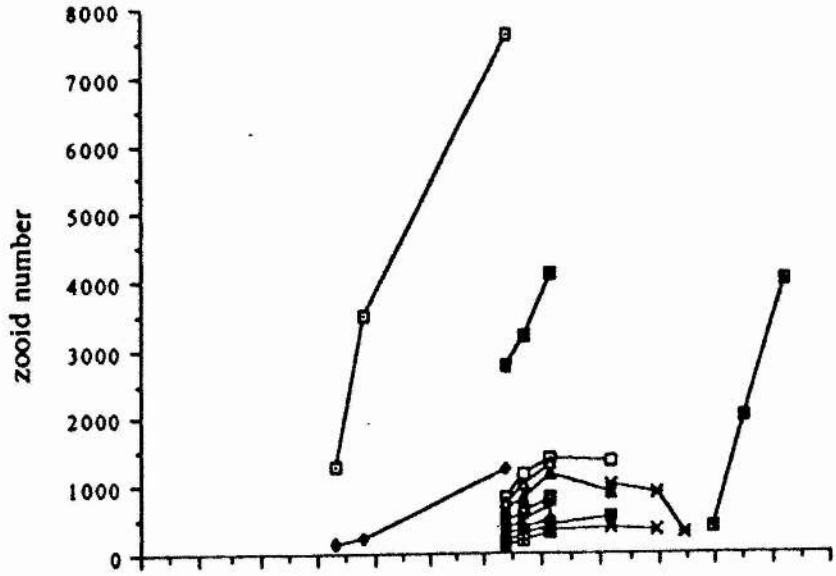
variables	coefficient	SE of coefficient	p
colony size	-0.0004	0.0000	0.000 ***
season	0.0152	0.0263	0.565

Figure 3.8. Growth trajectories of individual established colonies of *Electra pilosa* on *Fucus serratus* at a)St. Andrews and b) Clachan Seil.

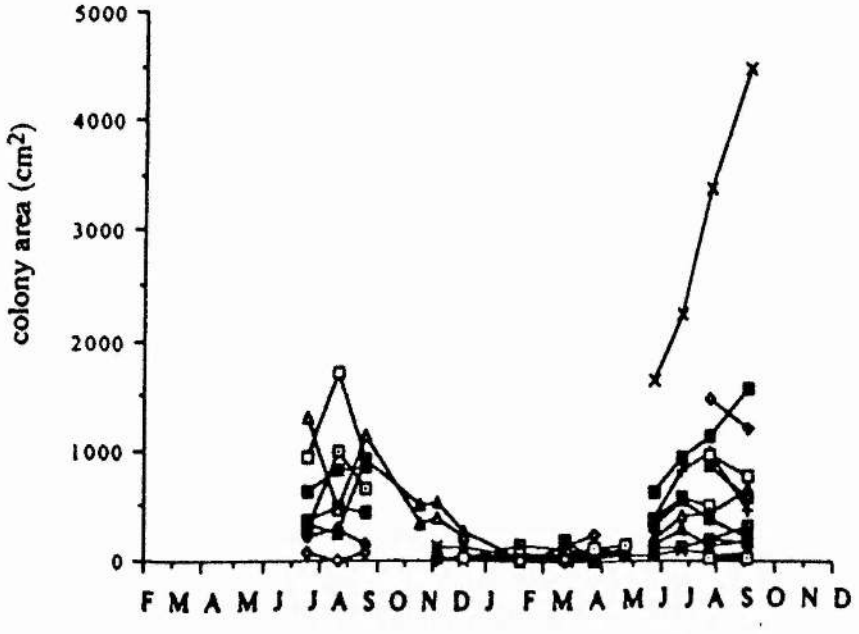
a. *Electra pilosa* on *Fucus serratus*



b. *Electra pilosa* on *Laminaria digitata*



b. *Electra pilosa* on *Fucus serratus*



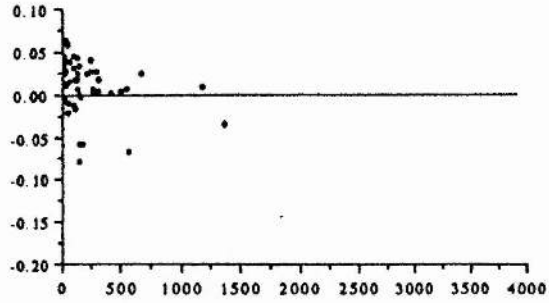
demonstrated that season had no significant influence on the aSGR of *Electra pilosa* on either algal species at either field site (table 3.12). Inspection of the growth trajectories of single colonies did, however, reveal a pattern (fig. 3.8a and b). However, the incidences of ≥3 continuous observations were very limited on *L. digitata* at St. Andrews and consequently cannot be considered. Colony growth of mature *E. pilosa* on *Fucus serratus* was positive in the summer months, but there were no data for the winter period. This latter omission was largely a result of markedly reduced colony longevity due to both colony and substratum mortality. The west coast populations, especially those on *F. serratus*, showed, in some individual cases, almost exponential growth during the summer (June to August 1990 and June to September 1991) with a cessation in growth over winter (October 1990 to May 1991). Colonies on *L. digitata* at Clachan Seil showed a less uniform pattern of growth, although again it seems that there was exponential growth in summer and a reduction of growth over the winter period of December 1990 to June 1991.

Colony size was very highly significant for colonies on *Laminaria digitata* at both Clachan Seil and St. Andrews. The significance of colony size can be seen in figs. 3.9a and 3.9b. Small colonies at St. Andrews had higher aSGRs than larger ones, whilst at Clachan Seil a large number of smaller colonies (less than 1000 zooids) had negative aSGRs. The same was true for colonies on *Fucus serratus* at this site.

Colony mortality was generally high (tables 3.5 to 3.8), although when percentage recovery was calculated exclusively for plants that were retrieved, rather than as a total percentage of all colonies deployed in the field, the retrieval percentage was improved to some extent on most occasions (figs. 3.10a and 3.10b). It is evident from these figures of colony mortality that the process of mortality is completely without pattern at both sites and on both plant species, with rates oscillating quite markedly from $time_1$ to $time_2$. As one can see from the graphed trajectories (figs. 3.8a and 3.8b) there were few colonies that had three or more continuous observations in the field and this cannot be attributed to plant loss alone.

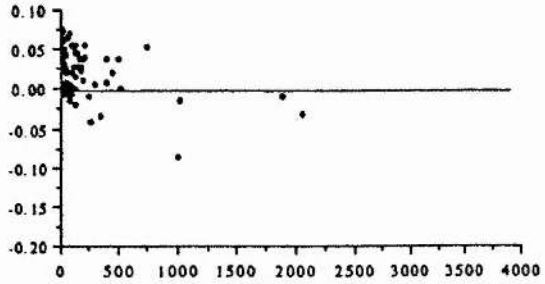
Figure 3.9. Variation in aSGR with colony size (zooid number) of *Electra pilosa* on *Fucus serratus* and *Laminaria digitata* at a) St. Andrews and b) Clachan Seil. Note that it is colony area (mm^2) and not zooid number that is the criterion assessed for colonies on *Fucus serratus*.

Electra pilosa on *Fucus serratus*



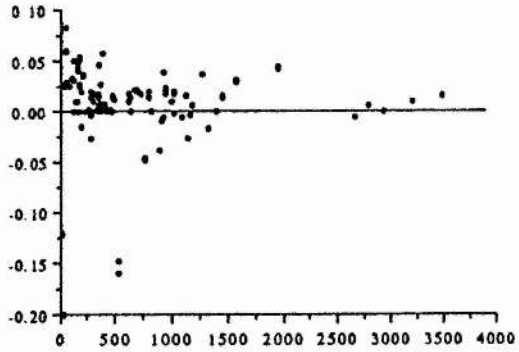
St. Andrews

Electra pilosa on *Laminaria digitata*



Electra pilosa on *Laminaria digitata*

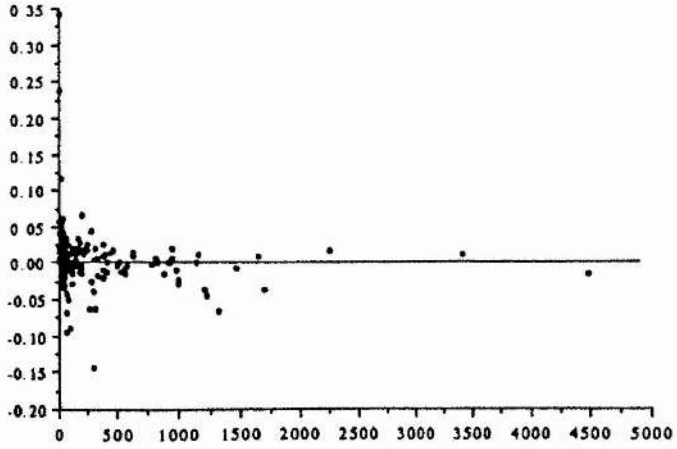
aSGR



zooid number

Clachan Seil

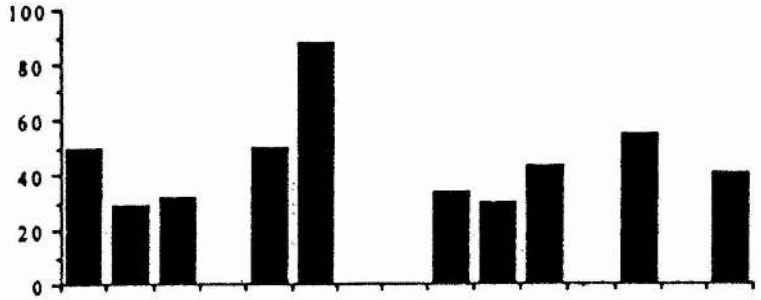
Electra pilosa on *Fucus serratus*



colony area (cm^2)

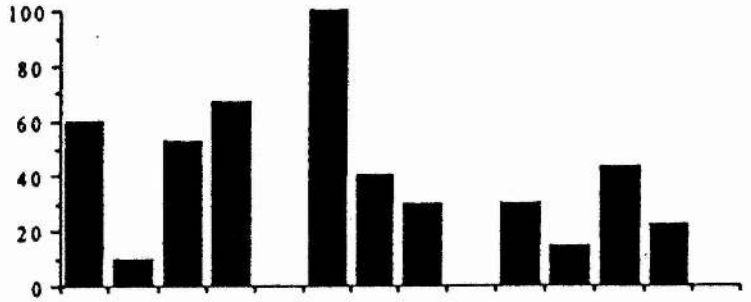
Figure 3.10. Percentage mortalities of *Electra pilosa* colonies on retrieved host plants at a) St. Andrews and b) Clachan Seil.

Electra pilosa on *Fucus serratus*



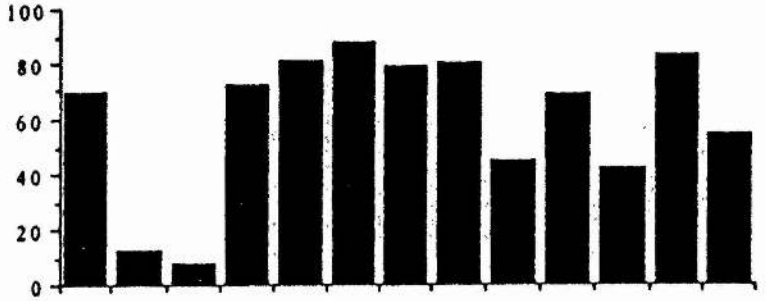
St. Andrews

Electra pilosa on *Laminaria digitata*



percentage monthly mortality

Electra pilosa on *Fucus serratus*



Clachan Seil

Electra pilosa on *Laminaria digitata*

Paired t-test analyses to assess the effects, deleterious or otherwise, of handling and retrieval of plants and colonies, was applied to data from the *in situ* studies on *Laminaria digitata* and *Fucus serratus* at St. Andrews. This indicated that recovery both of plants and bryozoan colonies was not significantly different between *in situ* and retrieved plants (table 3.13). Moreover, as seen for the retrieved plants studies, mean aSGR was independent of season, although significantly correlated with colony size on *F. serratus* (table 3.9).

Table 3.13. Paired t-test analyses of the effects of retrieval and handling on colonies of *Electra pilosa* on *Fucus serratus* and *Laminaria digitata* at St. Andrews.

Table 3.13

			p value
plants	*Fucus serratus* (*in situ*)	*Fucus serratus*	0.189
	Laminaria digitata (*in situ*)	*Laminaria digitata*	0.011 *
all colonies	*Fucus serratus* (*in situ*)	*Fucus serratus*	0.954
	Laminaria digitata (*in situ*)	*Laminaria digitata*	0.284
colonies on retrieved plants only	*Fucus serratus* (*in situ*)	*Fucus serratus*	0.905
	Laminaria digitata (*in situ*)	*Laminaria digitata*	0.292

3.4. Discussion

The life history of *Electra pilosa* on macroalgae is influenced by a complex of physical and biotic parameters, subsequently reflected in the patterns of settlement and growth exhibited by the bryozoan in the field as described in this study. The ephemeral "biologically dynamic" (Seed & O'Connor, 1981) and unstable nature of the macroalgae as substrata initiates adaptive and behavioural responses in their epiphytes to maximise their longevity and success (Buss, 1979a; Jackson, 1979a; Seed & O'Connor, 1981; Cancino, 1983, 1986; Williams & Seed, 1992).

The cyphonautes larvae of *Electra pilosa*, although present in the plankton all year round (Ryland & Hayward, 1977), were observed to settle in late summer continuing until December at both field sites. These observations concur quite closely with those presented by Ryland (1967) for settlement of the bryozoan on *Fucus serratus* at Menai Bridge, North Wales. He found continuous settlement between April and December with peaks in May and August to December. Settlement of *E. pilosa* was predominantly on the distal, concave surfaces of the fronds of *F. serratus* at both St. Andrews and Clachan Seil (figs. 3.1 and 3.2a). Such distribution of this cheilostome bryozoan has been previously recorded (Ryland, 1972; reviews in Buss, 1979a; Seed & O'Connor, 1981; Seed, 1985; Williams & Seed, 1992) and attributed to larval settlement of predictable refuges (Buss, 1979a) on a plant which grows by means of apical meristems (Knight & Parke, 1950). Ryland (1972) demonstrated a higher incidence of settlement of *E. pilosa* in the presence of established adult colonies. He attributed the aggregation of colonies to possible gregarious behaviour of the larvae. However, this factor is removed in this study by the cleaning of fronds before use in experimentation, and may thus place a greater emphasis on the influence of substratum than would otherwise occur. Selective settlement on the younger areas of macroalgae have also been reported for a number of bryozoa including *Membranipora membranacea* and *Scrupocelleria repens*

(Ryland & Stebbing, 1971; Stebbing, 1972), *Celleporella hyalina* (Cancino, 1983, 1986), *Lichenopora novae-zelandiae* (Durante & Chia, 1991) and *Bugula neritana* (Keough, 1986). However, at Clachan Seil settlement is more distal than expected, although the low frequency of settlement means that such larval selective behaviour may not be representative. This choice of older macroalgal substratum is contrary to the bryozoan's settlement behaviour on *F. serratus*, and to the behaviour exhibited by a number of other bryozoan species on *L. digitata* (Ryland, 1972). Ryland attributed aggregation of colonies on *L. digitata* primarily to the localised occurrence of breeding established colonies, but also to the non-uniform nature of the frond, competition with spirobids and possibly gregariousness rather than the age of the substratum. The utilisation of clean fronds in this investigation would have obviously negated the role of established colonies and competition and one can only surmise the effect this may have had on settlement at both field sites.

Previous studies of spatial distribution have failed to consider the actual area of substratum available for settlement, rather than the position along the frond, as a potential factor modifying larval spatial dispersion. It is demonstrated here that at St. Andrews, settlement of *Electra pilosa* on *Laminaria digitata* is significantly influenced by the available area of the frond (fig. 3.2b). The conflicting settlement strategies exhibited by *E. pilosa* may be attributed to a disparity in the need for the location of predictable spatial refuges on the two algal species. The epiphytic community of *Fucus serratus* is diverse and complex (Hagerman, 1966; Stebbing, 1973b; Boaden *et al.*, 1975; O'Connor *et al.*, 1979; Wood & Seed, 1980; Wood, 1983), and inter- and intra- specific competition is intense (Wood & Seed, 1980) such that the location of newly generated substrata is of paramount importance to such an opportunistic, competitively weak species as *E. pilosa* (O'Connor *et al.*, 1980). Indeed the ability of *E. pilosa* to orientate growth towards the younger, less encrusted regions of the plant enables *E. pilosa* to become an important and sometimes

dominant component of the bryozoan assemblages of *F. serratus* (O'Connor *et al.*, 1980).

By contrast *Laminaria digitata*, in common with other laminarians, is unattractive to a number of epiphytes (Cancino, 1983) and community diversity is reduced with *Electra pilosa* often being the sole occupier of the fronds at both sites (pers. obs.); the need to avoid potential competitors is minimal, if at all. It is relevant to note, however, that at St. Andrews the distribution of frond area of the laminarian decreases distally, as indeed does the frequency of settlement, such that maximum settlement occurs on the 10—20% increment of total plant length corresponding to the positioning of the basal intercalary meristem. It may be then that the relationship between area and spatial dispersion seen on *L. digitata* at St. Andrews is an artifact of larval selective settlement on the younger regions of the frond.

Whether any of the proposed cues of larval selective settlement for the detection of the age-gradient are responsible for the observed spatial distribution of *Electra pilosa* is uncertain. The mechanisms and roles of these cues is unclear and certainly not universal amongst the bryozoa: *Lichenopora novae-zelandiae* is believed to settle in relation to anti-herbivore compounds, polyphenol tannins (Durante & Chia, 1991), already identified for their role in the inhibition of bacterial and larval settlement (Hornsey & Hide, 1976; Al-Ogily & Knight-Jones, 1977). *Bugula flabellata* (Crisp & Ryland, 1960), *B. simplex*, *B. stolonifera*, *B. turrita* (Brancato & Woollacott, 1982) and *B. neritina* (Mihm *et al.*, 1981; Maki *et al.*, 1989) settled preferentially on microbially filmed surfaces, although the role of microbial films in the field is disputed by Durante & Chia (1991). Other authors (reviews in Seed & O'Connor, 1981; Seed, 1985; Williams & Seed, 1992) believed that physiological and chemical gradients, allied to the age-gradient of the fronds, influence spatial dispersion. The accumulation of storage carbohydrates, mannitol and laminarin, fated to become food reserves for winter or sporogenesis, present a concentration gradient along the frond of the kelps (Black, 1954); however, the

seasonality of this production precludes it from providing a year-round indication of the age gradient (Cancino, 1983, 1986). Antibiotic production, however, provides a constant gradient of age related activity (Hornsey & Hide, 1976); production of polyphenols is lowest in the younger regions of the fronds of laminarians (Al-Ogily & Knight-Jones, 1977). These antibiotic compounds have been shown to have anti-fouling properties (Sieberth & Conover, 1965; Al-Ogily & Knight-Jones, 1977; Durante & Chia, 1991; Hay, 1992) inhibiting larval settlement (Hay, 1992), but they are not deleterious to older established epiphytes (Al-Ogily & Knight-Jones, 1977). Similar chemical gradients, associated with their growth strategies, have been demonstrated in the fucoids (Moss, 1948; Knight & Parke, 1950; Conover & Sieberth, 1966). Photosynthetic gradients created by the suppression of photosynthetic capabilities of fronds by heavy epiphytic encrustation (King & Schramm, 1976; Oswald *et al.*, 1984) may provide another cue with the density of fouling related to the degree of reduction in photosynthetic activity (Oswald *et al.*, 1984). Whilst the cues operating for the selective settlement of *E. pilosa* on either of the macroalgae are unknown, the difference in the period of settlement and the location of that settlement can be attributed to the differing growth strategies of the two algae.

The seasonal pattern of growth of *Laminaria digitata* sporophytes at St. Andrews (fig. 3.5) was typical of previous growth studies of this alga (Sundene, 1964; Cosson, 1967; Perez, 1969, 1971; Mann, 1972; Lüning, 1979; Kain, 1979; Conolly, 1982) and others of the same genus (reviewed by Kain, 1979; Cancino, 1983). Frond growth became apparent in January, which was stimulated by the increasing photoperiod, and aided by the translocation of storage carbohydrates from the old frond to the growing meristem because light levels were still below the photosynthetic compensation point (Kain, 1979). The period of fast growth was superseded by slow growth which commenced in May, activated by the April seawater nutrient decline (Conolly, 1982). Settlement of *Electra pilosa* occurred during this period of slow growth, aggregating on the basal regions of the fronds,

either in response to the area available or to young tissue thereby ensuring maximum colony longevity (Cancino, 1983, 1986). This pattern of plant growth was not obvious at Clachan Seil (fig. 3.5), where growth appeared almost constant, a feature of the sublittoral kelps, and declined only in December. However, Cosson (1967) also noted such continuous growth in *Laminaria digitata*. Settlement of *E. pilosa* was minimal on the laminarian at this site, despite the large surface area available. This may perhaps be explained by the constant dynamic nature of this substratum, resultant from the continuous growth observed, acting as a possible anti-fouling mechanism.

Growth of *Fucus serratus*, mediated by apical meristems in common with the littoral fucoids and providing a contrast to the perennial *Laminaria digitata*, took place in the summer months, ceasing completely in winter (fig. 3.3). Cessation of growth has been shown to reflect the low, minimal levels of storage compounds present in the tissues, which are required only for respiration (Barrett & Yonge, 1985). In late autumn, following fruiting, a period of rapid defoliation in consequence of necrosis of reproductive tissue and the abrasion of the spent receptacles was reflected in the negative mean growth rates at both field sites. Concurrently, new frondage produced at the apical meristems (Knight & Parke, 1950) became conspicuous as new vegetative tissue. Aggregated settlement of *E. pilosa* on the distal regions of *F. serratus* occured during the summer months when uncolonised tissue was readily available. Apical defoliation after fruiting does, however, present a possible source of substratum loss but this must be less critical to *E. pilosa* than the avoidance of potential competitors.

Electra pilosa, with its potential for exponential indeterminate growth (Hughes & Hughes, 1986; Rubin, 1987; Silén, 1987), rarely realised that potential in the field. Several colonies, however, attained near exponential growth (figs. 3.8a and 3.8b). Although this fact was masked in the mean aSGRs which bear no resemblance to past growth rates quoted by various authors (Menon, 1972; Hughes & Hughes,

1986; Silén, 1987). At Clachan Seil, growth of individual colonies increased during the summer months before effectively ceasing in November (fig. 3.8b), in a manner similar to that previously described for *Flustra foliacea* (Stebbing, 1971). A decrease which was closely allied to the decline in surface seawater temperature below 10°C (fig. 2.3b). *E. pilosa* has been shown to respond to differing temperature regimes with a variation in the growth rate (Menon, 1972); 6°C effectively reduced the rate to a minimum, whilst rising seawater temperatures have been reported by Ryland (1970, 1976) as initiating growth rate increases in the Bryozoa. In considering growth rates of the Bryozoa in the field it is perhaps more pertinent then to consider individual colony growth trajectories in relation to laboratory produced optimal growth rates and life histories, rather than the mean aSGR of a field population.

The maintenance of aSGR with increasing colony size at Clachan Seil, both on *Fucus serratus* and *Laminaria digitata*, as opposed to the quite rapid decrease in aSGR with size at St. Andrews (figs. 3.9a and 3.9b) was born out by the colony morphologies of *Electra pilosa* observed at both field sites. The large stellate colonies at Clachan Seil enabled maintenance of a high aSGR (Hughes and Hughes, 1986) both in an environment of severe interspecific competition within the highly diverse epiphytic community of *F. serratus,* and on the very dynamic unstable substratum of *L. digitata,* despite the low diversity and complexity of epifauna on laminarian fronds (pers. obs.). Furthermore these extrinsic environmental factors operating at Clachan Seil were compounded by the high incidence of predation by the nudibranchs *Adalaria proxima* and *Onchidoris muricata* (pers. obs.). In contrast, the slow growth period of *L. digitata* at St. Andrews (fig. 3.5) enabled colonies to become established, and the less diverse epiphytic community (pers. obs.) on *F. serratus* resulted in the more regular, circular form of *E. pilosa.* Such plasticity of colony morphology is in accordance with the adaptive strategy model of Buss (1979a), but see Okamura (1992), and was viewed as a plastic developmental

response to changes in extrinsic environmental factors (McKinney & Jackson, 1991). Indeed, the life-history characteristics of the bryozoan at the two sites and on the two algal species matched the quantitative predictions made by the risk model of McKinney & Jackson (1991). A sheet-like morphology is associated with low levels of disturbance and predictability, whilst a uniserial growth form is indicative of a more unstable ephemeral environment. However, Hayward & Ryland (1975) noted that as the available space on the fronds of *F. serratus* diminished, growth of *Alcyonidium hirsutum* slowed. Furthermore, the point from which growth began to slow and the ultimate size of the colonies seemed to depend upon the density of settlement rather than on extraneous environmental factors. These factors have not, however, been investigated within the scope of this thesis.

Complete and partial colony mortality have complicated and distorted any pattern in the mean aSGRs (figs. 3.7a and 3.7b) and have profoundly influenced the dynamics of the bryozoan populations, mediated through the effects of physical and biological disturbance. Disturbance, in providing a means of space renewal, is one of the major structuring forces of the fundamentally space-limited communities on marine hard substrata (Dayton, 1971; Jackson, 1977; Osman, 1977; Sousa, 1979, 1984; Paine & Levin, 1981, Connell & Keough, 1985). Space is not limiting on macroalgae in consequence of the processes of constant regeneration of substrata during the growing season (Seed, 1985), but see Hayward & Ryland (1975). However, disturbance, presented as the constant attrition at the frond apices and the presence of disturbance gradients, is argued by Fletcher & Day (1983) to be a greater force of post settlement mortality than previously credited. Clearly, when the growth rate of a colonial animal which has the potential to realise exponential, indeterminate growth is suppressed, the factor instigating that suppression is of paramount importance, not only to the life history of that animal but also to the whole epiphytic community. The agents of disturbance, colony or frond abrasion and predation, have also been reported as the major forces influencing the growth of the phylactolaemate

bryozoan *Plumatella repens* (Bushnell, 1966) and *Alcyonidium hirsutum* (Hayward & Harvey, 1974), but consideration of these factors in the role of structuring epifaunal bryozoan assemblages has been limited (but see Seed, 1976; Todd & Havenhand, 1989) and will be discussed in Chapter 4 .

The intimate relationship between the epiphyte and its host may result in costs and consequences for both. This study has not considered the influence of *Electra pilosa* on the growth rate of either host plant species, whilst, aside from the roles of frond growth, attrition and abrasion, the influences of algal exudates and antifouling mechanisms have likewise not been investigated. There has, however, been recent interest in this area of algal epifaunal ecology. Whilst some believed that the fouling epifauna are inhibitory and deleterious to the plants (Wing & Clendinning, 1971; Woollacott & North, 1971; Bernstein & Jung, 1979; Dixon *et al.*, 1981; Oswald *et al.*, 1984) yet others considered that the costs to the plant are less severe (Cancino *et al.*, 1987; Muñoz *et al.*, 1991).

Chapter 4

The dynamics of nudibranch predation and colony damage on *Electra pilosa*

4.1. Introduction

Mortality and both intra- and inter-specific competition suppress the realisation of the growth potential in the Bryozoa. However, whilst the effects of competition have been well documented (Stebbing, 1973a, 1973b; Osman, 1977; Buss, 1979b; Jackson, 1979b; O'Connor *et al.*, 1980; Seed & O'Connor, 1981; Seed, 1985; Rubin, 1985; Rubin, 1987; Todd & Turner, 1988; Okamura, 1988, 1992), mortality, manifest as predation, colony abrasion and substratum loss, has been largely ignored with the exception of the studies by Seed (1976) and Palumbi & Jackson (1982).

4.1.1. Nudibranch predation of the Bryozoa

Nudibranchs are perhaps the most destructive predators of the Bryozoa (Ryland, 1976; review in Todd, 1981). This trophic group is dominated by the dorids, which subject the zooids to suctorial or rasping feeding action (Todd 1981). The nature of the buccal armature of these molluscs is indicative of the feeding technique adopted (Nybakken & McDonald, 1981). Raspers make use of the radula apparatus; the cartilaginous odontophore and the overlying radula with its long rows of chitinous teeth. The odontophore can be projected through the mouth causing erection of the radula teeth which are then used to scrape up the polypides and/or zooecium and transport it to the oesophagus (Barnes, 1980; Todd, 1981). Suctorial feeders, however, employ the extrinsic muscles of the buccal mass to enable polypides to be taken without radula action when the oral veil is pressed against the frontal membrane; here muscle action is sufficient to remove the polypide (Ryland & Hayward, 1977; Todd, 1981).

The feeding strategy adopted by most nudibranchs seems to be one of partial predation (Todd, 1981; Harvell, 1984b; Todd & Havenhand, 1989). Partial consumption of the prey is more accurately described as grazing (Begon *et al.*, 1986). The effect is harmful, but not necessarily lethal in the manner of true predators, and

consequently the term grazing is used here interchangeably with predation. Such incomplete destruction of cheilostome bryozoan colonies by the nudibranch predator has been observed for *Adalaria proxima* and *Onchidoris muricata* on *Electra pilosa* and *Polycera quadrilineata* on *Membranipora membranacea* (Todd, 1981; Todd & Havenhand, 1989), *Dirona albolineata, D. aurantea* and *Triopha catalinae* on *Dendrobeania lichenoides* (Harvell, 1986) and *Madrella sanguinea* on *Mucropetralliela ellerii* (Klempke & Keough, 1991). Such incomplete destruction of bryozoan colonies has inevitably provoked discussion as to the cause of this apparently strategical behaviour.

Zooids in a colony would be expected to be morphologically uniform due to their identical genetic composition. However, variations do occur and are ascribed by Boardman and Cheetham (1973) to the effects of zooidal ontogeny, colony astogeny, polymorphism or the microenvironment (thoroughly discussed in the text). Thus, bryozoan colonies are not homogeneous units, but are integrated collections of trophically, reproductively or defensively specialised zooids, whose intracolonial distribution varies with the age of the colony (Boardman & Cheetham, 1973; Ryland, 1979; Yoshioka, 1982; Harvell, 1984b). These differences in morphotype may present variation in resource quality for grazers on the colony. Harvell, (1984b) demonstrated that intra-individual differences in morphology of the bryozoan *Dendrobeania lichenoides*, a perennial cheilostome, influenced the feeding strategies of the nudibranchs *Dirona albolineata, D. aurantea* and *Triopha catalinae* . Individuals of all three species preferentially consumed the non-reproductive, younger, peripheral tissue. Harvell (1984b) attributed this preferential and partial predation to the decreased palatability of the older tissue, possibly the result of an aversion to the high N:C ratio of the brown bodies present. Best and Winston (1984), however, found that exoskeletal and frontal membrane strength of encrusting cheilostomes was greater in the centre of the colony. In five out of eight of the species tested the peripheral zooids were significantly weaker and hence would be expected to have a greater vulnerability to

predation. Whilst acknowledging that differential palatability alone may result in this partial predation, Harvell (1984b) also discussed the possibility that this strategy is one of "prudent predation" (Slobodkin, 1961, 1968, 1974). This concept is controversial, largely due to the differing interpretations of the prudent predation theory. Slobodkin (1974) suggested that prudent predation is an outcome of natural selection operating at the level of the prey, a situation which encourages development of defensive strategies or a lowering of the fitness of the preferred prey type, rather than one in which the predator consciously abstains from over-exploitation of the prey resource. Slobodkin (1974) argued that this does not involve group selection (Wynne-Edwards, 1962, 1986) but is a consequence of the evolutionary "arms race" between predators and prey in which the prey is always ahead (the life-dinner hypothesis: Dawkins & Krebs, 1979). This argument is contrary to that of Maynard-Smith and Slatkin (1973) and Krebs & Davies (1987) who believed that such a theory relies on group selection of the prudent predators, a theory they considered fundamentally flawed by "selfish" individuals. However it would seem logical that the presence of constitutive or induced prey defences would influence the foraging strategy of a predator.

4.1.2. Inducible defences of the Bryozoa

The Bryozoa employ an array of defensive mechanisms against predation and competition. Such defences are manifested in a number of behavioural and morphological strategies which have been extensively reviewed (Stebbing 1973a, 1973a; Ryland, 1976, 1979; Buss, 1979a; Jackson, 1979a; Osborne, 1984; review in Harvell, 1990; Harvell & Padilla, 1990). These defensive responses may be constitutive or inducible phenotypic adaptations mediated through cues from the biotic environment (Harvell, 1990). Such induced adaptations, prolific within the plant and animal phyla, have been shown to be structural or chemical, either through creation of new phenotypes or by modification of the existing form (Harvell, 1990). Within the

Bryozoa, recorded induced defences have all been structural, both through modification of existing zooids, and synthesis of new structures. The most comprehensively studied example of inducible defence is that of spine formation by the cheilostome, *Membranipora membranacea* .

Membranipora membranacea is observed to produce spines in response to predation by the trophically specialised nudibranch molluscs *Doridella steinbergae* and *Corambe pacifica* (Yoshioka, 1982). Yoshioka (1982) suggested that the two closely related species *Membranipora serrilamella* and *M. villosa* are in fact merely eco-phenotypic variants of *M. membranacea* mediated through this inducible response and should be recorded as such. He thus proposed a developmental sequence relating the three species: *M membranacea*, unspined, simple structure; *M. serrilamella* (intermediate) possessing a cryptocyst (the serrated inner extensions of the zooid wall) and *M. villosa* possessing both a cryptocyst and numerous spines. The presence of spines would appear to prevent complete decimation of the colonies, disrupting the feeding techniques employed by both nudibranchs. *C. pacifica*, normally a preferential consumer of the younger peripheral zooids was observed, in the presence of spines, to feed on the central, unspined regions of the colonies; *D. steinbergae* too, actively avoided consumption of spined zooids (Yoshioka, 1982). Thus, it was suggested that decimation of the field populations of *M. membranacea* was prevented, despite the resource potential being below that required by the high densities of nudibranchs observed by Seed (1976) and Yoshioka (1982).

Following these field observations Harvell (1984a) demonstrated that *Membranipora membranacea* rapidly produces permanent, chitinous straight corner spines and spines on the frontal membrane within 36 hour's of attack by *Doridella steinbergae*. The cue is water-soluble, triggered solely by the nudibranch and may be induced after only an hour exposure to the nudibranch extract; mechanical damage failed to initiate a response (Harvell, 1984a). Harvell (1991) suggested that spine induction in *M. membranacea* is an ontogenetic (zooidal) heterochronic response (in the

manner of the ontogenetic organisation of social hymenopterans). The response was confined to a peripheral band of zooids which possess the ontogenetic competence for spine formation, with spination evoked independently by local action at the level of the zooid. The slow nature of induction and the variable individual zooid response implies that the nervous system is not involved (Harvell, 1991).

It was proposed that the induced spines produced are composite zooids comprising an autozooid and four diminished interzooids (see Harvell, 1991 for details). The spines were effective in the disruption of grazing by *Doridella steinbergae*, significantly lowering feeding rates from 44 zooids.day^{-1} (unspined) to 8 zooids.day^{-1} (spined) (Harvell, 1984a): there is evidence also that they disrupt recruitment of the larval nudibranch (Harvell, 1986). Smaller colonies required greater threshold concentrations perhaps because of increased defensive costs with colony size. Whilst induced spines result in a 40 percent reduction in normal feeding rates, there is an associated cost to the colony; growth was 85 percent of the normal rate with a subsequent reduction in fecundity (Harvell, 1986) and colony longevity was decreased owing to the increased senescence of spined zooids (Harvell, 1990). Clarke and Harvell (1992) attributed the varying defensive response of *M. membranacea* colonies to a plastic optimal allocation of resources to defence, reproduction and growth by the respective colonies. The allocation to defence is demonstrated to vary temporally, with the colony condition and on the intensity of predation. As such, an inducible strategy will be favoured above a constitutive fixed-response strategy in an environment of unpredictable predation levels, whereby the conditions can be continually reassessed and resources allocated accordingly.

This partial consumption enables colony regeneration, seen in a number of modular colonial invertebrate phyla (Jackson & Palumbi, 1979; Ryland, 1979; Wahle, 1983; Harvell & Suchanek, 1987; Hoppe, 1988). Intracolonial variation in regeneration rate occurs in a number of encrusting cheilostome and ctenostome Bryozoa, to the extent whereby some exhibit little or no regenerative ability in the

colony centre (Bronstein, 1938, 1939; Gordon, 1968). However, both non-peripheral zooids and small fragments of peripheral zooids of *Membranipora membranacea* were observed to lack the ability to recommence growth after nudibranch predation (Todd & Havenhand, 1989). This will certainly be related to the direct relationship between area and colony fitness in encrusting bryozoans. Consequently, partial predation and variation in regeneration rates will have long-term net effects on bryozoan colonies.

Zooids of the cheilostome bryozoan, *Electra pilosa*, may exhibit polymorphism when threatened by colony overgrowth by competitors in the epiphytic bryozoan assemblages of *Fucus serratus* (Marcus, 1926; Stebbing, 1973a, 1973b). The normal zooid possess 4 to 12 spines encircling the frontal wall together with an enlarged proximal spine (Stebbing, 1973a; Ryland & Hayward, 1977). However, this proximal spine may elongate considerably, up to 5 times the length of the zooid; both Marcus (1926) and Stebbing (1973a, 1973b) observed this and deduced its role to be in overgrowth prevention. Stebbing (1973a, 1973b), in his studies of spatial competition of the epiphytes of *F. serratus*, noted that whilst *E. pilosa* often possessed these enlarged spines at the point of contact with other bryozoan species and at the colony extremities overgrowth was rarely prevented.

The spines might disrupt grazing of the predator *Adalaria proxima*, preventing the rasping feeding behaviour observed by Thompson (1958) and Todd (1979). Feeding is effected by intermittent action of the radula with some 3 to 8 strokes in each period of activity. The radula is projected through the mouth and used in a rasping manner with the effective stroke being forward and upward due to the posterior recurve of the radula teeth. Todd (1981) observed an efficient, methodical feeding habit in this nudibranch. However, feeding was noted to be discontinuous spatially and temporally, leading to incomplete destruction of the colony. Individual nudibranchs exhibit feeding rates far in excess of the growth rate of the bryozoan and yet the field populations of the bryozoan are clearly able to withstand this intense predation. However, this may be

due to the density of the nudibranch, isolation of colonies from predation effects or massive recruitment of the bryozoan (Klempke & Keough, 1991).

4.1.3. Aims of this chapter

In the light of the findings of Harvell and co-workers (Harvell, 1984a, 1984b, 1986, 1990, 1991; Harvell & Padilla, 1990; Clarke & Harvell, 1992) as to the phenotypic plasticity of *Membranipora membranacea*, and in view of the length of time since Stebbing's observations (1973a and 1973b) of proximal spine elongation in *Electra pilosa* in overgrowth interactions, it was decided to investigate the possible inducible properties of the proximal spine in *E. pilosa* in response to its nudibranch predator *Adalaria proxima*. Concurrently, the effect of colony quality on the predation behaviour adopted and the pattern of feeding in *A. proxima* were observed.

4.2. Materials and methods

4.2.1. Collection and maintenance of animals

Mature individuals of *Adalaria proxima* were collected by hand from fronds of *Fucus serratus* at Clachan Seil, Argyll (west coast, Scotland). Colonies of *Electra pilosa*, epiphytic on *F. serratus*, were collected at the same site. The nudibranchs were maintained in aerated seawater and constant illumination at T = 7—8°C, and fed *E. pilosa ad libitum*. *E. pilosa* on *F. serratus* was maintained in a flow-through (non-recirculating) aquarium tank, in unfiltered seawater to ensure an adequate food supply for the bryozoan, at ambient seawater temperature and constant illumination (to prevent the otherwise rapid decomposition of the algae). New colonies were collected on a monthly basis thereby providing a constant supply of healthy zooids. Before use in any experiment, individuals of *A. proxima* were starved for 24 hours.

4.2.2. Experimental protocol

4.2.2a. Experiment 1: observations of feeding by *Adalaria proxima*.

Small individual plants of *Fucus serratus* with a number of *Electra pilosa* ($n \approx 10$) colonies of varying size (200—2000 zooids) were suspended in separate transparent glass tanks containing aerated seawater. Each plant was mapped with respect to the position of the colony. The number of zooids of each colony was counted using a *camera lucida* attached to a Wild M8 stereomicroscope. A single nudibranch was placed on the holdfast of each plant and allowed to move freely for either 12 or 24 hours and its position was noted every 30 minutes during the experimental period. All nudibranchs used were weighed to the nearest milligram and measured to the nearest millimetre. Subsequent to completion of the experiment the nudibranch was removed and colonies which had been visited were examined and the number of zooids consumed, if any, were ascertained. Data obtained were plotted as

feeding rate (polypides consumed per hour) against individual body mass (mg). Observations of nudibranch movement were plotted as histograms representing time on and off the colonies, though it should not be inferred that feeding was continuous during the time spent on the colonies. Previous preliminary feeding observations of *Adalaria proxima* feeding on *E. pilosa* colonies that were pinned out in glass tanks for comparison with the more "natural" unpinned experiments, are also presented.

4.2.2b. Experiment 2: density-dependent effects.

Using the apparatus described in fig. 4.1, intact colonies of *Electra pilosa* of known zooid number, determined as previously described, were offered to individual *Adalaria proxima* in three different treatments i) 10 "small" colonies each of ≈ 250 zooids ii) 1 "small" colony and iii) 1 "large" colony of ≈ 2500 zooids.

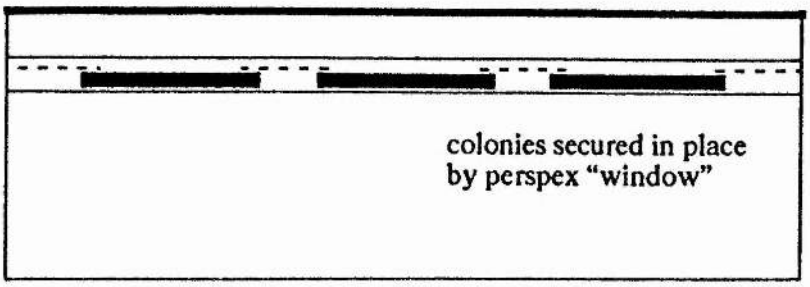


Figure 4.1. Apparatus for the observation of *Adalaria proxima* feeding on 10 "small" colonies of *Electra pilosa.* The colonies were held in place by the presence of a perspex window. A lid above the window prevented nudibranchs becoming dislodged from the substrata. The entire apparatus, constructed from transparent perspex, which facilitated the monitoring of the nudibranch during the experimental period. The apparatus was filled with aerated seawater at ambient temperature.

Thus, an assessment of the behaviour of *A. proxima* on being presented with colonies of different quality could be made. The nudibranchs were allowed to graze for 6 hours. Each slug was placed on the edge of the intact colony (see fig. 4.1). Treatments were replicated five times. Upon completion of the experiment the nudibranchs were removed and colonies were examined for any consumption of zooids. From these data histograms of nudibranch movement were plotted against time and an analysis of variance (Sokal & Rohlf, 1981) was applied to the data to examine the relationship between the treatment and the number of zooids consumed.

4.2.2c. Experiment 3: regeneration after predation.

Colonies from experiment 2 were used to ascertain the effect of actual predation upon "small" and "large" colonies and exposure to the nudibranch predator. Consequently control colonies, one "small" and one "large" per replicate were also used. After completion of each replicated predation experiment, the colonies were assessed for damage, zooid consumption and enlargement of proximal spines. The colonies, together with the control colonies were then maintained in an aerated flow-through aquarium tank in unfiltered seawater at ambient seawater temperature and constant illumination. Growth of these colonies was recorded every three or four days for one month noting any instances of zooid formation and spine induction. Growth was expressed as the specific growth rate (SGR). This was calculated using the geometric mean rather than the arithmetic mean which is a more pertinent mean to the pattern of colony growth in *Electra pilosa* (see chapter 3). One-way analysis of variance (ANOVA) (Sokal & Rohlf, 1981) was applied to the data to assess the effects of the respective treatments: i) predation ii) exposure to the nudibranch predator and iii) control (no predation or exposure) on the subsequent SGR of *E. pilosa* colonies. This statistical test assumes that the differences observed in the SGR, if any, are due to the treatments applied.

4.2.2d. Experiment 4: mechanical simulation of predation.

Intact colonies of known zooid number were manually damaged, in a manner simulating the action of known predators of *Electra pilosa*. Matched pairs of colonies of the same zooid number and similar shape were damaged in two ways: 1) the frontal membrane was punctured (mimicking *Adalaria proxima* and *Onchidoris muricata*) using a fine tungsten needle that had been electro-chemically etched in potassium hydroxide. Care was taken not to pierce the underlying algal substratum, 2) the whole zooid was removed (mimicking *Polycera quadrilineata*) again taking care not to damage either the alga or neighbouring zooids. Each of the pair was subjected to purely peripheral or central damage. The same number of zooids were damaged in each case to ensure a consistency in the level of attack between the two pairs. The growth subsequent to this mechanical damage was followed fortnightly over a 2 month period in these pairs and control colonies of the same zooid number and shape. Pairs, together with controls, were placed either in the field between July and September, 1991 in labelled small nylon mesh cages (Toby "teaboys", Aldridge Plastics Ltd.) attached via twine to nails embedded in the sandstone substratum at St. Andrews (east coast, Scotland), or maintained in a flow-through aquarium tank, between May and June, 1991. The teaboy mesh size is 0.025mm which is much greater than the food size range of the bryozoan ($\approx$ 8—20 μm) (Okamura, 1992), and thus would not be expected to compromise the feeding capabilities of the colonies. Paired t-test analyses (Sokal & Rohlf, 1981) which test the hypothesis that the true difference between the paired observations is zero, were applied to the data to assess whether growth rates differed between the damaged pairs and control colonies. *A priori* unpaired t-tests were applied to compare the specific growth rates of the matched pairs and comparable data of *E. pilosa* apparent specific growth rates (aSGR) on *Fucus serratus* in the field (section 3.2.3.) to assess any "teaboy" effect.

4.3. Results

4.3.1. Feeding rates

The feeding rates of 15 individuals on "unpinned" *Fucus serratus* were determined over a total experimental period of 300 hours (fig 4.2b). The mean feeding rate obtained was 20.8 polypides.h^{-1} with a standard deviation of 12.6 polypides.h^{-1}. This was in marked contrast to the results obtained for feeding rates on "pinned" *Electra pilosa* (fig. 4.2a). Rates were determined for 36 individuals over a period of 538 hours and a mean rate of only 12.58 polypides.h^{-1} with a standard deviation of 3.8 polypides.h^{-1} was recorded. An *a priori* paired t-test analysis showed that there was indeed a significant difference between the two data sets ($t = 2.61$, $p = 0.022$). All the nudibranchs monitored were observed to have an initial search phase, though of differing duration. During the 24 hour experiment all feeding individuals spent at least 6 hours on the bryozoan colonies (fig. 4.3) and despite spending different amounts of time on these colonies, all fed at the same rates. However, it should be noted that a large number of nudibranchs failed to feed at all in the time period of these experiments.

Electra pilosa colonies utilised in all the predation experiments were subsequently examined with respect to the shape index (S.I.) of the colony and the percentage of the colony consumed (fig. 4.5). The S.I. index is the perimeter-area ratio of the colony. Data obtained from the percentage of the colonies consumed were arc-sine transformed. This transformation stretches out both tails and compresses the middle of the distribution, and in that way yields normally distributed data which meets the assumptions of statistical analyses (Sokal and Rohlf, 1981). Simple regression analysis (Sokal and Rohlf, 1981) of the colonies with respect to these two criteria enabled an assessment of possible functional relationship between the morphology of an *E. pilosa* colony and its susceptibility to nudibranch predation. A colony with a runner-like configuration will have a higher S.I. value for a given number of zooids than one with a sheet morphology (fig. 4 6). As figure 4.5 demonstrates, there is no

Figures 4.2. Feeding rate observations (polypides.h^{-1}) of individuals of *Adalaria proxima* of different body size on a) "pinned" and b) "unpinned" *Electra pilosa.*

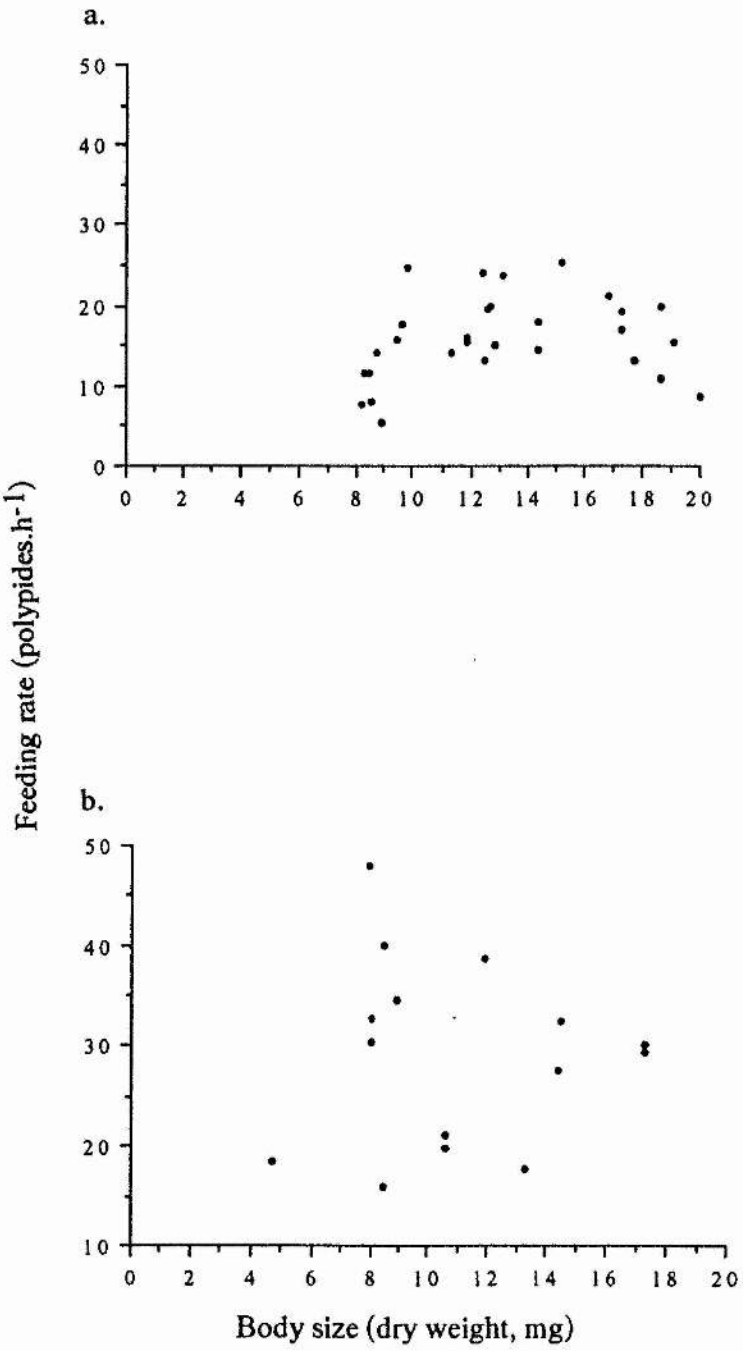
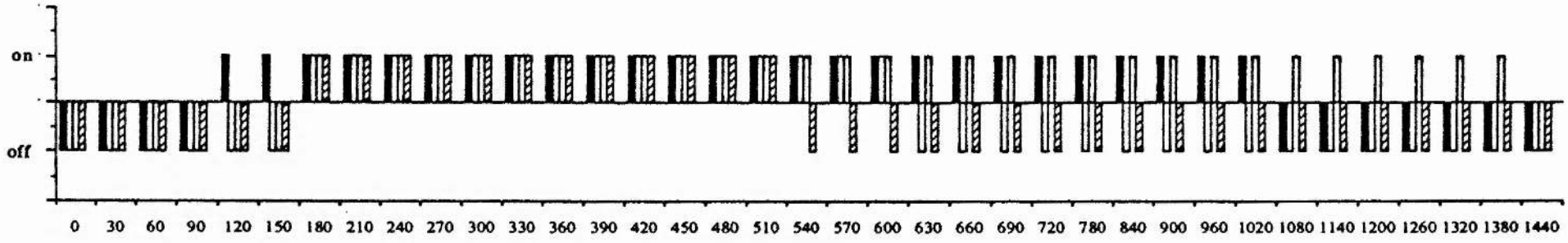
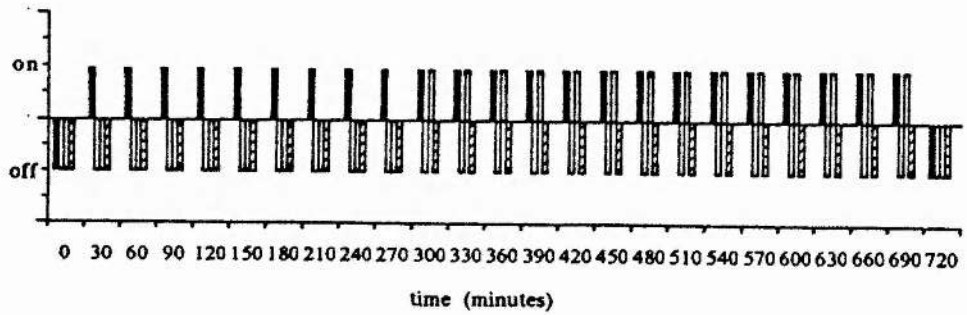

a.
b.
Feeding rate (polypides.h-1)
Body size (dry weight, mg)
0
10
20
30
40
50
0
2
4
6
8
10
12
14
16
18
20

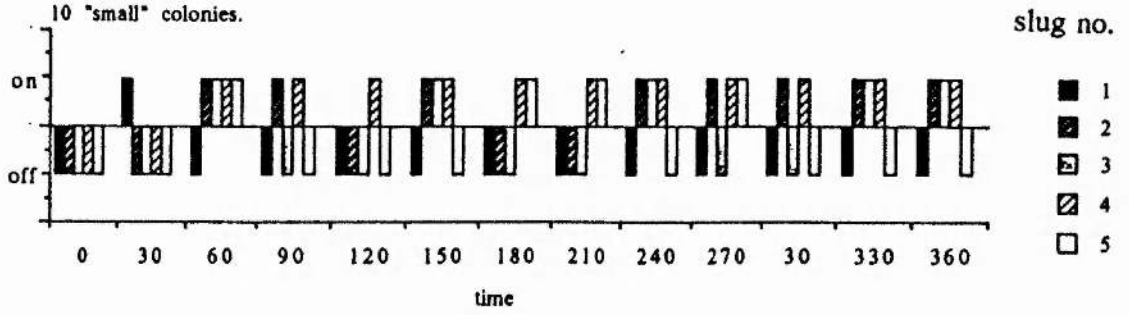
Figure 4.3. Histogram plots showing the discontinuous behaviour of *Adalaria proxima* on different patch sizes of *Electra pilosa* over two experimental periods of a) 24 hours and b) 12 hours.

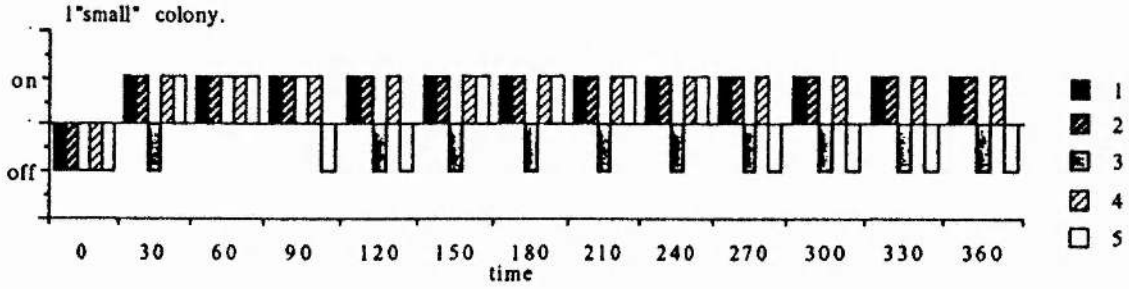




slug 1
slug 2
slug 3
slug 4

Figure 4.4. Histogram plots showing the discontinuous behaviour of *Adalaria proxima* on a different patch sizes of *Electra pilosa* over an experimental period of 6 hours.


10 "small" colonies.
slug no.
on
off
1
2
3
4
5
0 30 60 90 120 150 180 210 240 270 30 330 360
time


1 "small" colony.
on
off
1
2
3
4
5
0 30 60 90 120 150 180 210 240 270 300 330 360
time

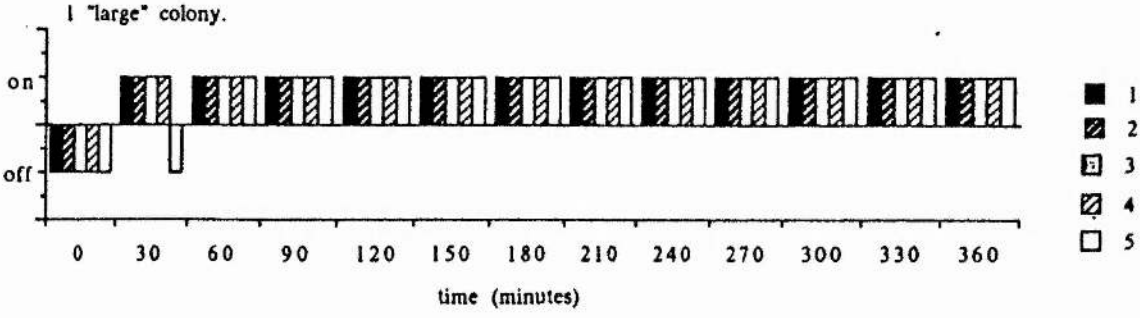
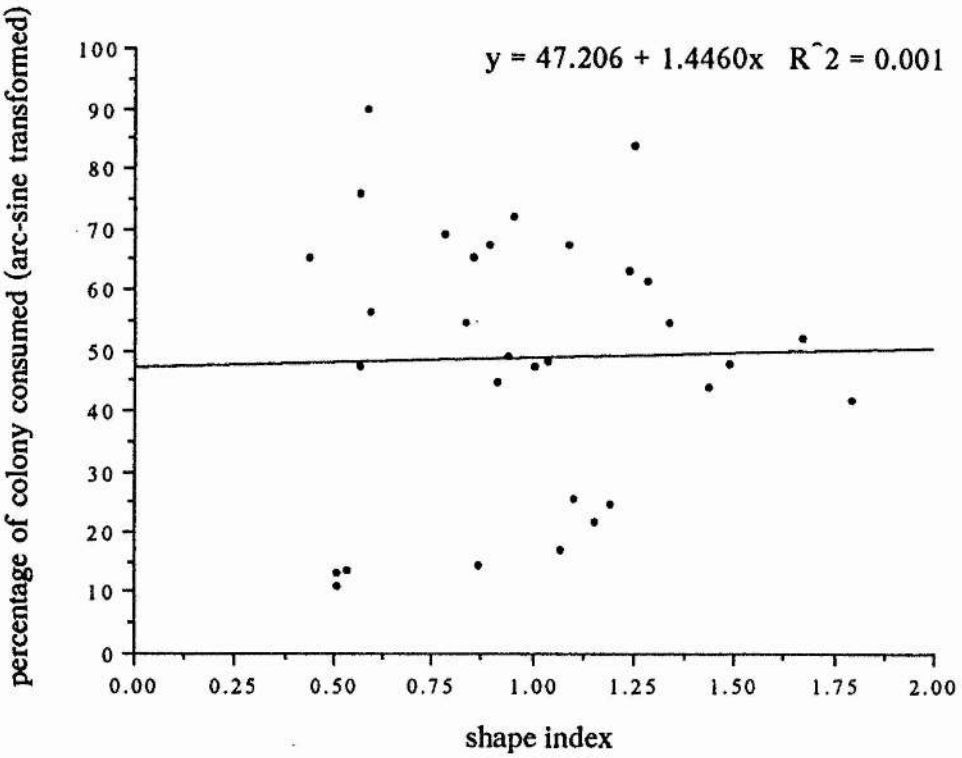

1 "large" colony.
on
off
1
2
3
4
5
0 30 60 90 120 150 180 210 240 270 300 330 360
time (minutes)

Figure 4.5. Scatter plot showing the relationship between the proportion of the *Electra pilosa* colony consumed by *Adalaria proxima* and the shape index of the colony. The values are presented for the arc-sine transformed data obtained from the percentage of the colony consumed against the S.I. of the colonies. Simple regression analysis of the data points demonstrates the lack of a relationship between susceptibility to predation and colony S.I.


y = 47.206 + 1.4460x R^2 = 0.001
percentage of colony consumed (arc-sine transformed)
shape index
100
90
80
70
60
50
40
30
20
10
0
0.00
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00

significant relationship between the S.I. and the proportion of the colony consumed by *Adalaria proxima* ($t = 0.1261$, $p = 0.901$).

4.3.2. Density dependent experiment

One-way ANOVA ($F = 1.798$, $p = 0.207$) showed that there was no significant difference in the number of zooids consumed between experiments and, it was not possible to do a multiple comparison test owing to the lack of variance in two of the replicates. Fig. 4.4, however, suggests that the nudibranchs on "large" colonies tend to remain on those colonies for the duration of the experimental period: individuals on the "small" colonies showed evidence of leaving and, in the case of the single "small" colony treatments, returning to the colony.

The effects of predation or exposure to the nudibranch predator had no significant effect on the subsequent SGR of the bryozoan colonies (table 4.1). Enlargement of the proximal spine was not observed in any of the replicates after experimentation.

4.3.3. Mechanical Damage

Paired t-test analyses (table 4.2) showed that over a period of three months there were no differences in the increase in zooid number between colonies damaged either at the periphery or in the centre and the controls. This was true irrespective of whether the bryozoan was maintained in the aquarium at an ambient temperature or on the shore at St. Andrews. It should be noted that a number of the colony pairs were subject to substratum decomposition. In the event of such a loss of substratum the results for the pair were removed from the data set.

Table 4.1. Analysis of variance of the effects of damage and exposure to the nudibranch predator *Adalaria proxima* on the subsequent SGR of *Electra pilosa* colonies of two size classes. It should be noted that p values were significant.

Table 4.1.

	"Small" colonies		"Large" colonies	
	F-statistic	p	F-statistic	p
Between times	0.5811	0.714	0.6171	0.689
Between treatments	0.0693	0.933	0.1120	0.895

One way ANOVA on the differences between "small" and "large" colonies

	F-statistic	p
Between times	0.529	0.753
Between treatments	0.078	0.995

Table 4.2. Paired t-test analyses of the effects of damage to the central or peripheral zooids of *Electra pilosa* colonies of the same zooidal number and dimensions. $T+_n$ denotes the time from initial damage, in months. It should be noted that no p values were significant.

Table 4.2.

Aquarium-maintained colonies

Treatment		t-statistic	p
T+1 edge	centre	0.2437	0.830
T+1 edge	control	0.1754	0.889
T+1 centre	control	-0.2707	0.832
T+2 edge	centre	2.3239	0.146
T+2 edge	control	0.2966	0.816
T+2 centre	control	-0.4879	0.711
T+3 edge	centre	3.5905	0.070
T+3 edge	control	0.3034	0.813
T+3 centre	control	-1.1257	0.462

Field-maintained colonies

Treatment		t-statistic	p
T+1 edge	centre	-0.6406	0.567
T+1 edge	control	-1.3528	0.218
T+1 centre	control	-2.0916	0.128
T+2 edge	centre	-1.2455	0.259
T+2 edge	control	0.3393	0.767
T+2 centre	control	-0.0229	0.984

A priori unpaired t-test analyses showed that the SGRs of aquarium-maintained colonies were not significantly different from comparable aSGRs of *Electra pilosa* colonies in the field ($t = 1.3$, $p > 0.05$). There was, however, a significant difference between the aSGRs of shore-maintained colonies and those of field populations ($t = -2.776$, $p < 0.05$). This latter result may well be a consequence of restraining the shore-maintained colonies in "teaboys".

4.4. Discussion

The feeding rates of *Adalaria proxima* were highly variable with some individuals failing to feed at all despite having been starved for 24 hours prior to the experiment. Perhaps the most striking feature was the significant difference between the feeding rates on *Electra pilosa* on "pinned" and "unpinned" *Fucus serratus*. The range of feeding rates obtained on "pinned" *F. serratus* in this study, 5.4 to 25.2 polypides.h^{-1} (fig. 4.2a) corresponded to the values of Todd (1981), 2.3 to 17.8 polypides.h^{-1}. However, no report of animal size is given by Todd. The maximum feeding rate on "pinned" *E. pilosa* here is 48.2 polypides.h^{-1} for an 8 mg damp weight individual. Todd and Havenhand (1989) found an asymptotic relationship between body size and mean feeding rate, predicting a 39 mg dry weight equivalent individual (Havenhand and Todd, 1988) to feed at an average rate of 18 polypides.h^{-1}. However, despite recording a maximum feeding rate of ≈ 50 polypides.h^{-1} the means obtained from their observations were much lower, due to periodic cessation of feeding associated with loading of the gut. The range of "unpinned" rates recorded in this investigation, 15.9 to 47 polypides.h^{-1} (fig. 4.2b) seem to correspond with the findings of Todd and Havenhand (1989), although not with their model of predicted ingestion. Thus, one must assume that feeding rates in the field are greater than previously believed, and certainly exceed the predictions of Todd and Havenhand (1989).

Adalaria proxima appears to have a strategy of indiscriminate feeding. Preference for any region of the colony was not exhibited. Rather, a methodical feeding pattern in the manner described by Todd (1981) was adopted. From the point of contact with the colony the nudibranch moves from side to side along the colony ensuring that all zooids are consumed efficiently and predated areas are not reworked (fig. 4.6). A pattern of optimal foraging is not adopted (*sensu* the Marginal Value Theorem, Charnov, 1976). Rather, a nudibranch will follow the lobe/arm of a colony

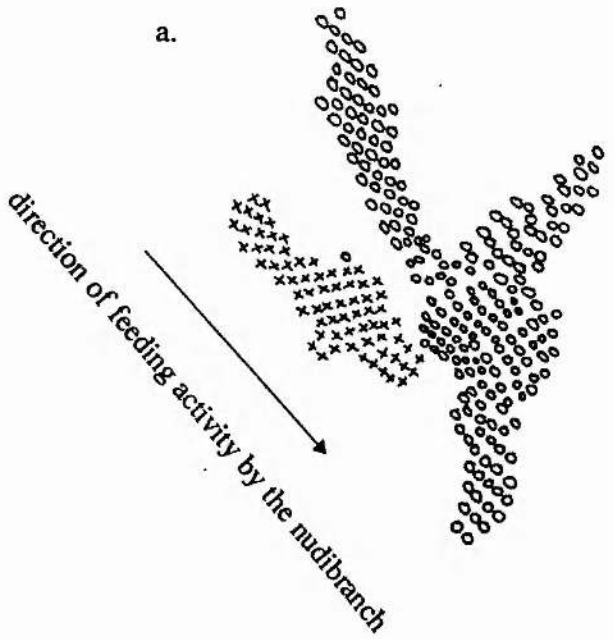
until it reaches the periphery, and then move off the colony irrespective of the number of zooids consumed. It is initially surprising then to observe the apparent lack of a relationship between the colony S.I. and the proportion of the colony consumed (fig. 4.5). Adoption of a uniserial growth form has previously been conceived as more susceptible to predation than a colony with a sheet morphology (Buss, 1979a; Jackson, 1979; McKinney and Jackson, 1991). However, it may be that *Electra pilosa* never truly achieves a completely multiserial morphology on *Fucus serratus*, such that the contrast between sheet and runner morphologies is negligible. The maintenance of a high SGR after predation/mechanical damage by all colonies attacked may be a more useful adaptation (table 4.1). Colonies in the field may be revisited and more than one nudibranch may attack a single colony (pers. obs). Sheet forms may not confer a definite advantage since it seems that the nudibranch feeds on a narrow front (fig. 4.6), perhaps because it pivots from side to side through a limited arc as it feeds. This may be the reason that a nudibranch will work its way up runners when these are available. Thus, an individual nudibranch may be just as likely to miss areas of a multiserial colony during feeding. The region of the colony attacked will depend on the initial angle of approach of the nudibranch and the point at which feeding commences (fig. 4.6). The plastic morphology displayed on macroalgae would appear to result from the ephemeral nature of the substrata rather than as response to colony damage. If predation is intense the colony may adopt a uniserial growth strategy in a search for refuges.

However, patch quality would seem important. Although two-way ANOVA showed no significant difference between the number of zooids eaten in each density treatment, which was shown to be a consequence of the high variability recorded, nudibranchs did stay longer on larger colonies (fig. 4.4). Once on, they tended to stay for the duration of the experimental period, whether for 6 hours or for 24 hours (fig. 4.3). When confronted with 10 "small" colonies, nudibranchs moved between colonies feeding occasionally, although at the same overall rate. Confronted with a

Figure 4.6. *Electra pilosa* colonies of contrasting Shape Indices, drawn with the aid of a *camera lucida*, to illustrate the feeding behaviour of *Adalaria proxima*. Colony a) possesses a uniserial morphology and characteristic high value for S.I., whilst colony b) is of a more sheet-like form, demonstrated by the lower S.I. value.

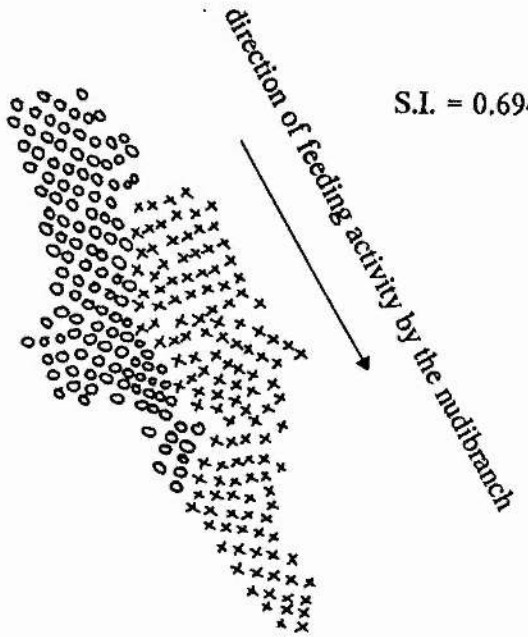
S.I. = 1.314

a.



S.I. = 0.694

b.



single "small" colony, animals did not remain on the colony for two of the five occasions, but moved off and on again a number of times. Feeding did, however, eventually take place by one of the two individuals. It seems, therefore, that partial predation of *Electra pilosa* by *Adalaria proxima* is a consequence of the characteristic stellate growth form displayed by the bryozoan, to maintain near exponential growth (Hughes & Hughes, 1986) rather than by any strategy of prudent predation (Slobodkin, 1961) or optimal foraging.

The indiscriminate feeding behaviour of *Adalaria proxima* is reflected in the observed subsequent SGRs of the damaged colonies. Such damage, whether through mechanical or predatory action, had no effect on the SGR when compared to control colonies (table 4.1). This is contrary to the findings of Bronstein (1939). A decrease in regeneration and the speed of wound repair was proportional to the increase in age of the zooid tissue. Certainly, a physiological gradient of colloidal proteins exists across the colony, with high levels of amino acids present in the growing margins (Ryland, 1979), which itself is a direct consequence of the higher physiological allocation to the budding zooids. One would, therefore, expect a gradation in SGR with astogeny. However, the observed maintenance of a high SGR in all regions of the colony might be interpreted as a constitutive defence. Certainly no incidence of visible induced defences was observed during this investigation. However, this might be anticipated for *Electra pilosa* which, unlike *Membranipora membranacea*, possesses constitutive defences in the form of the 4—12 chitinous spines, including the larger proximal spine, circling the frontal membrane and which are simple evaginations of the gymnocyst, containing extensions of the coelom (Stebbing, 1973a, 1973b; Ryland & Hayward, 1977; Ryland, 1979). This contrasts to the composite zooid structure of *M. membranacea* spines (Harvell,1991). *E. pilosa* clearly exhibits phenotypic plasticity in the number of spines present, so that it might be that a situation of a constitutive defence with a plastic allocation strategy occurs (Clarke & Harvell, 1992). Stebbing (1973a, 1973b) did not actually observe spine formation, and merely commented on the

presence of the elongated spines. More importantly he made the observation that such spines are not successful in the prevention of overgrowth by competitively superior bryozoans of the epifaunal assemblages on *Fucus serratus*.. Furthermore, the rasping feeding method adopted by *Adalaria proxima* contrasts with that observed in *Doridella steinbergae* which is a suctorial feeder (McBeth, 1968). *D. steinbergae* presses its mouth against the frontal membrane of *M. membranacea* and its oral veil is protruded to form a water-tight seal to the membrane. After an initial breach of the membrane by the radula the polypide is sucked out by pulsating dilations of the buccal pump. Consequently, spine formation by the bryozoan inhibits this mode of feeding (Harvell, 1984a). In contrast, *A. proxima* does not require such close contact with the frontal membrane, as the radula is pushed through the mouth and in a rasping manner removes the frontal membrane and the polypide (Todd, 1979, 1981). Elongation of the proximal spine would probably not interfere with this rasping behaviour of *A. proxima*. *E. pilosa* might then adopt a plastic optimal resource allocation strategy, preferring, in the event of nudibranch predation, to allocate more resources to the maintenance of a high SGR at the cost of defence or reproduction.

The morphological plasticity exhibited by *Electra pilosa*, whilst fundamentally associated with the maintenance of a high SGR (Rubin, 1987), enables the cheilostome to respond to inimical ambient conditions (Rubin, 1987) or to poor nutritional regimes (Jebram, 1980a, 1980b). The plastic morphology displayed by *E. pilosa* on *Fucus serratus* would appear to result from the ephemeral nature of the substrata, rather than as a response to colony damage. Proximal spine formation may well occur in situations of intense competition whereby an allocation of redirected growth or high SGR is not suitable. Stebbing (1973b), although having observed enlarged proximal spines, argued that the high SGR and the orientation of growth towards the younger regions of the fronds enabled *E. pilosa* to maintain its presence on the fronds of *F. serratus* despite being competitively inferior. However, the sublethal effects of resource allocation are not understood (Harvell & Suchanek, 1987). Whilst rapid regeneration

precludes settlement and subsequent overgrowth, the drain on energy budgets or future resources for defence, growth and reproduction may be high (Hoppe, 1988). However, *E. pilosa* is a short-lived, opportunistic species that inhabits largely ephemeral substrata whereby rapid regeneration and the maintenance of high SGRs is the main priority in resource allocation to ensure high fecundity. The susceptibility to predation is not of sufficient consequence to influence the adoption of a specific growth form. Rather, predation and colony damage merely serve to further complicate and distort the life history strategies of individual colonies.

Chapter 5

Larval metamorphosis of *Adalaria proxima*

5.1. Introduction

Larval metamorphosis, a critical process in the development of most benthic marine invertebrates, is the conversion of an organism whose body plan is specialised for a pelagic larval existence to a form specialised for a benthic adult existence (Bonar, 1978), an event which normally occurs in conjunction with settlement (Hirata & Hadfield, 1986). The terms settlement and metamorphosis have been variously interpreted in the literature. However, using Burke's (1983a) terminology, settlement is defined here as a repeatable behavioural phase, and metamorphosis is an irreversible developmental phenomenon, combining morphogenetic, histolytic and histogenic processes, only once in the life-history of an individual (but see Richmond, 1985).

5.1.1. Natural cues of metamorphic induction

Activation of this phenomenon, which is arrested in the larval stage, has, for all species where substratum-specific metamorphosis has been investigated, been shown to depend upon recognition of exogenous (environmental) cues associated with the recruiting substrata (reviews by Meadows & Campbell, 1972; Crisp, 1974, 1976, 1984; Scheltema, 1974; Chia & Rice, 1978; Burke, 1983a; Morse, 1985 and 1990; Hadfield, 1977, 1978, 1986; Bonar *et al.*, 1990; Pawlik, 1990a and 1990b; Pechenik, 1990). Indeed, many competent larvae can delay metamorphosis for a considerable length of time (Birkeland *et al.*, 1971; Kempf, 1981; Pechenik & Eyster, 1989; Coon *et al.*, 1990a; Miller & Hadfield, 1990; Pechenik & Cerulli, 1991; review by Pechenik, 1990) with little or no postmetamorphic cost (but see Highsmith & Emlet, 1976; Woollacott *et al.*, 1989) and in extreme cases can delay it indefinitely, eventually dying as larvae (Hadfield, 1977; Coon *et al.*, 1990a) or undergoing spontaneous metamorphosis (Coon *et al.*, 1990a). However, in the absence of an appropriate

stimulus, the competent larvae do not proceed through metamorphosis (Kempf 1981; Chia & Rice 1978; Hadfield 1978).

Perception of the exogenous stimulus can involve chemical, tactile and visual cues (Meadows & Campbell, 1972; Crisp 1974, 1976, 1984; Scheltema, 1986; Hadfield, 1977; Chia & Rice, 1978; Burke, 1983a; Pawlik, 1990a). Such a sensory basis of induction would imply that the larval nervous system is involved in the initiation of metamorphosis (Hadfield 1978; Baloun & Morse 1983; Burke 1983a, 1983b; Morse, 1985; Bonar *et al.*, 1990). A profusion of neurotransmitters and their derivatives have been demonstrated to effect a metamorphic response in the manner of natural inducers and are comprehensively reviewed and tabulated by Pawlik (1990b). This has culminated, certainly in the Mollusca, with a number of hypotheses as to the role of the nervous system in metamorphosis: *Crassostrea* sp. by catecholamines (Bonar *et al.*, 1990); *Haliotis* sp. by g-aminobutyric acid (GABA) (Morse, 1985, 1990); *Phestilla sibogae* by choline (Hadfield & Scheuer, 1985; Hirata & Hadfield, 1986).

5.1.1.1. Metamorphosis in *Crassostrea* sp; independent induction of settlement and metamorphosis

The ability to promote independent settlement and metamorphosis in oyster larvae,*Crassostrea* sp., led Bonar *et al.*, (1990) to propose a "conceptual model of behavioural and morphogenetic control pathways for oyster metamorphosis". L-dihydroxyphenylalanine (L-DOPA) was observed to induce settlement and metamorphosis in *C. gigas*, whilst adrenaline and noradrenaline promoted metamorphosis without settlement behaviour. Coon *et al.* (1985) perceived that either L-DOPA, or a mimetic molecule, was the natural exogenous cue for settlement and metamorphosis, a hypothesis seemingly verified by the work of Weiner *et al.* (1985). The oyster larvae exhibited preferential settlement and metamorphosis on substrata

fouled by the marine gram-negative bacterium, *Alteromonas colwelliana* (previously termed LST), which produced an oxidative product of L-DOPA, an exopolymeric melanin-like pigment, in active culture. However, inhibition of the inductive capacity of L-DOPA by selective DOPA antagonists and aromatic amino acid decarboxylases implies that exogenously applied L-DOPA is endogenously converted into dopamine, thus interacting with dopaminergic receptors to effect a response (Coon & Bonar, 1987). It is interesting to note, however, that exogenously applied DOPA is not a successful inducer of settlement or metamorphosis of oyster larvae (Coon & Bonar, 1986). Adrenaline and noradrenaline, unlike L-DOPA, induce metamorphosis without previous settlement behaviour. The presence of a_1-adrenergic receptors (Coon & Bonar, 1987) and noradrenaline present in homogenates of competent *C. gigas* larvae (Coon & Bonar, 1986) would suggest that noradrenaline and adrenaline act endogenously, mediating metamorphosis during or subsequent to the terminal phases of settlement, either by direct stimulation of a_1-adrenergic receptors or by causing the release of a morphogenic agent (Bonar *et al.*, 1990). Metamorphic induction by bacterial supernatants was also mediated by un-ionised ammonium (NH_3) (Coon *et al.*, 1988). Larval habituation to this soluble cue after only 15 minutes, but not to the supernatants themselves, suggested that NH_3 induces settlement behaviour but additional supernatant factors associated with the bacterial film maintain settlement and initiate subsequent metamorphosis (Coon *et al.*, 1988). Indeed, NH_3 has since been clearly demonstrated as the natural cue for settlement behaviour in oyster larvae (Fitt & Coon, 1992). Ammonia is released, as an excretory product, in high enough concentrations by adult congeners in oyster reefs and beds to trigger the settlement response in the larvae of both *C. gigas* and *C. virginicas*, although the release of ammonium as an excretory product of most marine organisms is not addressed. However, the cues for cementation and metamorphosis have still to be determined.

The separate induction of settlement and metamorphosis is also found in the dorid nudibranch, *Onchidoris bilamellata* (Todd, 1979; Chia & Koss, 1988;

Havenhand, 1991). Settlement of the veliger larvae is induced by a water-soluble product of the adult prey, the barnacle *Chthamalus dalli* (*B. balanoides* in the British Isles, Todd, 1979) whilst metamorphosis occurs only on contact with barnacle-associated substrata. The two events are seen to be distinct but may overlap temporally.

5.1.1.2. Metamorphosis in *Haliotis rufescens*; substratum-contact induction

Larvae of the marine gastropod mollusc *Haliotis rufescens*, the large Eastern Pacific red abalone, are induced to settle and undergo metamorphosis to the adult form by metabolites of specific "recruiting crustose red algae" including species of *Lithothamnion, Lithophyllum* and *Hildenbrandia* (Morse & Morse, 1984a); a characteristic which is shared with eleven of its congeners and the larvae of a number of other molluscan species (Morse *et al.*, 1984; Morse, 1992). The larvae must make direct contact with the algal surface, where inducing algal molecules are available to chemosensory receptors on the larvae, thus triggering the transitional developmental processes bridging the planktonic larval and benthic juvenile stages of the mollusc (Morse & Morse, 1984b).

Whilst other Rhodophyta and cyanobacteria contain similar inducers intracellularly, these molecules are not available to the larvae on the intact foliose red algae or cyanobacteria (Morse & Morse, 1984a, 1984b; Morse *et al.*, 1984). This unique availability of inductive molecules at the surface of the recruiting algae may be correlated with vegetative epithelial sloughing from the surfaces of the crustose red algae. The process by which the pigmented cells undergo regular degeneration and transport to the surface, would make possible their replacement from beneath (Giraud & Cabloch, 1976) and thereby expose the inducers of settlement in a non-diffusible form (Morse & Morse, 1984a). The larvae do not exhibit chemotaxis and recognise the

inducer only as a result of chance contact with the algal surface facilitated by exploratory behaviour (Morse *et al.*, 1980). The work of Morse and colleagues (see review by Morse, 1985) has shown that the molecules capable of inducing metamorphosis of *Haliotis rufescens* are found only in the Rhodophyta and cyanobacteria and are physically complexed with phycobiliproteins (the characteristic accessory photosynthetic pigments present only in red and blue-green algae). Analyses of the amino acid composition from the hydrolysates of an active extract from *Lithothamnion* sp. showed the presence of an amino acid structurally related to g-aminobutyric acid (GABA) or its analog d-aminolevulinic acid. Furthermore, GABA, an important inhibitory neurotransmitter in the mammalian brain, and several of its analogs including d-aminolevulinic acid, d-aminovaleric acid and e-aminocarproic acid, are themselves potent inducers of metamorphosis in *H. rufescens*. Furthermore, Troxler and Lester (1967) showed d-aminolevulinic acid to be a direct precursor in the biosynthesis of the tetrapyrrole chromophores of the algal phycobiliproteins. It is apparent, therefore, that the natural inducer of *H. rufescens* larvae may include moieties related to precursors of metabolites of the phycobiliproteins (Morse, 1985).

Induction of metamorphosis in *Haliotis rufescens* is stereochemically specific, the larvae possessing chemosensory receptors responsible for the recognition of GABA-mimetic signals that initiate the metamorphic response. Both GABA and the natural inducer purified from *Lithothamnion* sp. compete for receptors, thereby demonstrating the common mode of receptor activation of the natural and artificial agents (Morse, 1992). Like the GABA-receptors within the nervous system of vertebrates and invertebrates, the larval receptors, located externally on the larval epithelium (Morse, 1984; Baloun & Morse, 1984), possess the ability to be regulated (Trapido-Rosenthal & Morse, 1985, 1986a). Up-regulation (enhancement/facilitation) is brought about in a concentration-dependent manner by L-a,b-diaminoproprionic acid (L-DAPA) and other diamino acids (facilitating compounds). This is characterised by a decrease in the concentration of the inducing compound, though the simultaneous

presence of facilitating and inducing compounds is not required. Down-regulation (habituation) occurs in the event that precompetent larvae are exposed to GABA, and is slowly reversible. The neurological implications and modes of action are discussed in detail by Trapido-Rosenthal and Morse (1985, 1986a) and Morse (1985).

The ecological role of both up-regulation and down-regulation in *Haliotis rufescens* is not completely certain. Trapido-Rosenthal and Morse (1985, 1986a) propose that the up-regulation of induction by diamino acids present in dissolved organic matter could serve as a means of "priming" larvae for enhanced settlement in nutrient-rich regions, or a means by which the inducer-producing crustose red algae could regulate grazing intensity. Habituation may prolong the planktonic stage to ensure larval dispersal. This hypothesis culminated in the model of a complex regulatory pathway involving a GTP-binding protein (G protein)-diacylglycerol cascade (the "regulatory" or "amplifier" pathway) (Baxter and Morse, 1987, but see also Pawlik,1990b).

However, the relevance and interpretation of the role of GABA and coralline encrusting red alga by Morse and colleagues has been questioned by Pawlik (1990b). This is in the light of the undoubted preference of *Haliotis rufescens* and the Japanese abalone, *H. discus hannai* for diatom films and/or the mucus of juvenile and adult conspecifics for the processes of settlement and metamorphosis (Akashige *et al.*, 1981; Slattery, 1987; Leighton, 1988). The use of diatom films and not GABA in the commercial cultivation of abalone in the U.S.A. and Japan would appear to confirm the ambiguity of Morse and colleagues' results. Moreover, the apparent settlement response of *H. rufescens* to GABA is perceived to be a narcotic effect in which the larvae fall out of suspension. Pawlik (1990b) suggested that, contrary to the belief of Morse and co-workers (Morse, 1985; Trapido-Rosenthal & Morse, 1986b), GABA acts on an epithelial chemoreceptor to induce metamorphosis. The reality of the response is in consequence of the arrest of the larval velar cilia by GABA such that larvae are unable to swim and fall. Attachment, settlement and metamorphosis may

follow but "not necessarily as a result of any natural sequence of events." (Pawlik, 1990b).

5.1.1.3. Metamorphosis in *Phestilla sibogae*; induction by a water-soluble substance

The larvae of *Phestilla sibogae*, a Pacific tropical nudibranch, in common with many nudibranch species, are induced to metamorphose by the presence of its live adult prey, the scleractinian coral *Porites compressa* (Hadfield, 1977). However, unlike the larvae of *Haliotis rufescens*, and contrary to Crisp's suggestion that all invertebrate settling substances are encountered as adsorbed layers (Crisp, 1974), metamorphosis in the competent veligers of *P. sibogae* is initiated by a water-soluble substance. This is more specifically, a component of the coral mucus, released by the coral heads into the surrounding water (Hadfield, 1977). The larvae, competent shortly after hatching, will metamorphose in seawater previously exposed to *P. compressa* and, in a dose-dependent manner, to seawater containing lyophilised-distilled water extracts of the coral (Hadfield, 1977). 0.1 percent solutions of the crude inducer are maximally effective to initiate metamorphosis in the competent larvae, indicating that the inducer is active to a few parts per billion (Hadfield, 1978). The chemical structure of this compound is still unknown, though the molecular weight (about 500 Da) has been determined by ultrafiltration (Hadfield and Scheuer, 1985). Larvae withheld from exposure to *P. compressa* or its extracts will continue to live a planktonic existence for several weeks but eventually die without metamorphosing (Hadfield, 1977). Precompetent larvae, however, become refractory to the inductive capacity of the coral if exposed to it before attaining competence. If they are removed from the inducer for a period of time, as little as 1 to 2 hours, they will then readily respond to treatments in a manner suggesting facilitation after habituation. From these observations it is proposed that upwardly diffusing substances could influence the behaviour of the planktonic

veliger larvae so as to bring about site-specific settlement (Hadfield, 1980; Hadfield & Scheuer, 1985).

Bonar (1976) discovered the potency of succinylcholine chloride to induce metamorphosis in the larvae of *Phestilla sibogae*, even in the absence of the natural inducer. Other choline-containing compounds cause a metamorphic response in the competent larvae, to varying degrees, which prompted Hadfield (1978) to suggest that it is the choline moiety which is active; choline chloride alone is as equally effective as succinylcholine chloride in inducing metamorphosis in the nudibranch larvae. In contrast to the observed response of the competent veligers to the coral inducer, the metamorphic response to choline and associated compounds typically shows a latency of 48 to 72 hours. Moreover, the maximal effective concentration of choline (10^{-2} to 10^{-3}M) is several orders of magnitude greater than that of the natural inducer (Hadfield, 1978). Precompetent *P. sibogae* larvae do not habituate to choline as they do to the natural, coral-produced metamorphic inducer, whilst precompetent larvae exposed to choline are not refractory to the effects of the coral inducer if exposed to it subsequent to becoming competent and *vice versa* (Hirata & Hadfield, 1986).

Choline therefore acts at a different site from the natural inducer in *Phestilla sibogae*. The absence of any consistent interaction between choline and the coral inducer implies that choline normally has no external role in metamorphic induction. Rather, it acts endogenously in its more normal capacity as a precursor for neurologically active substances within the larval nervous system (Hirata & Hadfield, 1986). Hirata and Hadfield (1986) proposed a simple model for the events occurring in the metamorphic induction of *P. sibogae*. The coral product, a small water-soluble molecule interacts stereochemically (see Morse *et al.*, 1980) with a specific cell receptor on the larval epithelium. The receptor cell is neurally connected to the CNS from which the component processes of metamorphosis are initiated, probably individually. More recently, however, metamorphosis in *P. sibogae* has been demonstrated to occur only after active uptake of choline by the larvae resulting in millimolar internal concentrations

of choline (Hadfield & Pennington, 1990). This process, taking some 48 hours, is consistent with the latency observed (Hirata & Hadfield, 1986). Choline is perceived to serve in the neurological events of typical metamorphic activation by 1) acting on internal receptor sites usually specific for acetylcholine (Ach), a common neurotransmitter 2) serving as a precursor in the biosynthesis of Ach or 3) stimulating the synthesis and release of catecholamine neurotransmitters. These possibilities are more thoroughly discussed in the texts (Hirata & Hadfield, 1986; Pennington & Hadfield, 1989).

The role of catecholamines as inductive agents of the metamorphic event has recently been questioned. Catecholamines rapidly autoxidize in seawater to quinones, a multistep reaction, the by-product of which is hydrogen peroxide (H_2O_2). H_2O_2, in common with solutions of aged catecholamines, induces velar loss (partial metamorphosis) in the veliger larvae of *Phestilla sibogae*, without a subsequent loss of metamorphic competence (Pires & Hadfield, 1991). It is argued by Pires and Hadfield (1991) that the inductive capacity of catecholamines is, therefore, the result of either H_2O_2 or the quinone oxidation products rather than the catecholamines themselves. Certainly, molecules containing quinone (or quinone-like units) or that are precursors of quinones (catecholamines or phenolic proteins), have also been implicated in the settlement and metamorphosis of a number of marine invertebrate larvae; melanin forming LST, an oxidation product of L-DOPA, induces metamorphosis in *Crassostrea gigas* (Weiner *et al.*, 1985); quinone-tanned proteins of the periostracum promote preferential settlement on adult shells by oyster larvae (Crisp, 1967); cross-linked quinoid derivatives of DOPA residues in proteins are implicated in the metamorphosis of the polychaete *Phragmatopoma californica* (Jensen & Morse, 1990); and Jacarone, a simple quinone isolated from aqueous extracts of the marine red alga *Delessaria sanguinea*, induces metamorphosis in the scallop *Pecten maximus* (Chevolot *et al.*, 1991).

5.1.2. The role of the potassium ion in metamorphic induction

Whilst many of the external chemical stimuli capable of inducing larvae to metamorphose are restricted in activity by species-specificity, potassium appears to be a notable exception. Elevated external concentrations of potassium chloride (KCl) have been found to induce metamorphosis in several marine invertebrate phyla (but see Rittschoff *et al.*, 1986 and Eyster & Pechenik, 1987); the molluscs *Haliotis rufescens*, *Phestilla sibogae*, *Astrea undosa*, *Crepidula fornicata*, *Adalaria proxima* (Baloun & Morse, 1984; Yool *et al.*, 1986; Pechenik & Heyman, 1987; Todd *et al.*, 1990) the hydrozoans *Hydractinia echinata* and *Agaricia* sp. (Müller, 1973; Müller I Buchal, 1973; Morse, 1988), the echinoderm *Arbacia punculata* (Cameron & Hinegarder, 1974), the polychaete *Phragmatopoma californica* (Yool *et al.*, 1986) and the bryozoan *Membranipora membranacea* (Stricker, 1988), all with distinctly dissimilar metamorphic inducers. In all responsive species it is the potassium ion that is the active agent rather than the increased osmolarity or increased concentrations of associated anions (Pechenik & Heyman, 1987).

Müller and colleagues (Spindler & Müller, 1972; Müller & Buchal, 1973), demonstrated the inductive effect of external cations on larval metamorphosis in the planula larvae of *Hydractinia echinata* (Cnidaria) using elevated concentrations of K^+, Li^+, Rb^+ and Cs^+ in the absence of the natural bacterial inducer. It may be then that elevation of the external K^+ concentration results in the excitatory depolarisation of the larval chemosensory membrane (Baloun & Morse, 1984; Morse, 1985). Indeed it is well known that membrane potential in excitable cells is influenced by changes in the potassium ion (K^+) electrochemical gradient (Hodgkin & Horowitz, 1952; Hagiwara *et al.*, 1961). Certainly, depolarising electrical stimulation elicits complete and normal metamorphosis in competent larvae of the Pacific sand dollar *Dendraster excentricus* (Burke, 1983a, 1983b) and those of the sea urchin *Arbacia punculata* (Cameron & Hinegarder, 1974), which would suggest that membrane depolarisation is indeed involved. The broad effectiveness of K^+ as a metamorphic inducer implies that the

depolarisation of external, accessible, excitable cells may be a generally operative mechanism in marine invertebrate larvae, although not ubiquitous. The metamorphosis of the acorn barnacle, *Balanus amphitrite*, is inhibited by elevated K^+ (Rittschoff *et al.*, 1986) whilst competent larvae of *Mytilus edulis* are insensitive to the inductive properties of the potassium ion (Eyster & Pechenik, 1987).

However, the indifferent response to the potassium channel blocker TEA by two of the species investigated by Yool *et al.* (1986) suggests that the action of K^+ as an inducer may be mediated through one of a number of physically distinct potassium channels, although the effectiveness of the potassium ion does not imply that K^+ currents are necessarily involved in transducing signals for metamorphosis. Depolarisation of larval receptor cells may be accomplished by a cation influx or anion efflux involving any of the physiologically relevant ions. Whilst transduction of chemical or other stimuli by receptor cell depolarisation is a common occurrence in the initiation of metamorphosis, the mechanisms driving and responding to this event are likely to vary amongst species (Baloun & Morse, 1984; Yool *et al.*, 1986).

However, recent publications by Pawlik (1990a, 1990b) refute the roles of neurotransmitters in larval settlement and metamorphosis and question the techniques of bioassay of these bioactive compounds and the subsequent validity of models of metamorphic induction pathways. Indeed, it seems that the increasing number and array of compounds, inorganic or organic, that induce settlement behaviour or a metamorphic response strongly commend such wariness (Pennington & Hadfield, 1989). The active uptake of amino acids by larvae (Jaeckle & Manahan, 1989) cautions against presumptive interpretations of the role of GABA and DOPA, both water soluble amino acids, as exogenous stimulators of larval metamorphosis. Catechol oxidation (Pires & Hadfield, 1991) provokes ambiguity in situations of catecholamine induced metamorphosis. Moreover, "bath-applied" (Pires & Hadfield, 1991) bioassays are not specific in their application, concentration or location of action, and the response, positive or otherwise cannot be completely conclusive. Certainly the role of

neurotransmitters in the settlement and metamorphosis of marine invertebrate larvae will remain ambiguous unless it can be shown at a neurological level, by means of isolation of larval chemosensory organs, cells or receptors by electrophysiological or chemical approaches (see Chia & Koss, 1989; Arkett *et al.*. 1989; Chia *et al.*, 1992).

5.1.3. Aims of this chapter

Whilst high settling specificity by larvae for natural substrata has long been recognised (Scheltema 1961), the molecular structure of the natural inducers, with few exceptions, remains largely unknown (Kato *et al.*, 1975). Within the Opisthobranchia elucidation of the molecular structure of the natural inducer, whether from the prey of stenophagous species or more generalistic cues, is far from complete (review in Havenhand, 1991). Since the observations of Thompson (1958) on the ability of the cheilostome bryozoan *Electra pilosa* to induce metamorphosis in the small dorid nudibranch *Adalaria proxima* , little has been done to investigate the manner and nature of the inductive properties of the bryozoan (but see Todd *et al.*, 1991).

After an obligate planktonic phase of one to two days, the lecithotrophic larvae attain competence and are stimulated to metamorphose only on contact with live colonies of *Electra pilosa*, the preferred adult prey: neither dead colonies of the bryozoan, nor other intertidal bryozoan species are reported as suitable, and metamorphosis is delayed in the absence of the natural inducer (Thompson, 1958; Kempf & Todd, 1989; Todd *et al.*, 1991). In the light of the comprehensive study of artificial induction by choline and elevated potassium of *A. proxima* (Todd *et al.*, 1991) and the extraordinary length of time since Thompson's original work (1958), it was decided to further investigate the nature of the inductive properties of the bryozoan, and to propose a possible role for choline by the use of betaine as a possible metamorphic inducer. Choline is perceived to serve in the neurological events of metamorphic activation in *Adalaria proxima* in a similar role to that proposed by Hirata

and Hadfield (1986). Betaine, an oxidation product of choline, is often a participant in the activated methyl cycle, in which it is transmethylated during the synthesis of substances such as creatine, adrenaline and the methylated bases in RNA (Datta & Ottaway, 1976). Induction of the metamorphic process in *A. proxima* by betaine would support the role of choline in catecholamine synthesis.

5.2. Materials and Methods

5.2.1. Collection and maintenance of animals

Mature *Adalaria proxima* were collected by hand from fronds of *Fucus serratus* and holdfasts of *Laminaria digitata* at the two field sites, Clachan Seil, Argyll (west coast, Scotland), and East Sands, St. Andrews, Fife (east coast, Scotland). These adults were maintained in aerated seawater (T= 5 to 7 °C), in constant illumination, and fed *ad libitum* the cheilostome bryozoan *Electra pilosa* (epiphytic on *F. serratus*) (Kempf & Todd, 1989).

5.2.2. Collection and maintenance of spawn-masses

Spawn masses deposited on the fucoid fronds or the walls of the glass aquaria were transferred to small bowls and incubated in aerated, filtered seawater until hatching. The water was replaced on alternate days and Parafilm "M"® (American National Can, Greenwich, CT) placed over the bowls to prevent dust contamination. Upon hatching, larvae were collected and concentrated by pouring the hatching water through a 40μm nitex® filter. The larval concentrate was then used in larval assays of induction.

5.2.3. Manipulations of artificial seawater media, choline chloride and betaine concentrations

Solutions of choline chloride (10^{-1} M—10^{-4} M) and betaine (10^{-1} M—10^{-6} M) (Sigma Chemicals, Ltd.) were made fresh for every bioassay as a single dilution series.

Increased external potassium was substituted as chloride salts with an apposite reduction in sodium chloride, thereby maintaining the total ionic content and

osmolarity, to artificial seawater (ASW). Preparations of ASW, hereafter referred to with respect to the concentration of K^+ present, were based on the Woods Hole Marine Biological Laboratory (MBL) recipe (Cavanaugh 1956, in Baloun & Morse 1984)[1]. All salts were "Analar" grade (BDH chemicals) and all solutions were made up with millipore Milli-Q deionised water (Todd *et al*, 1991). The high survivorship of larvae precluded the necessity of antibiotics in the experimental solutions. Results are presented for four experiments which assess the validity of K^+, choline and betaine (a metabolic precursor of choline) for use as positive controls in the bioassays of natural induction:

Experiment 1; comparison of elevated [K^+] by substitution. Treatments were of (i) 9mM K^+ ASW, (ii) 19mM K^+ ASW, (iii) 29mMK^+ ASW and (iv) 39mMK^+ ASW.

Experiment 2; the effect of choline chloride concentration. Choline chloride concentrations used were (i) 10^{-1} M, (ii) 10^{-2} M, (iii) 10^{-3} M and (iv) 10^{-4} M.

Experiments 3 and 4; the effect of betaine concentration. Concentrations used were (i-iv) 10^{-1}—10^{-4} M (v) 10^{-5} M, (vi) 5 x 10^{-6} M, (vii) 2 x 10^{-6} M and (viii) 10^{-6} M.

---

[1] Salt concentration of 9.00mM K^+ ASW

Salt	Concentration (mM)	
NaCl	423.00	
KCl	9.00	
$CaCl_2$	9.27	
$MgCl_2$	22.94	added as $MgCl_2$ 2M solution
$MgSO_4$	25.50	
$NaHCO_3$	2.13	

The success of 19mM K^+ ASW and 10^{-2} M choline chloride solution as inductive agents of metamorphosis enabled their use as positive controls in the assessment of natural induction .

5.2.4. Natural induction

Competent veligers of *Adalaria proxima* will metamorphose following contact with the live adult prey bryozoan *Electra pilosa* (Thompson, 1958; Todd *et al.*, 1991). To attempt to further elucidate the nature of the natural inducer a number of criteria of assessment were employed in various combinations and with appropriate controls. All experiments were conducted in twice filtered seawater (TFSW) unless otherwise specified. Results are presented for seven experiments:

Experiment 1; natural induction by *Electra pilosa.*: the treatments were (i) $2cm^2$ intact colony of the bryozoan on *Fucus serratus*, veligers being put back onto the colony if dislodged, (ii) $2cm^2$ *F. serratus* (control), (iii) TFSW (control), (iv) 10^{-2} M choline chloride (positive control) and (v) 19mM K^+ ASW (positive control).

Experiment 2; *Electra pilosa* pellet/supernatant: $2cm^2$ section *E. pilosa* was removed, by the blunt side of a scalpel blade, from *Fucus serratus*, homogenised in 1ml ASW then pelleted at 2000rpm (Todd *et al*, 1990). The treatments used were (i) the supernatant, (ii) the pellet, (iii) live *E. pilosa* on *F. serratus*, (iv) *F. serratus* (control), (v) TFSW (control), (vi) 10^{-2} M choline chloride (positive control) and (vii) 19mM K^+ ASW (positive control).

Experiment 3; *Electra pilosa* "skeleton": (i) $2cm^2$ of colony whose polypides have been consumed by adult *Adalaria proxima*, leaving the zooid walls intact, (ii) $2cm^2$ intact colony of *E. pilosa* frozen in isopentane in liquid nitrogen (this method preserves the cell structure and thus enables assessment of the inductive qualities of the bryozoan when physiologically inactive, (iii) live *E. pilosa* on *F. serratus*, (iv) *F.*

serratus (control), (v) TFSW (control) and (vi) 10^{-2} M choline chloride (positive control).

Experiment 4; *Electra pilosa* conditioned seawater (ECSW), (i) large quantities of *E. pilosa* on *Fucus serratus* fronds were placed in a polythene bag containing 100mls TFSW and left for 24 hours. The water was then filtered through Whatman filter paper to remove detritus, (ii) *F. serratus* conditioned water (FCSW) was obtained in the same manner and provided a further control treatment, (iii) live *E. pilosa*, (iv) TFSW (control) and (v) 10^{-2} M choline chloride (positive control).

Experiment 5; reassessed the combinations of experiments 2 and 4. Treatments used were (i) ECSW + pellet, (ii) TFSW + pellet, (iii) live *Electra pilosa*, (iv) TFSW (control) and (v) 10^{-2} M choline chloride (positive control).

Experiment 6; trapped larvae whereby veliger larvae were kept suspended in the surface film of the solution for the duration of the experiment. The treatments used were (i) live *Electra pilosa* on *Fucus serratus*, (ii) TFSW (control), (iii) 10^{-2} M choline chloride (positive control) and (iv) 19mM K^+ ASW (positive control).

Experiment 7; the treatments were (i) $2cm^2$ intact colony of *Alcyonidium* sp. on *Fucus serratus* and (ii) *Flustrellidra hispida* on *F. serratus* (two coexisting epiphytes in the bryozoan assemblages of *F. serratus* which would be encountered by veligers of *Adalaria proxima* in the field) larvae being replaced on the colonies if dislodged (iii) live *Electra pilosa* on *F. serratus* (iv) TFSW (control), (v) 10^{-2}M choline chloride (positive control). To assess competence of the veligers, larvae were placed in 10^{-2} M choline chloride after 4 days.

5.2.5. Experimental protocol and statistical analysis

The bioassays were conducted in 30ml lidded Stender dishes. These were filled with 20ml of the test solution and 10 sibling larvae added to each dish. Each solution was replicated four times and each assay accompanied by at least three of four possible controls: 10 larvae placed in a) 20ml twice filtered seawater (TFSW) and live *Electra pilosa* on *Fucus serratus* to test the metamorphic competence of the larvae, b) 20ml TFSW to test for the frequency of spontaneous metamorphosis, c) 20ml 10^{-2} M choline chloride (positive artificial control) and d) 20ml 19mM K^+ ASW (positive artificial control).

Dishes were kept in constant illumination, in incubators kept at T ≥ 7 to 8 °C. All experiments were scored at the same time of day and on a daily basis by examination with a Wild M8 stereomicroscope. Larvae that had become trapped in the surface film were resuspended by pipetting water onto them from above, and dead larvae were removed. Larvae that had spontaneously evacuated their shells ("hopped-out") were scored as such. Although most continued to swim for a few days before dying, some did go on to metamorphose. Metamorphs were recorded once the individual had evacuated the shell and detorsion of the gut had occurred (Todd *et al,* 1991).

The observations were recorded as percentages, necessitating arc-sine transformation of the data (Sokal & Rohlf, 1981) before statistical analysis. The arc-sine, or angular, transformation stretches out both tails and compresses the middle of the distribution, and in that way yields normally distributed data. Thus, the percentage data in the figures are for back-transformed means and standard errors. For the $\log_{10}$ (N/N-x) transformation, N is the number of larvae at the start of the experiment, and x the cumulative number of metamorphs on each day. This is plotted against time as cumulative metamorphosis for the sum of the replicates. This transformation is more pertinent to the data producing a straight line plot for a constant *per capita* rate of

metamorphosis, rather than the curved numerical plot, a consequence of the diminishing number of unmetamorphosed larvae as the experiment progresses (Todd *et al.*, 1991).

5.3. Results

5.3.1. Elevated potassium ion dose-response

The inductive effect of elevated potassium is optimal in the 19mM treatments (fig. 5.1) Metamorphosis was not observed in either 9mM, 29mM or 39 mM K^+. Overall mortality was low in all the prepared ASW solutions, 0% in both 29mM and 39mM and 3.33% in both 9mM and 19mM K^+. The observed delay of 1 to 2 days before metamorphosis was recorded in the 19mM K^+ ASW is attributable to the attainment of competence in the veliger larvae.

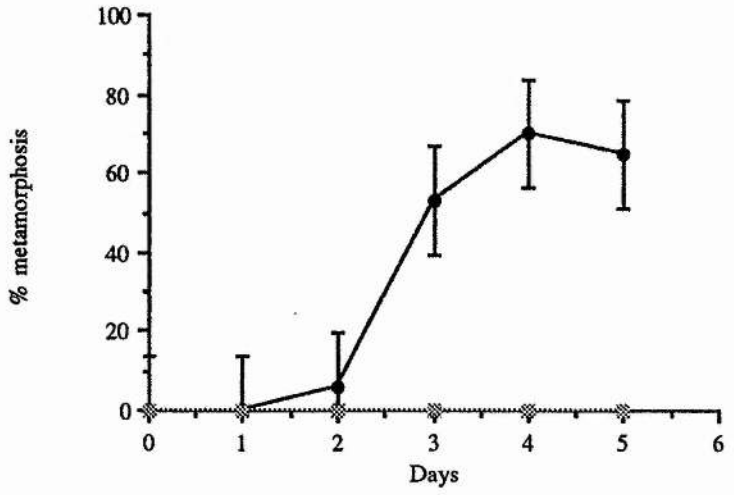
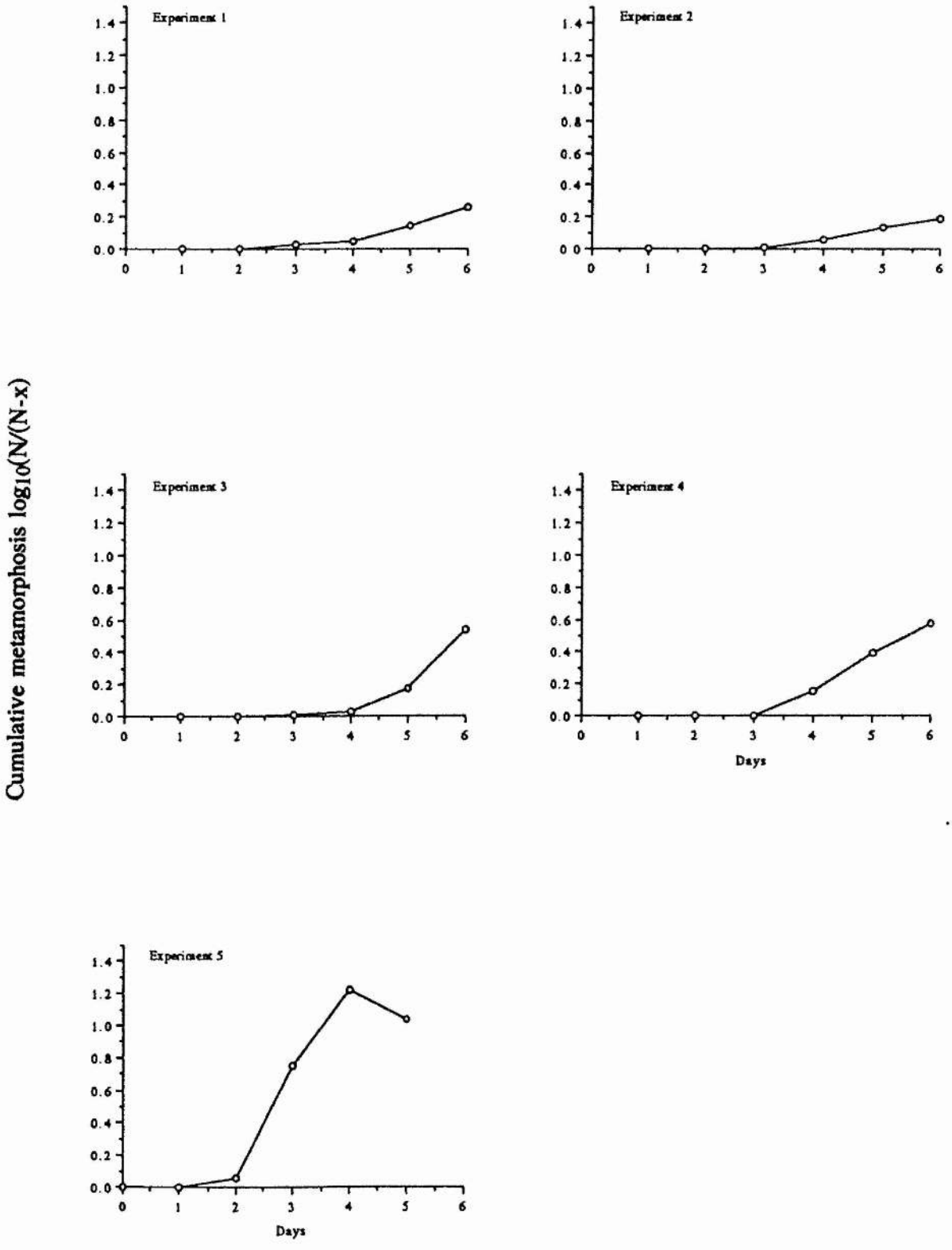


Figure 5.1. The response to elevated potassium ion concentrations (experiment 1). Data are for back-transformed means and standard errors of each quadruplicated treatment. Closed circles, 19mM K^+ ASW. Metamorphosis was not observed in either 9mM K^+ ASW, 29mM K^+ ASW or 39mM K^+ ASW and as such the plots for these solutions are indistinguishable from one another and the abscissa.

Figure 5.2. Cumulative metamorphosis in response to 19 mM K^+ ASW for five experiments. Data, for the summed quadruplicates, are presented as the $\log_{10}(N/N\text{-}x)$ transformation against time.


Experiment 1
Experiment 2
Experiment 3
Experiment 4
Experiment 5
Cumulative metamorphosis log10(N/(N-x))
Days
Days

The response to 19mM K^+ in the five quadruplicated treatments (fig. 5.2) demonstrates a certain degree of variability, with a delay of 1 to 3 days. Bearing in mind the obligate phase of 1 to 2 days, this would indicate a delay of about 24 hours before a visible metamorphic response. The *per capita* rate of metamorphosis induced would seem constant for all experiments, though different between them. Experiment 3, however, shows a greater number of metamorphs in day 6 than would be expected.

5.3.2. Choline chloride dose-response

The inductive capacity of choline chloride is optimal at 10^{-2} M (fig. 5.3), with zero metamorphosis in all the other concentrations. The 10^{-1} M concentration proved highly toxic to the larvae, manifested in the noticeable swelling of larval tissue, producing an overall mortality of 66.66%. Concentrations ≤ 10-3 M were neither toxic nor inductive to the veligers with an observed 0% mortality. 10^{-2} M choline chloride proved to be a much more effective artificial agent of metamorphosis than did 19mM K^+. Indeed, in all but one (experiment 5) of the seven quadruplicated treatments (fig. 5.4) 100% metamorphosis was induced. A delay of 1 to 5 days was observed, the competence of the larvae again being a factor in that delay. Once initiated, however, the metamorphic response of the larvae was total; on three occasions 100% metamorphosis was obtained in one 24 hour period, with no observable mortality. This extreme response meant that 10^{-2} M choline chloride provided a suitable positive indicator of competence in the assays of natural induction.

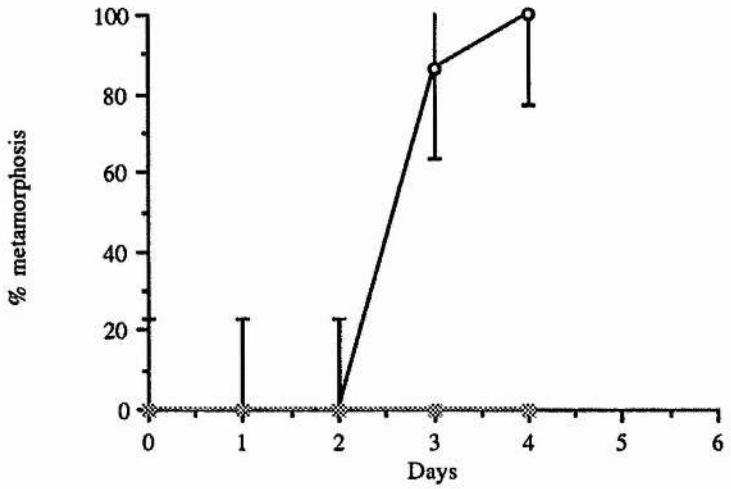


Figure 5.3. The response to various concentrations of choline chloride (experiment 2). Data are for back-transformed means and standard errors for the quadruplicated treatments. Open circles, 10^{-2} M choline chloride. Metamorphosis was not observed in either 10^{-1} M choline chloride, 10^{-3} M choline chloride or 10^{-4} M choline chloride. The plots of these are thus indistinguishable from one another and the abscissa.

5.3.3. Betaine dose-response

Betaine produced highly variable results (fig. 5.5) and cannot be considered a reliable or highly inductive source of metamorphosis. The 10^{-1} M solution was highly toxic, inducing 100% mortality after just one day. Toxicity decreased with a concurrent decrease in concentration, although toxic effects (swelling of larval tissue) were still observable in 10^{-4} M betaine. However, 10^{-6} M, 2 x 10^{-6} M, 5 x 10^{-6} M and 10^{-5} M concentrations did produce on several occasions, a few metamorphs, and in the case of the 10^{-5} M concentration this was some 35.5% of the treated larvae.

Figure 5.4. Cumulative metamorphosis in response to 10^{-2} M choline chloride for seven experiments. Data, for the summed quadruplicates, are presented as the $\log_{10}(N/N-x)$ transformation against time.

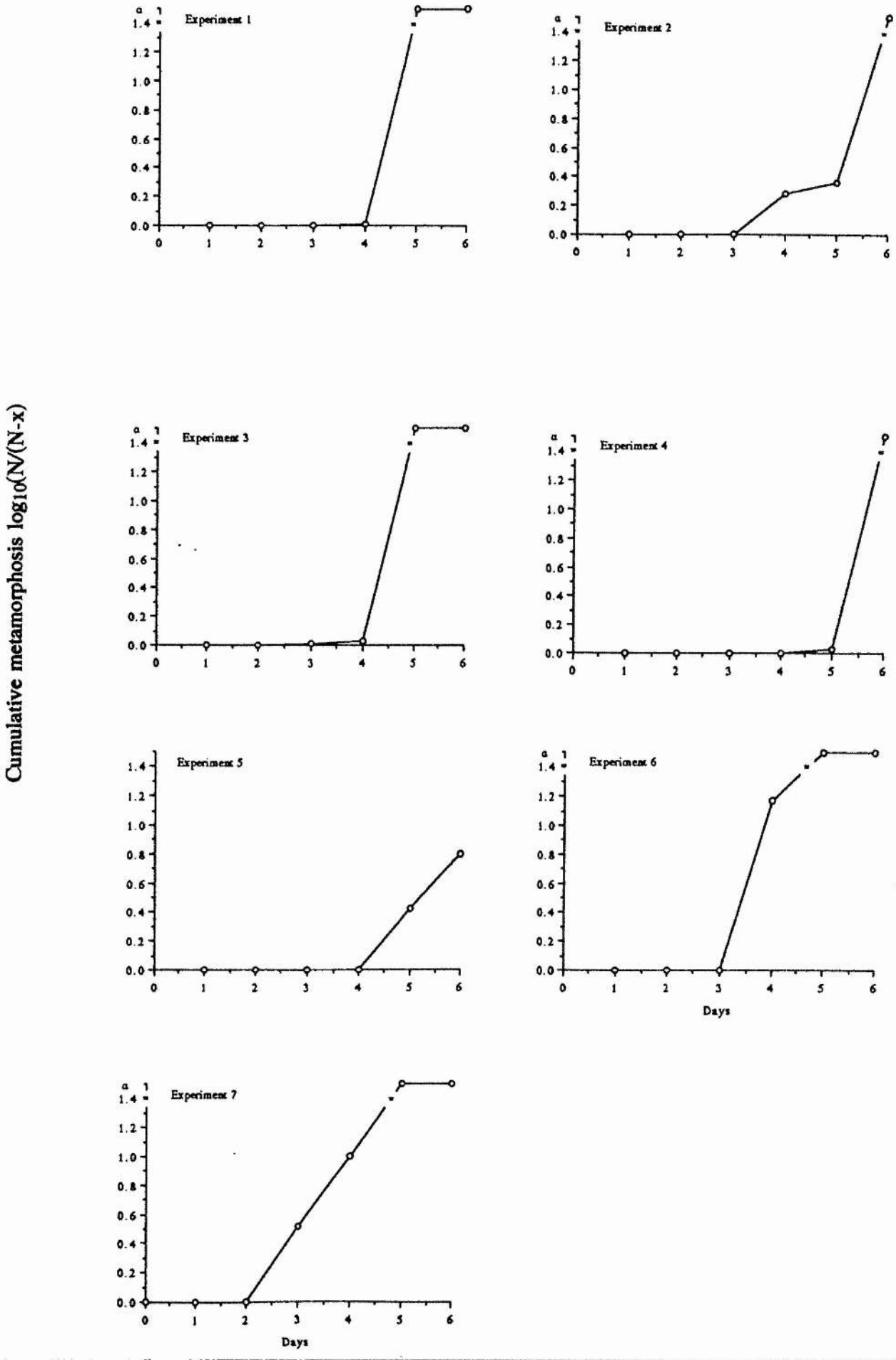
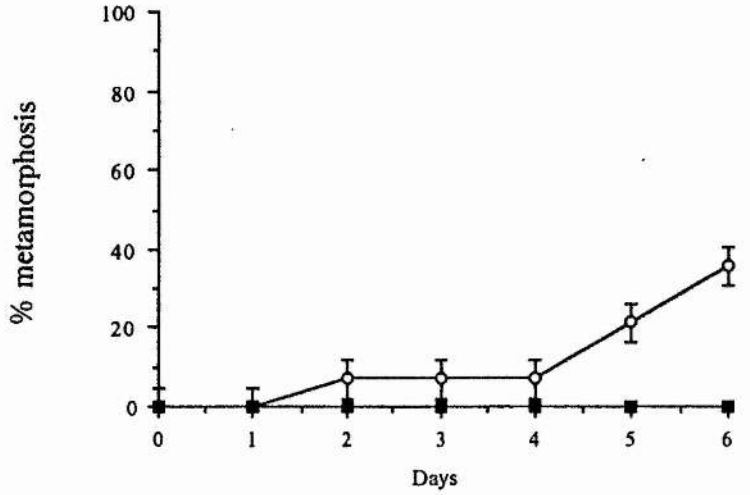
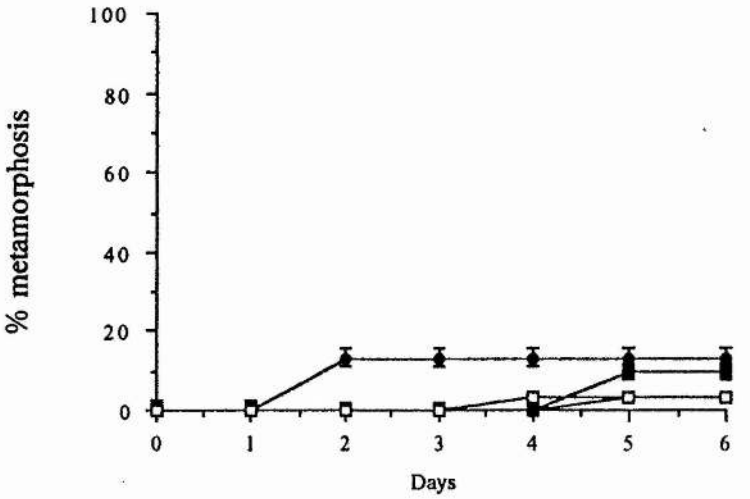

Experiment 1
Experiment 2
Experiment 3
Experiment 4
Experiment 5
Experiment 6
Experiment 7
Cumulative metamorphosis log10(N/(N-x))
Days
Days

Figure 5.5. The response to varying concentrations of betaine (experiments 3 and 4). Data are for back-transformed means and standard errors for each replicated treatment. Experiment 3: zero metamorphosis was observed in all the treatments, 10^{-1} M to 10^{-6} M betaine and, as such, the plots are indistinguishable from one another and the abscissa. Experiment 4: open circles, 10^{-5} M betaine; closed circles, 5×10^{-6} M betaine; closed squares, 2×10^{-6} M betaine; open squares, 10^{-6} M betaine.

Experiment 3



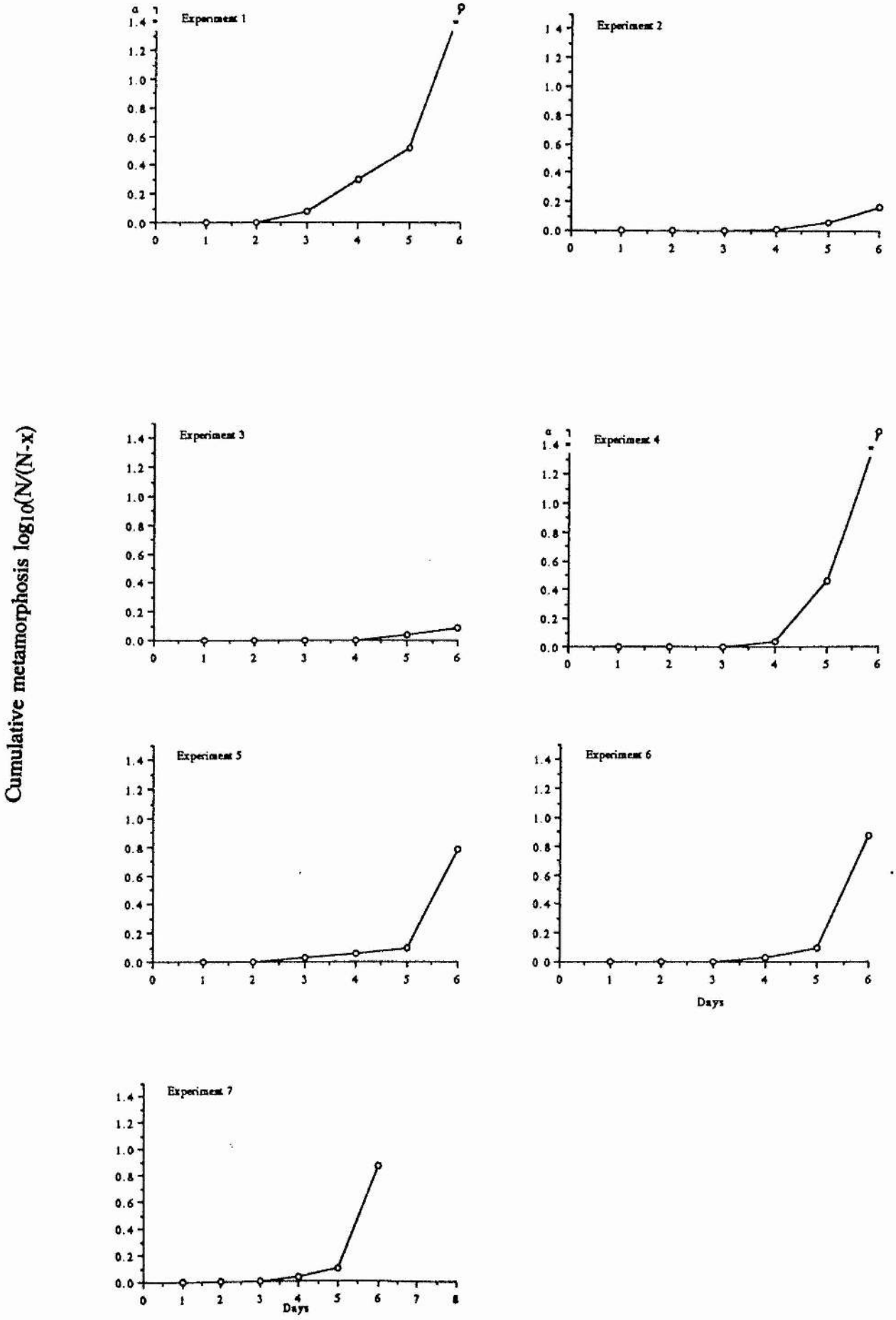
Experiment 4



5.3.4. Natural induction of metamorphosis

From all the assays of natural induction metamorphosis was induced only by contact with live colonies of *Electra pilosa*. The *per capita rate* of metamorphosis was variable (fig. 5.6), and mortality was zero, but what is apparent from the figure is that, whilst some larvae metamorphosed immediately after the initial delay phase, the majority of veligers required a further delay. During this time it may be that there was a build-up of the necessary cue, perhaps a bryozoan excretory product, or that active or passive uptake of the cue occured which was required to reach a threshold level before metamorphosis was initiated. Those larvae undergoing more immediate metamorphosis may be more susceptible to the metamorphic cue or may have had a shorter obligate phase. The lack of a metamorphic response to anything other than physical contact with *E. pilosa* clearly demonstrated that metamorphosis of *Adalaria proxima* was by some component of the live, intact bryozoan. This component is not water-soluble or extractable and the inductive property is eliminated by homogenisation and freezing. Metamorphosis occurred in all live *E. pilosa* treatments, in 10^{-2} M choline chloride and in 19mM K^+ ASW, with values ranging from 50-100%.

Figure 5.6. Cumulative metamorphosis in response to live *Electra pilosa* on *Fucus serratus* for seven separate experiments. Data are for the quadruplicates summed. Cumulative metamorphosis is plotted as the $\log_{10}(N/N-x)$ transformation against time.


Cumulative metamorphosis log10(N/(N-x))
Experiment 1
Experiment 2
Experiment 3
Experiment 4
Experiment 5
Experiment 6
Experiment 7
Days
Days

5.4. Discussion

Morse and Morse (1984b) suggested that the natural GABA-mimetic inducer of metamorphosis in *Haliotis rufescens* may be made available to the larvae by vegetative epithelial sloughing of crustose red algae, the adult prey. Mucus production in the coral *Porites compressa*, seen to contain the water-soluble diffusible active inducer of *Phestilla sibogae* (Hadfield, 1977), also serves as a sloughing mechanism, preventing fouling and silt accumulation of the coral surface. However, a similar sloughing/cleansing process has not been described for the Bryozoa. Rather, bryozoan distribution is directly influenced by habitat characteristics and the local nature of the physical environment: *Electra pilosa* shows a marked avoidance of silty conditions (Kitching & Ebling, 1967; O'Connor *et al.*, 1979). These observations would imply that bryozoans are not capable of sloughing to remove accumulated debris and as such are unlikely to release an inducer in the manner of either *P. compressa* or coralline red algae.

The fact that physical contact with live *Electra pilosa* appears to be largely responsible for the metamorphic process in the competent veliger larvae of *Adalaria proxima* suggests that the inductive substance may be something inherently associated with the exposed frontal surface of *E. pilosa*. In common with other anascan cheilostome bryozoans, the frontal surface of *E. pilosa* is dominated by the frontal membrane, consisting of the periostracum (Tavener-Smith & Williams, 1972) (or pellicle: Ryland, 1970, 1976) and an underlying epithelium. The periostracum is comprised of an external cover fully developed as a triple-unit membrane, the external membrane of which bears a dense filamentous coat. Beneath this membrane the main layer of the periostracum is composed of mucopolysaccharides, proteins and chitinous filaments (bryozoan chitin is a stereoisomer of true chitin, specifically the b-polymer of N-acetylglucosamine [Ryland, 1970]). Such frontal membranes are characteristic of the cheilostomes, though the nature of the periostracum varies somewhat in thickness,

texture and structure between species. In *E. pilosa* the frontal membrane is reduced to an oval shape, and is protected by a calcified exterior frontal wall (gymnocyst), a continuation of the calcified exterior walls (Sandberg, 1977). This frontal wall is composed mainly of vertical calcite crystals secreted onto the periostracal matrix which impart a granular texture to the zooecial surfaces of *E. pilosa* (Tavener-Smith & Williams, 1972). Thus, whilst most anascan cheilostome bryozoans possess essentially similar frontal surfaces, subtle differences in surface texture, structure and composition may be perceived by those organisms closely associated with them. One Ghanaian nudibranch found on *Anguinella palmata* feeds on the bacterial film and other detritus which accumulates on that bryozoan. It is totally specific, refusing to clean large colonies of *Nolella* sp. which appear indistinguishable from *A. palmata* with regard to surface flora and fauna (Cook, as cited by Ryland, 1976).

The importance of surface texture and bacterial films in the settlement and metamorphosis of benthic marine invertebrates has been demonstrated for a number of phyla; the Cnidaria, Polychaeta, Mollusca, Echinodermata and Bryozoa all have been extensively reviewed (Meadows & Campbell, 1972; Crisp. 1974, 1976, 1984; Scheltema, 1974; Mihm *et al.*, 1981; Bonar *et al.*, 1986; Maki *et al.*, 1989; Wahle, 1989; Bonar *et al.*, 1990; Pawlik, 1990a). Settlement of larvae of the oyster, *Crassostrea gigas* is mediated by the surface film produced by the gram-negative, melanin-forming bacterium, *Alteromonas colwelliana* (LST) (Weiner *et al.*, 1985). Whilst unionised ammonium (NH_3) present in the bacterial supernatant induced the settlement response (Coon *et al.*, 1990b), Bonar *et al.*, (1990) argued that additional supernatant factors may be responsible for the maintenance of settlement behaviour. Such supernatants, dominated by highly adsorptive exopolymeric polysaccharides covalently linked to proteins or peptides, may provide additional exogenous tactile or chemical cues that maintain settlement behaviour and ultimately induce metamorphosis (Bonar *et al.*, 1990). Maki and Mitchell (1985) proposed a biochemical model for settlement and metamorphosis of larvae of the spirobid polychaete *Janua* (*Dexiospira*)

brasiliensis, mediated by the bacterial film of *Pseudomonas marina*. Lectins (proteins or glycoproteins with two or more binding sites that recognise a specific sequence of sugar residues (Alberts *et al.*, 1983) on the surface of the polychaete larvae bind to a D-glucose containing molecule in the exopolymer of the bacterium. Yeaton (1981) noted that certain invertebrate lectins have a greater affinity for glycoprotein sugars than for monosaccharides, so it is interesting to note that the principal components of the frontal membrane and indeed the frontal wall of *Electra pilosa* are mucopolysaccharides; these are long unbranched polysaccharide chains composed of repeating disaccharide units, one of which is always an amino sugar (N-acetylglucosamine or N-acetylgalactosamine) and covalently linked to proteins. Furthermore, N-acetylglucosamine, the basic amino sugar unit of chitin, is known to be recognised by the commonly used, commercially available wheat germ lectin (Alberts *et al.*, 1983).

It is perhaps of interest then to refer to the recent work of Bahamondes and colleagues with the nudibranch *Eubranchus doriae*. Competent larvae of the nudibranch are induced to metamorphose not only by the specific adult prey, the hydrozoan *Kirchenpaueria pinnata*, but also by certain artificial agents, adrenaline, acetylcholine chloride, KCl, and the amino sugars asparagine and galactosamine (Bahamondes-Rojas & Tardy, 1988). Furthermore, the metamorphic response is stereochemically specific, mediated by solutions of purified hexoses or galactosamine, with the hydroxyl group bound to carbons 3 and 4 in the *Cis* position (Bahamondes-Rojas & Dherbomez, 1990). The isolated inductive agent of the hydrozoan is a molecule of some 1000 Da, with one or more a-D-galactose residues, which Bahamondes-Rojas and Dherbomez (1990) suggested may be recognised by lectins of the competent veligers.

It may be that lectins present on the surface of competent veliger larvae of *Adalaria proxima* bind to either a stereo-isomer of the N-acetylglucosamine molecule, or some constituent mucopolysaccharide (unique to *Electra pilosa*) on the frontal surface of the bryozoan, thereby initiating the metamorphic response.

However, recent experiments (Lambert & Todd, in prep.) have shown that *Adalaria proxima* is induced to metamorphose without actual physical contact with *Electra pilosa*. Competent larvae trapped in the surface layer above live colonies of *E. pilosa* and larvae exposed to *E. pilosa* conditioned seawater (produced by a different method to that of this investigation) undergo true and complete metamorphosis. It may be that *E. pilosa* releases an inductive substance as a by-product of its excretory process. Unfortunately, the nature of bryozoan excretory products has not been considered to any extent in the literature. There are no excretory organs in the Bryozoa; gas exchange occurs across the exposed body surface, and waste products, in the form of faecal pellets are expelled from the rectum by peristalsis (Ryland, 1970, 1976). Whilst phytoplankton and bacteria are the principal diet, there is some evidence that the Bryozoa are, to some extent, discriminating in feeding habit. The range of particle size being utilised as a food source probably varies with lophophore size and behavioural differences (Winston, 1978). Certainly, the nature and abundance of the food consumed by bryozoans, under laboratory conditions, affects the form of both zooids and colonies (Jebram, 1973) and the type of food ingested will ultimately influence the composition of the excretory products.

Alternatively the inductive agent may be a factor associated with the bacterial film of the bryozoan surface. Neumann (1979) described settlement of the planula larvae of the scyphozoan, *Cassiopaea andromeda* in response to filtrates from a culture of the gram-negative marine bacterium, *Vibrio* sp. Whilst Müller (1973) observed the settlement of larvae of *Hydractinia echinata* (Hydrozoa), mediated by "leakage-products" osmotically shocked from *Vibrio* sp., the bacterial film of *Alteromonas colwelliana* (LST) is implicated in the induction of settlement and metamorphosis of the oyster *Crassostrea gigas* (Bonar *et al.*, 1990). Some of these contrary observations may be reconciled with the results presented in this study. Veliger larvae of *Adalaria proxima* clearly show a latency of response to *Electra pilosa* (fig. 5.6). Hadfield and Pennington (1990) demonstrated that metamorphosis in *Phestilla sibogae* occurred

only after active uptake of choline to a threshold concentration, above which metamorphosis was initiated. It may be that *A. proxima* requires the attainment of a threshold concentration by the natural inducer, which would not have been realised by the preparative method of ECSW in this investigation.

Whilst the inductive capacities of choline and K^+ were included in this study solely as potential positive controls of metamorphosis, betaine, a metabolic precursor of choline, provided a new criterion of assessment for the role of choline. However, unlike Hadfield (1978), who found betaine HCl to be non-inductive, and Taurobetaine to be highly toxic, metamorphosis of *Adalaria proxima* was initiated by low concentrations (10^{-5} M to 10^{-6} M) of betaine (fig. 5.5). The low level of induction observed would, however, suggest that it is not involved at a neurological level. This can only be implied from the results, but it may be that like dopamine in oyster larvae (Coon & Bonar, 1986) betaine has no obvious exogenous role in metamorphosis but is involved endogenously. Furthermore, the result would indicate that the neurological role of choline in metamorphosis is not in the synthesis and release of catecholamines. The roles of choline and K^+ in the metamorphic process of *A. proxima* have been discussed comprehensively by Todd *et al.*, (1991).

As with the majority of investigations into the induction, natural or artificial, of benthic marine invertebrates, further work must continue into the identification of the natural inducer *Adalaria proxima*. Bath-applied bioassays present too many ambiguities which are difficult to resolve. Only through the isolation and identification of the natural inducer through chemical isolation techniques can one begin to explain the neurological events that occur during the phenomenon of metamorphosis. Subsequent identification of the larval chemosensory organs and receptors by electrophysiological and biochemical techniques may follow.

Chapter 6

General discussion

General Discussion

The work outlined in this thesis has examined aspects of the relationship between the nudibranch *Adalaria proxima* and its bryozoan prey, *Electra pilosa*. The role of larval settlement (chapter 3), predation (chapter 4) and the dynamics of the substratum (chapter 3) as putative influences on the life history of *E. pilosa* have been discussed and the natural induction of the metamorphic process in *A. proxima* by live *E. pilosa* has been investigated (chapter 5).

Adalaria proxima is a generalist predator, consuming not only the cheilostome *Electra pilosa* but also the ctenostomes *Flustrellida hispida* and *Alcyonidium* sp. However, Todd & Havenhand (Todd & Havenhand, 1988; Havenhand & Todd, 1988a, 1988b) have demonstrated that laboratory reared *A. proxima* fed solely *E. pilosa* are able to acquire sufficient energy from the diet to produce comparable numbers and quality of progeny as field populations of the nudibranch. Furthermore, individuals will undergo true and complete metamorphosis in the presence of that bryozoan alone. The reasons for, and the nature of this close association, together with the inductive capacity of *E. pilosa* still remains unclear. The year round presence of the bryozoan in the field, unlike *Flustrellida hispida* and *Alcyonidium* sp. (Seed, 1985), may be important to the recruitment of the nudibranch larvae to the sessile macroalgal assemblages by providing a constant and essential point of reference to the settling larvae.

Electra pilosa has the potential for exponential indeterminate growth (Hughes & Hughes, 1986; Rubin, 1987; Silén, 1987) but was observed at both field sites studied to rarely realise that potential in the field. Complete and partial mortality complicated and distorted the discernible patterns of colony growth and profoundly influenced the dynamics of the *E. pilosa* populations. Such suppression of the growth potential may be mediated through the effects both of physical (colony and

frond abrasion) and biological (predation by nudibranchs) disturbance and interspecific and intra specific competition.

A sessile organism cannot separate spatially and temporally its requirements for space and food (Buss, 1979b; Best & Thorpe, 1986a, 1986b; Okamura, 1992). Therefore, spatial and nutritive competition are not mutually exclusive and the demonstration of competition for space did not preclude the possibility of competition for food or *vice versa* (Jackson & Winston, 1982). In the light of this, Yodzis (1986) proposed a continuum of intermediate strategies between the two extremes of consumptive and spatial competition.

Competition for space in epifaunal assemblages occurs in the event that substratum availability is sufficiently reduced, to the extent, whereby, the lateral margins of the colonies come into contact (Buss, 1979b). Subsequent growth may cease along the region of juxtaposition and any further growth is re-directed, or overgrowth may occur. The extent and outcome of overgrowth by one or both competitors may vary (Todd & Turner, 1988). Whilst death may occur, interruption of growth and a reduction in colony size and thus, potential fecundity, is more frequent (Buss, 1986).

Species differ in their abilities to overgrow other competitors and become locally dominant. The frequency of particular outcomes between species provides a measure of the competitive ability of the species. Ranking these frequencies creates patterns of hierarchies and networks (Gilpin, 1975; Jackson & Buss, 1975; Buss, 1979a; Buss & Jackson, 1979; Wood and Seed, 1980; Karlson & Jackson, 1981; Karlson & Buss, 1984; Karlson, 1985). There is increasing evidence, however, that competitive networks and hierarchies are not the only possibilities for competitive dominance relationships. Rather, they represent the extremes of a continuum of relationships that can be produced by contingent interactions and are rarely encountered (Connell, 1976; Kay & Keough, 1981; Quinn, 1982; Russ, 1982). The

fewer the factors determining competitive superiority, the more likely are hierarchies. Conversely, more numerous and complex interactions produce networks. The greater the indeterminacy or stochasticity of competition arising from the "competitive equivalence" (Kay & Keough, 1981) or "symmetry" (Connell, 1983) of the interacting competitors then the more likely it is that an intermediate pattern of relationships will arise. Such variation in outcome, leading to disruption of hypothetical networks or hierarchies, has been attributed to a number of factors. These include a difference in size of competing colonies (Buss, 1980; Russ, 1982; Sebens, 1986b), the condition of the colony surfaces at the region of contact (Jackson, 1979b), spatial heterogeneity (Walters & Wethy, 1986), the directionality of growth and therefore the encounter angle (Jackson, 1979b; Harris & Irons, 1982; Liddell & Brett, 1982; Rubin, 1982; Quinn, 1982; Turner, 1988), seasonality (Turner, 1988; but see Schoener, 1983) and site (Connell, 1983; Sebens, 1986b; Todd & Turner, 1988; Turner, 1988).

Within the bryozoan assemblages on *Fucus serratus* "direct competition is the exception rather than the rule" (O'Connor *et al.*, 1980). The spatial resource is "partitioned" by the species specific settlement and growth responses of the Bryozoa present. These include the time interdependence of the recruitment of *Electra pilosa*, *Membranipora membranacea* and *Celleporella hyalina* which allows year-round settlement and establishment of these competitively inferior but opportunistic species (Seed, 1985). Temporal heterogeneity in life-history pattern permits the co-existence of the dominant competitive ctenostomes *Flustrellidra hispida* and *Alcyonidium* sp., on *Fucus serratus* whilst spatial heterogeneity of the host plant is reflected in the distal reversal of competitive superiority of *F. hispida* over *Alcyonidium* sp on this fucoid (O'Connor *et al.*, 1979). The orientated growth of *E. pilosa* towards algal meristematic tissue (Ryland & Stebbing, 1971) is an adaptative opportunistic response which allows the rapid colonisation of newly generated substratum. However, a degree of opportunism coupled with the inevitable temporal and spatial

overlap of favourable abiotic and biotic cues presents a breakdown in resource partitioning and the subsequent occurrence of interspecific competition (O'Connor *et al.*, 1980). Competition within the bryozoan assemblages of marine macroalgae is a complex of hierarchies and networks, mediated by the ephemeral and "biologically dynamic" nature of the substratum (Seed, 1985).

Competitive ability and, indeed colony growth, will be suppressed further by the action of biological and physical disturbance. The observed maintenance of a high specific growth rate by *Electra pilosa* in response to predation and mechanical damage may be of critical importance to this competitively weak species, preventing colonisation by competitively superior species in the patch (type 1 *sensu* Connell & Keough, 1985) created. Palumbi and Jackson (1982) recorded 50% colonisation of artificially produced 4mm diameter holes, in two species of tropical, encrusting Bryozoa, *Steginoporella* sp. and *Reptadeonella* sp. after only six days. Through the rapid colony regeneration typical of many Bryozoa (Lutaud, 1961; Menon, 1972; Jackson & Palumbi, 1979; Ryland, 1979; Palumbi & Jackson, 1981), colonies would be able to overgrow newly settled larvae (potential competitors), thereby precluding further damage to the colony (Connell & Keough, 1985). However, the extent of damage and the location and type of damage (polypide or zooecium removal) within the colony may have profound effects both for the colony and the newly settled colonising larvae. The rate of regeneration and degree of larval settlement will depend upon patch size, the damaged species and the position within that species (Palumbi & Jackson, 1982). Although larger colonies are able to survive partial mortality (Jackson, 1977; Jackson & Winston, 1981; Palumbi & Jackson, 1982), mortality of even a few zooids in the initial stages of astogeny may not be tolerated. D'Hondt (1976) inhibited further growth of young colonies of *Alcyonidium polyoum*, by killing the ancestrula. Silén (1977), however, argued that the ancestrula has no special function in the life of a colony except in its occasional role as the primary or only holdfast of a number of arborescent colonies. Certainly, *E. pilosa* was observed

to undergo one or more splits of the colony during its lifetime in consequence of the tearing of the fronds of the *Laminaria digitata* substratum (pers. obs.), thereby removing portions of the colony from communication with the ancestrula. It may be that a minimum size exists before the colony can become independent of the ancestrula. The manner of colony damage may also have implications for continued colony survival. Colony abrasion, or predation by nudibranchs such as *Polycera quadrilineata* (in which the zooecium and polypide are removed) contrasts to the feeding technique adopted by *Adalaria proxima* and *Onchidoris muricata* (the polypide alone is sucked out) (Todd, 1979a, 1981, 1983; Todd & Havenhand, 1989). Removal of the whole zooid precludes fouling of the damaged tissue remaining (pers. obs.) which may culminate in the death of the colony.

Patch colonisation may reflect a variety of adult-larval interactions such as, allelopathy, modification of the physical condition of the substratum, larval site-specific selectivity, temporal and spatial variability of larvae and larval-larval interactions (discussed in Connell & Keough, 1985). Palumbi & Jackson (1982) suggested that the strong feeding currents generated by *Steginoporella* sp., or the use of patches for excurrent flow (analogous to "chimneys", Banta, 1974) would push larvae away, whilst Okamura (1988), observed zooids of *Bugula neritina* to ingest cyprid larvae. Even if larvae are not ingested, they can incur damage during the rejection process (Mileikovsky, 1974).

The variation in morphology of *Electra pilosa* colonies observed in this study and in previous accounts (Jebram, 1980a; Ryland & Hayward, 1977; Rubin, 1987; Silén, 1987) is perhaps then counterintuitive. In inimical conditions the adaptive phenotypic formation of the stellate growth form perceived by Buss (1979a) and Jackson (1979a) to be a fugitive strategy, presents opportunity for the colonisation of competitors within the indentations of the projected axes. Indeed, the extreme uniserial growth form adopted in severe conditions (Silén, 1987) would be expected to be susceptible to overgrowth. In fact the uniserial runner successfully overgrows

other colonies (Silén, 1987). It may be that the high specific growth rate associated with such fugitive forms may temporarily elevate the competitive ability of the bryozoan, presenting an "asymmetry" in competitive encounters with other species.

As demonstrated in chapter 4, the shape index (S.I.) of the *Electra pilosa* colony is not indicative of susceptibility to predation by *Adalaria proxima*. Rather, it is the maintenance of a high specific growth rate that enables the colonies to recover rapidly from damage. It would appear that the outcome of predatory attack is, in a manner similar to competition success, related to the angle of encounter, the point at which feeding commences and the subsequent feeding behaviour of the nudibranch.

One may conclude from the observations in this study, therefore, that *Electra pilosa,* in consequence of its phenotypic plasticity and ability to maintain a high specific growth rate, despite often quite detrimental biotic and abiotic interactions, is able to persist in the essentially ephemeral environment presented by marine macroalgae. The variation in form is proposed to be due to the high specific growth rate encountered in colonies inhabiting dynamic ephemeral substrata not as a response to predation. The adoption of a stellate growth form enables *E. pilosa* to achieve larger colonies and an associated elevated fecundity than would be possible in purely multiserial forms under such extrinsic environmental conditions. This is clearly demonstrated in this thesis by the differing growth forms promoted at the two study sites and on the two species of macroalgae.

References

Akashige, S. T., Seki, T., Kan-no, H. & Nomura, T., (1981). Effects of γ-aminobutyric acid and certain neurotransmitters on the settlement and the metamorphosis of the larvae of *Haliotis discus hannai* Ino (Gastropoda). *Bulletin of the Tohoku Regional Fisheries Research Laboratory*, **43**: 37—45

Al-Ogily, S. M. & Knight-Jones, E. W., (1977). Antifouling role of antibiotics by marine algae and bryozoans. *Nature, London*, **265**: 728—729.

Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. & Watson, J. D., (1983). *Molecular Biology of the Cell.* Garland Publishing Inc., New York. 1145 pp.

Arkett, S. A., F-S., Chia, J. I., Goldberg & Koss R., (1989). Identified settlement receptor cells in a nudibranch veliger respond to specific cue. *Biological Bulletin*, **176**: 155—160

Atkins, D., (1955a). The cyphonautes larvae of the Plymouth area and the metamorphosis of *Membranipora membranacea* (L.). *Journal of the Marine Biological Association of the United Kingdom*, **34**: 441—449.

Atkins, D., (1955b). The ciliary feeding mechanisms of the cyphonautes larvae (Polyzoa Ectoprocta). *Journal of the Marine Biological Association of the United Kingdom*, **34**: 451—466.

Bahamondes, I. & Tardy, J., (1988). Induction of metamorphosis of *Eubranchus doriae* (Trinchèse 1879) (Mollusc Nudibranchiata) by various bioactive substances. *Haliotis*, **18**: 121—130.

Bahamondes-Rojas, I. & Dherbomez, M., (1990). Purification partielle de substances glycoconjugeés capable d'induire la metamorphose des larves compétentes d'*Eubranchus doriae* (Trinchèse, 1879) mollusque nudibranche. *Journal of Experimental Marine Biology and Ecology*, **144**: 17—27.

Baloun, A. & Morse, D.E., (1984). Ionic control of settlement and metamorphosis in larval *Haliotis rufescens* (Gastropoda). *Biological Bulletin*, **167**: 124—138.

Banta, W. C., McKinney, F. & Zimmer, R., (1974). Bryozoan monticules: excurrent water outlets? *Science*, **185**: 783—784.

Barnes, R. D., (1980). *Invertebrate Zoology.* Fourth edition. Saunders College/Holt, Rinehart and Winston, Philadelphia, PA. 1089 pp.

Barrett, J. & Yonge, C. M., (1985). *Collins Pocket Guide to the Seashore*. Collins, London. 272 pp.

Baxter, G. & Morse, D. E., (1987). G protein and diacylglycerol regulate metamorphosis of planktonic molluscan larvae. *Proceedings of the National Academy of Sciences of the United States of America*, **84**: 1867—1870.

Begon, M., Harper, J. L. & Townsend, C. R., (1986). *Ecology: Individuals, Populations and Communities*. Blackwell Scientific Publications, Oxford. 876 pp.

Bernstein, B. B. & Jung, N., (1979). Selective pressures and coevolution in a kelp canopy community in Southern California. *Ecological Monographs*, **49**: 335—355.

Best, M. A. & Thorpe, J. P., (1986a). Effects of food particle concentration on feeding current velocity in six species of marine Bryozoa. *Marine Biology (Berlin)*, **93**: 255—262.

Best, M. A. & Thorpe, J. P., (1986b). Feeding-current interactions and competition for food among the bryozoan epiphytes of *Fucus serratus*. *Marine Biology (Berlin)*, **93**: 371—375.

Best, B. A. & Winston, J. E., (1984). Skeletal strength of encrusting cheilostome bryozoans. *Biological Bulletin*, **167**: 390—409.

Birkeland, C., Chia, F-S. & Strathmann, R. R., (1971). Development, substratum selection, delay of metamorphosis and growth in the sea star, *Mediaster aequalis* Stimpson. *Biological Bulletin*, **141**: 99—108.

Bishop, J. D. D., (1989). Colony form and the exploitation of spatial refuges by encrusting Bryozoa. *Biological Reviews. Cambridge Philosophical Society*, **64**: 197—218.

Black, W. A. P., (1954). Concentration gradients and their significance in *Laminaria saccharina* (L.) Lamour. *Journal of the Marine Biological Association of the United Kingdom*, **33**: 49—60.

Boaden, P. J. S., O'Connor, R. J. & Seed, R., (1975). The composition and zonation of a *Fucus serratus* community in Strangford Lough, Co. Down. *Journal of Experimental Marine Biology and Ecology*, **17**: 111—136.

Boaden, P. J. S., O'Connor, R. J. & Seed, R., (1976). The fauna of a *Fucus serratus* L. community: ecological isolation in sponges and tunicates. *Journal of Experimental Marine Biology and Ecology*, **21**: 249—267.

Boardman, R. S. & Cheetham, A. H., (1973). Degrees of colony dominance in stenolaemate and gymnolaemate Bryozoa. In: *Animal Colonies*. R. S. Boardman, A. H. Cheetham and W. A. Oliver (eds.). Dowden, Hutchinson and Ross, Stroudsberg, Pennsylvania. pp. 121—220.

Bobin, G., (1968). Morphogenèse du termen et des éspines dans les zoécies d'*Electra verticillata* (Ellis et Solander) (Bryozoaire Chilostome, Anasca). *Cahiers de Biologie Marine*, **9**: 53—68.

Bonar, D. B., (1976). Molluscan metamorphosis: a study in tissue transformation. *American Zoologist*, **16**: 573—561.

Bonar, D. B., (1978). Morphogenesis at metamorphosis in opisthobranch molluscs. In: *Settlement and Metamorphosis of Marine Invertebrate Larvae*. F-S. Chia, and M. E. Rice, (eds.). Elsevier, New York. pp. 177—196.

Bonar, D. B., Coon, S. L., Walch, M., Weiner, R. M. & Fitt, W., (1990). Control of oyster settlement and metamorphosis by endogenous and exogenous chemical cues. *Bulletin Marine Science*, **46**: 484—498.

Boney, A. D., (1966). *A Biology of Marine Algae*. Hutchinson Educational Ltd., London. 216 pp.

Brancato, M. S. & Woollacott, R. S., (1982). Effect of microbial films on settlement of bryozoan larvae (*Bugula simplex*, *B. stolonifera* and *B. turrita*). *Marine Biology (Berlin)*, **7**: 51—56.

Bronstein, G., (1938). Note sur le croissance residuelle des fragments de zoarium chez un bryozoaire *Membranipora membranacea* (L.). *Comptes Rendus Hebdomadaires des Séances de la Societe de Biologie*, **126**: 65—68.

Bronstein, G., (1939). Sur les gradients physiologiques dans une colonie de bryozoaires. *Comptes Rendus Hebdomadaires des Séances de l'Academie des Sciences, Paris*, **209**: 602—603.

Burgh, M. E., de & Fankboner, P. V., (1979). A nutritional association between the bull kelp *Nereocystis luetkeana* and its epizooic bryozoan *Membranipora membranacea*. *Oikos*, **31**: 69—72.

Burke, R. D., (1983a). The induction of metamorphosis of marine invertebrate larvae: stimulus and response. *Canadian Journal of Zoology*, **61**: 1701—1719.

Burke, R. D., (1983b). Neural control of metamorphosis in *Dendraster excentricus*. *Biological Bulletin*, **164**: 176,188.

Bushnell, J. A., (1966). Environmental relations of Michigan Ectoprocta, and the dynamics of natural populations of *Plumatella repens*. *Ecological Monographs*, **36**: 95—123.

Buss, L. W., (1979a). Habitat selection, directional growth, and spatial refuges: why colonial animals have more hiding places. In: *Biology and Systematics of Colonial Organisms*. G. P. Larwood and B. R. Rosen (eds.). Academic Press, London. pp. 459—497.

Buss, L. W., (1979b). Bryozoa overgrowth interactions— the interdependence of competition for space and food. *Nature, London* , **281**: 475—477.

Buss, L. W., (1980). Competitive intransitivity and site-frequency distributions of interacting populations. *Proceedings of the National Academy of Sciences of the United States of America*, **77**: 5355—5359.

Buss, L. W., (1986). Competition and community organisation on hard surfaces in the sea. In: *Community Ecology*. J. Diamond and T. J. Case (eds.). Harper and Row Publishers, New York. pp. 517—536.

Buss, L. W. & Jackson, J. B. C., (1979). Competitive networks: nontransitive competitive relationships in cryptic coral reef environments. *American Naturalist*, **113**: 223—234.

Cameron, R. A. & Hinegardner, R. T., (1974). Initiation of metamorphosis in laboratory cultured sea urchins. *Biological Bulletin*, **146**: 335—342.

Cancino, J. M., (1983). *Demography of animal modular colonies*. Ph.D. thesis. University College North Wales, Bangor.

Cancino, J. M., (1986). Marine macroalgae as a substratum for sessile invertebrates : a study of *Celleporella hyalina* (Bryozoa) on fronds of *Laminaria saccharina* (Phaeophyta). *Monografias Biologicas*, **4**: 279—308.

Cancino, J. M., Muñoz, J., Muñoz, M. & Orellana, M. C., (1987). Effects of the bryozoan *Membranipora tuberculata* (Bosc) on the photosynthesis and growth of *Gelidium rex* Santelices et Abbott. *Journal of Experimental Marine Biology and Ecology*, **113**: 105—112.

Charnov, E.L., (1976). Optimal foraging: the marginal value theorem. *Theoretical and Experimental Biology*, **9**: 129—136.

Chevolot, L., Cochard, J-C. & Yvin, J-C., (1991). Chemical induction of larval metamorphosis of *Pecten maximus* with a note on the nature of naturally occurring triggering substances. *Marine Ecology Progress Series*, **74**: 83—89.

Chia, F-S. & Koss, R., (1988). Induction of settlement and metamorphosis of the veliger larvae of the nudibranch, *Onchidoris bilamellata*. *International Journal of Invertebrate Reproduction and Development*, **14**: 53—70.

Chia, F-S. & Koss, R., (1989). The fine structure of the newly discovered propodial ganglia of the veliger larva of the nudibranch, *Onchidoris bilamellata*. *Cell Tissue Research*, **256**: 17—26.

Chia, F-S., Koss, R., Stevens, S. & Goldberg, J. I., (1992). Isolation of neurons of a nudibranch veliger. *Biological Bulletin*, **182**: 66—76.

Chia, F-S. & Rice, M. E., (eds.) (1978). *Settlement and Metamorphosis of Marine Invertebrate Larvae*. Elsevier, New York.

Clarke, C. W. & Harvell, C. D., (1992). Inducible defenses and the allocation of resources: a minimal model. *American Naturalist*, **139**: 521—539.

Colman, J., (1940). On the fauna inhabiting intertidal seaweeds. *Journal of the Marine Biological Association of the United Kingdom*, **24**: 129—183.

Connell, J. H., (1961). Effects of competition, predation by *Thais lapillus* and other factors on natural populations of the barnacle *Balanus balanoides*. *Ecological Monographs*, **31**: 61—103.

Connell, J. H., (1966). Studies on the interactions of populations of intertidal animals. *Summary Report to the Office of Naval Research, Washington.*

Connell, J. H., (1972). Community interactions on marine rocky intertidal shores. *Annual Review of Ecology and Systematics*, **3**: 169—192.

Connell, J. H., (1976). Competitive interactions and the species diversity of corals. In: *Coelenterate Eology and Behaviour*. G. O. Mackie (ed.). Plenum Press, New York. pp. 51—58.

Connell, J. H., (1983). On the prevalence and importance of interspecific competition: evidence from field experiments. *American Naturalist*, **122**: 661—696.

Connell, J. H. & Keough, M. J., (1985). Disturbance and patch dynamics of subtidal marine animals on hard substrata. In: *The Ecology of Natural Disturbance and Patch Dynamics*. T. A. Pickett and P. S. White (eds.). Academic Press, London. pp. 125—151.

Conolly, N. J., (1982). *Seasonal growth of L. saccharina and L. digitata: relation with inorganic nutrients.* Ph. D thesis. University of St. Andrews.

Conolly, N. J. & Drew, E.A. (1985). Physiology of *Laminaria*. III. Effect of a coastal eutrophication gradient on seasonal patterns of growth and tissue composition in *L. digitata* Lamour. and *L. saccharina* (L.) Lamour. *Pubblicazioni della Stazione Zoologica di Napoli: Marine Ecology*, **6**: 181—195.

Conover, J. T. & Sieberth J. McN., (1966). Effects of tannins excreted from Phaeophyta on planktonic animal survival in tide pools. *Proceedings of the Fifth International Seaweed Symposium* , 99—100.

Coon, S. L. & Bonar, D. B., (1986). Norepinephrine and dopamine content of larvae and spat of the Pacific oyster, *Crassostrea gigas*. *Biological Bulletin*, **171**: 632—639.

Coon, S. L. & Bonar, D. B., (1987). Pharmacological evidence that alpha$_1$-adrenoreceptors mediate metamorphosis of the Pacific oyster, *Crassostrea gigas*. *Neuroscience* , **23**: 1169—1174.

Coon, S. L., Bonar, D. B. & Weiner, R. M., (1985). Induction of settlement and metamorphosis of the Pacific oyster, *Crassostrea gigas* (Thunberg), by L-DOPA and catecholamines. *Journal of Experimental Marine Biology and Ecology*, **94**: 211—221.

Coon, S. L., Bonar, D. B. & Weiner, R. M., (1986). Chemical production of clutchless oyster spat using epinephrine and norepinephrine. *Aquaculture*, **58**: 255—262.

Coon, S. L., Walch, M., Fitt, W. K., Bonar, D. B. & Wiener, R. M., (1988). Induction of settlement behaviour in oyster larvae by ammonia. *American Zoologist*, **28**: 70A.

Coon, S. L., Bonar, D. B. & Fitt, W. K. (1990a). Competence and delay of metamorphosis in the Pacific oyster *Crassostrea gigas*. *Marine Biology (Berlin)*, **106**: 379—387.

Coon, S. L., Walch, M., Fitt, W. K., Weiner, R. M. & Bonar, D. B., (1990b). Ammonia induces settlement behaviour in oyster larvae. *Biological Bulletin*, **179**: 297—303.

Cosson, J., (1967). Étude d'une population de *Laminaria digitata* Lam. (biométrie, croissance, régénération). *Bulletin de la Societe Linneenne de Normandie. 10^e Serie*, **8**: 246—281.

Crisp, D. J., (1967). Chemical factors inducing settlement in *Crassostrea virginica* Gmelin. *Journal of Animal Ecology*, **36**: 329—335.

Crisp, D. J., (1974). Factors influencing the settlement of marine invertebrate larvae. In: *Chemoreception in Marine Organisms.* P. T. Grant and A. M. Mackie (eds.). Academic Press, London. pp. 177—265.

Crisp, D. J., (1976). Settlement responses in marine organisms. In: *Adaptation to Environment. Essays on the Physiology of Marine Animals.* R. C. Newell (ed.). Butterworth and Co. (publishers) Ltd., London. pp. 83—124.

Crisp, D. J., (1984). Overview of research on marine invertebrate larvae, 1940—1980. In: *Marine Biodeterioration: an Interdisciplinary Sudy.* J. D. Costlow and R. C. Tipper (eds.). Navel Institute Press, Annapolis, Maryland. pp. 103—106.

Crisp, D. J. & Ryland, J. S., (1960). Effects of extracts from fucoids in promoting settlement of epiphytic Polyzoa. *Nature, London*, **186**: 1206—1207.

Datta, S. P. & Ottaway, J. H., (1976). *Biochemistry* . Bailliere Tindall, London. 504 pp.

Dawkins, R. & Krebs, J. R., (1979). Arms race between and within species. *Proceedings of the Royal Society of London. Section B. Biological Sciences*, **205**: 489—511.

Dayton, P. K., (1971). Competition, disturbance and community organisation: the provision and subsequent utilization of space in a rocky intertidal shore. *Ecological Monographs*, **41**: 351—389.

Dixon, J., Schroeter, S. C. & Kastendiek, J., (1981). Effects of *Membranipora membranacea* the encrusting baldes and fronds by the giant kelp *Macrocystis pyrifera* (Laminariales). *Journal of Phycology*, **17**: 341—345.

Durante, K. M. & Chia, F-S., (1991). Epiphytism on *Agarum fimbriatum*: can herbivore preferences explain distributions of epiphytic bryozoans? *Marine Ecology Progress Series*, **77**: 279—287.

Eyster, L. S. & Pechenik, J. A., (1987). Attachment of *Mytilus edulis* L. on algae and byssal filaments is enhanced by water agitation. *Journal of Experimental Marine Biology and Ecology*, **114**: 99—110.

Fitt, W. K. & Coon, S. L., (1992). Evidence for ammonia as a natural cue for recruitment of oyster larvae to oyster beds in a Georgia salt marsh. *Biological Bulletin*, **182**: 401—408.

Fitt, W. K., Coon, S. L., Walch, M., Weiner, R. M., Colwell, R. R.. & Bonar, D. B., (1990). Settlement behavior and metamorphosis of oyster larvae (*Crassostrea gigas*) in response to bacterial supernatants. *Marine Biology (Berlin)*, **106**: 389—394.

Fletcher, W. J. & Day, R. W., (1983). The distribution of epifauna on *Ecklonia radiata* (C. Agardh) J. Agardh and the effect of disturbance. *Journal of Experimental Marine Biology and Ecology*, **71**: 205—220.

Gilpin, M. E., (1975). Limit cycles in competition communities. *American Naturalist*, **109**: 51—60.

Giraud, G. & Cabloch, J., (1976). Étude ultrastructurale de l'activitie des cellules superficielles du thalle des corallinaceés (Rhodophyceés). *Phycologia*, **15**: 405—414.

Gordon, D. P., (1968). Zooidal dimorphism in the polyzoan *Hippodinella adpressa* (Busk). *Nature, London* , **219**: 633—634.

Gordon, D. P., (1976). The ageing process in bryozoans. In: Biology of Bryozoans. R. M. Woollacott and R. L. Zimmer (eds.). Academic Press, New York. pp. 335—376.

Hadfield, M.G., (1977). Chemical interactions in larval settling of a marine gastropod. In: *Marine Natural Products Chemistry* . D.J . Faulkner and W. H. Fenical (eds.). Plenum Press, New York. pp. 403—413.

Hadfield, M. G., (1978). Metamorphosis of marine molluscan larvae: an analysis of stimulus and response. In: *Settlement and Metamorphosis of Marine Invertebrate Larvae*. F-S. Chia and M. E. Rice (eds.). Elsevier, New York. pp. 165—175.

Hadfield, M. G., (1980). Habituation in metamorphic induction of larvae of *Phestilla sibogae* (Gastropoda). *American Zoologist*, **20**: 955.

Hadfield, M. G., (1986). Settlement and recruitment of marine invertebrates: a prespective and some proposals. *Bulletin of Marine Science*, **39**: 418—425.

Hadfield, M. G. & Pennington, J. T., (1990). Nature of the metamorphic signal and its internal transduction in larvae of the nudibranch *Phestilla sibogae*. *Bulletin of Marine Science*, **46**: 455—464.

Hadfield, M. G. & Scheuer, D., (1985). Evidence for a soluble metamorphic inducer in *Phestilla* : ecological, chemical and biological data. *Bulletin of Marine Science*, **37**: 556—566.

Hagerman, L., (1966). The macro- and microfauna associated with *Fucus serratus* L., with some ecological remarks. *Ophelia*, **3**: 1—-43.

Hagiwara, S., Kusan, K. & Saito, N., (1961). Membrane changes of *Onchidium* nerve cells in potassium-rich media. *Journal of Physiology (London)*, **155**: 470—489.

Harris, L. G., (1973). Nudibranch associations. *Current topics in Comparative Pathobiology*, **2**: 213—315.

Harris, L. G. & Irons, K.P., (1982). Substrate angle and predation as determinants in fouling community succession. In: *Artificial Substrates*. J. Cairns (ed.). Ann Arbor Science Publishers, Ann Arbor, Michigan. pp. 131—174.

Harvell, C. D., (1984a). Predator-induced defense in a marine bryozoan. *Science*, **224**: 1357—1359.

Harvell, C. D., (1984b). Why nudibranchs are partial predators: intracolonial variation in bryozoan palatability. *Ecology*, **65**: 716—724.

Harvell, C. D., (1986). The ecology and evolution of inducible defenses in a marine bryozoan: cues, costs and consequences. *American Naturalist*, **128**: 810—823.

Harvell, C. D., (1990). The ecology and evolution of inducible defenses. *Quarterly Review of Biology*, **65**: 323—340.

Harvell, C. D., (1991). Coloniality and inducible polymorphism. *American Naturalist*, **138**: 1—14.

Harvell, C. D. & Padilla, D.K., (1990). Inducible morphology, heterochrony, and size hierarchies in a colonial invertebrate monoculture. *Proceedings of the National Academy of Sciences of the United States of America*, **87**: 508—512.

Harvell, C. D. & Suchanek, T.H., (1987). Partial predation on tropical gorgonians by *Cyphona gibbosum* (Gastropoda). *Marine Ecology Progress Series*, **38**: 37—44.

Havenhand, J. N., (1991). On the behaviour of opisthobranch larvae. *Journal of Molluscan Studies*, **57**: 119—131.

Havenhand, J. N., Thorpe, J. P. & Todd, C.D., (1986). Estimates of biochemical genetic diversity within and between the nudibranch molluscs *Adalaria proxima* (Alder and Hancock) and *Onchidoris muricata* (Müller) Doridacea: Onchidoridídae. *Journal of Experimental Marine Biology and Ecology*, **95**: 105—111.

Havenhand, J. N. & Todd, C. D., (1988a). Physiological ecology of *Adalaria proxima* (Alder and Hancock) and *Onchidoris muricata* (Müller) (Gastropoda: Nudibranchia). I. Feeding, growth and respiration. *Journal of Experimental Marine Biology and Ecology*, **118**: 151—172.

Havenhand, J. N. & Todd, C.D., (1988b). Physiological ecology of *Adalaria proxima* (Alder and Hancock) and *Onchidoris muricata* (Müller) (Gastropoda: Nudibranchia). II. Reproduction. *Journal of Experimental Marine Biology and Ecology*, **118**: 173—189.

Havenhand, J. N. & Todd, C.D., (1989). Reproductive effort of the nudibranch molluscs *Adalaria proxima* (Alder and Hancock) and *Onchidoris muricata* (Müller): an evaluation of techniques. *Functional Ecology*, **3**: 153—163.

Hay, M., (1992). In: *Plant-animal interactions in the marine benthos*. D. M., John, S. J. Hawkins and J. H. Price, (eds.). Systematics Association Special Volume, No. 46. Clarendon Press, Oxford.

Hayward, P. J., (1980). Invertebrate epiphytes of coastal marine algae. In: *The shore environment, Vol. 2: Ecosystems*. J. H. Price , D. E. G. Irvin and W. F. Farnham (eds.). Academic Press, London. Systematics Association Special Volume No. 17. pp. 761—787.

Hayward, P. J. & Harvey, P. H., (1974). Growth and mortality of the bryozoan *Alcyonidium hirsutum* (Fleming) on *Fucus serratus* (L.). *Journal of the Marine Biological Association of the United Kingdom*, **54**: 677—684.

Hayward, P. J. & Ryland, J. S., (1975). Growth, reproduction and larval dispersal in *Alcyonidium hirsutum* (Fleming) and some other Bryozoa. *Pubblicazioini della Stazione zoologica di Napoli*, **39**: 677—684.

Hayward, P. J. & Ryland, J. S., (1979). *British Ascophoran Bryozoans. Synopses of the British Fauna. No. 14*. Academic Press, London. 312 pp.

Highsmith, R. C. & Emlet, R. B., (1986). Delayed metamorphosis: effect on growth and survival of juvenile sand dollars (Echinoidea: Clypeasteroida). *Bulletin of Marine Science*, **39**: 327—361.

Hirata, K. Y. & Hadfield, M. G., (1986). The role of choline in metamorphic interaction of *Phestilla* (Gastropoda, Nudibranchia). *Comparative Biochemistry and Physiology. C. Comparative Pharmacology*, **84**: No1: 15—21.

Hodgkin, A. L. & Horowitz, P., (1952). The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *Journal of Physiology*, **148**: 127—160.

Hondt, J-L. d', (1976). Effects sur l'ablation de l'ancestrula de la première zooecie-fille sur le développement d'une jeune colonie du Bryozoaire ctenostome *Alcyonidium polyoum* (Hassal). *Bulletin. Societe Zoologique de France*, **101**: 993—995.

Hoppe, W. F., (1988). Growth, regeneration and predation in three species of large coral reef sponges. *Marine Ecology Progress Series*, **50**: 117—125.

Hornsey, I. S. & Hide, D., (1974). The production of antimicrobial compounds by British marine algae. I. Antibiotic-producing marine algae. *British Phycological Journal*, **9**: 353—361.

Hornsey, I. S. & Hide, D., (1976). The production of antimicrobial compounds by British marine algae. III. Distribution of antimicrobial activity within the algal thallus. *British Phycological Journal*, **11**: 175—181.

Hughes, D. J. & Hughes, R. N., (1986). Metabolic implications of modularity: studies on the respiration and growth of *Electra pilosa*. *Philosophical Transactions of the Royal Society of London. Series B. Biological Sciences*, **313**: 23—29.

Hurlbutt, C. J., (1991). Larval substratum selection and postsettlement mortaltiy as determinants of the distribution of two bryozoans. *Journal of Experimental Marine Biology and Ecology*, **147**: 103—119.

Hyman, L. H., (1959). *The Invertebrates, 5: Smaller Coelomate Groups*. McGraw-Hill, New York. 783 pp.

Jackson, J. B. C., (1977). Competition on marine hard substrata: the adaptive significance of solitary and colonial strategies. *American Naturalist*, **111**: 743—767.

Jackson, J.B.C., (1979a). Morphological strategies of sessile animals. In: *Biology and Systematics of Colonial Organisms*. G. P. Larwood and B. R. Rosen (eds). Academic Press, London. pp. 499—555.

Jackson, J. B. C., (1979b). Overgrowth competition between encrusting cheilostome ectoprocts in a Jamaican cryptic reef environments. *Journal of Animal Ecology*, **48**: 805—823.

Jackson, J. B. C. & Buss, L. W., (1975). Allelopathy and spatial competition among coral reef invertabrates. *Proceeedings of the National Academy of Sciences of the United States of America*, **72**: 5160—5163.

Jackson, J. B. C. & Palumbi, S. R., (1979). Regeneration and partial predation in cryptic coral reef environments on sponges and ectoprocts. *Colloques Internationaux du Centre National de la Recherche Scientifique*, **291**: 303—308.

Jackson, J. B. C. & Winston, J. E., (1981). Modular growth and longevity in bryozoans. In: *Recent and Fossil Bryozoa.* G. P. Larwoodand C. Neilsen (eds.). Olsen and Olsen, Fredensborg, Denmark. pp. 121—126.

Jackson, J. B. C. & Winston, J. E., (1982). Ecology of cryptic coral communities. I. Distribution and abundance of major groups of encrusting organisms. *Journal of Experimental Marine Biology and Ecology*, **57**: 135—147.

Jaeckle, W. B. & Manahan, D.T., (1989). Feeding by "non-feeding" larvae: uptake of dissolved amino acids from sea water by lecithotrophic larvae of the gastropod *Halotis rufescens* Swainson. *Marine Biology (Berlin),* **103**: 87—94.

Jebram, D., (1973). Preliminary observation on the influences of food and other factors on the growth of Bryozoa: with the description of a new apparatus for cultivation of sessile plankton feeders. *Kieler Meeresforschungen*, **29**: 50—57.

Jebram, D., (1980a). Prospection for a sufficient nutrition for the cosmopolitic marine bryozoan *Electra pilosa* (Linnaeus). *Zoologische Jahrbücher (Systematik)*, **107**: 368—390.

Jebram, D., (1980b). Influences of the food on the colony forms of *Electra pilosa* (Bryozoa, Cheilostomata). *Zoologische Jahrbücher (Systematik)*, **108**: 1—14.

Jensen, R. A. & Morse, D. E., (1990). Chemically induced metamorphosis of polychaete larvae in both the laboratory and ocean environment. *Journal of Chemical Ecology*, **16**: 911—930.

Kain, J. M., (1979). A view of the genus *Laminaria*. *Oceanography and Marine Biology. An Annual Review*, **17**: 101—161.

Karlson, R. H., (1985). Competitive interactions among sessile colonial invertebrates: a comparison of stochastic and phenotypic variation. *Ecological Modelling*, **27**: 299—312.

Karlson, R. H. & Buss, L. W., (1984). Competition, disturbance and local diversity patterns of substratum-bound clonal organisms: a simulation. *Ecological Modelling*, **23**: 243—255.

Karlson, R. H. & Jackson, J. B. C., (1981). Competitive networks and community structure: a simulation study. *Ecology* , **62**: 670—678.

Kato, T., Kumanireq, A. S., Icninose, I., Kitihara, Y., Kakinuma, Y., Nishihira, M., & Kato, M., (1975). Active components of *Sargassum tartile* effecting settlement of swimming larvae of *Coryne urchidai*. *Experimentia* , **31**: 433.

Kaufmann, K. W., (1970). A model for predicting the influence of colony morphology on reproductive potential in the phylum Ectoprocta. *Biological Bulletin*, **139**: 426.

Kaufmann, K. W., (1973). The effects of colony morphology on the life history parameters of colonial animals. In: *Animal Colonies*. R. S. Boardman, A. H. Cheetham and W. A. Oliver (eds.). Dowden, Hutchinson and Ross, Stroudsberg, Pennsylvania. pp. 221—222.

Kaufmann, K. W., (1976). Gompertzian growth in cheilostome bryozoans. *Biological Bulletin*, **151**: 416.

Kay, A. M. & Keough, M.J., (1981). Occupation of patches in the epifaunal communities on pier pilings and the bivalve *Pinna bicolor* at Edithburgh, South Australia. *Oecologia (Berlin)*, **48**: 123—130.

Kempf, S. C., (1981). Long-lived larvae of the gastropod *Aplysia juliana* : do they disperse and metamorphose or just slowly fade away? *Marine Ecology Progress Series*, **6**: 61—65.

Kempf, S. C. & Todd, C. D., (1989). Feeding potential in the lecithotrophic larvae of *Adalaria proxima* and *Tritonia hombergi*: an evolutionary prespective. *Journal of the Marine Biological Assiociation of the United Kingdom*, **69**: 659—682.

Keough, M. J., (1986). The distribution of a bryozoan on seagrass blades: settlement, growth, and mortality. *Ecology* , **67**: 846—857.

Keough, M. J. & Downes, B. J., (1982). Recruitment of marine invertebrates: the role of active larvae and early mortality. *Oecologia (Berlin)*, **54**: 348—352.

King, R. J. & Schramm, W., (1976). Determination of a photosynthetic ratio for the marine algae *Fucus vesiculosus* and *Laminaria digitata*. *Marine Biology (Berlin)*, **37**: 209—213.

Kirchman, D., Graham, S., Reish, D. & Mitchell, R., (1982). Lectins may mediate the settlement and metamorphosis of *Janua* (*Dexiospira*) *brasiliensis* Grube (Polychaeta: Spirobidae). *Marine Biology Letters*, **3**: 131—142.

Kitching, J. A. & Ebling, F. J., (1967). Ecological studies at Lough Ine. *Advances in Ecological Research*, **4**: 197—291.

Klempke, J. E. & Keough, M. J., (1991). Relationships between the nudibranch *Madrella sanguinea* and its broadly-distributed prey, the bryozoan *Mucopetraliella ellerii*. *Journal of Molluscan Studies*, **57**: 23—30.

Knight, M. & Parke, M., (1950). A biological study of *Fucus vesiculosus* L. and *F. serratus* L. *Journal of the Marine Biological Association of the United Kingdom*, **29**: 439—514.

Krebs, J. R. & Davies, N. B., (1987). *An Introduction to Behavioural Ecology*. Second edition. Blackwell Scientific Publications. 389 pp.

Laverack, M. S. & Blackler, M., (eds.), (1974). *Fauna and Flora of St. Andrews Bay*. Scottish Academic Press, Edinburgh. 310 pp.

Leighton, D. L., (1988). Abalone mariculture in California and related San Diego-based research. *Annual Report of the Western Society of Malacologists*, **20**: 21—25

Lewis, J. R., (1964). *Ecology of Rocky Shores*. English Universities Press, London. 323 pp.

Liddell, W. D. & Brett, C. E., (1982). Skeletal overgrowth among epizoans from the Silurian (Wenlockian) Waldron Shale. *Paleobiology*, **8**: 67—78.

Lüning, K., (1979). Growth strategies of three *Laminaria* species (Phaeophyceae) inhabiting different depth zones in the sublittoral region of Helgoland (North Sea). *Marine Ecology Progress Series*, **1**: 195—207.

Lutaud, L., (1961). Contribution a l'étude du bourgeonnement et de la croissance des colonies chez *Membranipora membranacea* (Linne), Bryozoaire chilostome. *Annales de la Société royale Zoologique de Belgique*, **91**: 157—300

Maki, J. S. & Mitchell, R., (1985). Involvement of lectins in the settlement and metamorphosis of marine invertebrate larvae. *Bulletin of Marine Science*, **37**: 675—683.

Maki, J. S., Rittschoff, D., Schmidt, A. R., Snyder, A. G. & Mitchell, R., (1989). Factors controlling attachment of bryozoan larvae: a comparison of bacterial films and unfilmed surfaces. *Biological Bulletin*, **177**: 295—302.

Mann, K. H., (1972). Ecological energetics of the sea-weed zone in a marine bay on the Atlantic coast of Canada. II. Productivity of the seaweeds. *Marine Biology (Berlin)*, **14**: 199—209.

Mann, K. H., (1973). Seaweeds: their productivity and strategy for growth. *Science*, **182**: 975—981.

Marcus, E., (1926). Beobachtungen und Versuche an lebenden Meeresbryozoen. *Zoologische Jahrbücher (Systematik)*, **52**: 1—102.

Maynard-Smith, J. & Slatkin, M., (1973). The stability of predator prey systems. *Ecology*, **54**: 384—391.

McBeth, J. W., (1968). Feeding behaviour of *Corambella steinbergae*. *Veliger*, **11**: 145—146.

McKinney, F. K. & Jackson, J. B. C., (1991). *Bryozoan Evolution.* The University of Chicago Press, Chicago. 238 pp.

Meadows, P. S. & Campbell, J. I., (1972). Habitat selection by aquatic invertebrates. *Advances in Marine Biology*, **10**: 271—361.

Menge, B. A. & Sutherland, J. P., (1976). Species diversity gradients: synthesis of the role of predation, competition and temporal heterogeneity. *American Naturalist*, **110**: 351—369.

Menon, N. R., (1972). Heat tolerance, growth and regeneration in three North Sea bryozoans exposed to different constant temperatures. *Marine Biology (Berlin)*, **15**: 1—11.

Mihm, J. W., Banta, W. C. & Loeb, G. I., (1981). Effects of adsorbed organic and primary fouling films on bryozoan settlement. *Journal of Experimental Marine Biology and Ecology*, **54**: 167—179.

Mileikovsky, S. A., (1974). On predation of pelagic larvae and early juveniles of marine bottom invertebrates and their passing through their predators. *Marine Biology (Berlin)*, **26**: 303—311.

Miller, S. A. & Hadfield, M. G., (1990). Developmental arrest during larval life and life-span extension in a marine mollusc. *Science* , **248**: 356—357.

Morse, A. N. C., (1992). Role of alga in the recruitment of marine invertebrate larvae. In: *Plant-Animal Interactions in the Marine Benthos*. D. M., John, S. J. Hawkins and J. H. Price, (eds.). Systematics Association Special Volume, No. 46. Clarendon Press, Oxford. pp. 385—403.

Morse, A. N. C. & Morse, D. E., (1984a). GABA-mimetic molecules from *Porphyra* (Rhodophyta) induce metamorphosis of *Haliotis* (Gastropoda) larvae. *Hydrobiologia*, **117**: 155 —158.

Morse, A. N. C. & Morse, D. E., (1984b). Recruitment and metamorphosis of *Haliotis* larvae induced by molecules uniquely available at the surface of crustose red algae. *Journal of Experimental Marine Biology and Ecology*, **75**: 191—215.

Morse, A. N. C., Froyd, C. A. & Morse, D. E., (1984). Molecules from cyanobacteria and red algae that induce settlement and metamorphosis in the mollusc *Haliotis rufescens*. *Marine Biology (Berlin)*, **81**: 293—298.

Morse, D. E., (1984). Biochemical and genetic engineering for improved production of abalones and other valuable molluscs. *Aquaculture*, **39**: 263—283.

Morse, D. E., (1985). Neurotransmitter-mimetic inducers of larval settlement and metamorphosis. *Bulletin of Marine Science*, **37**: 697—706.

Morse, D. E., (1988). Trigger and amplifier pathways: sensory receptors, transducers, and molecular mechanisms controlling larval settlement, adhesion, and metamorphosis in response to environmental chemical signals. In: *Marine Biodeterioration. Advanced Techniques Applicable to the Indian Ocean.* M. F. Thompson, R. Sarojini and R. Nagabhushanam (eds.). Oxford and IBH, New Delhi. pp. 453—462.

Morse, D. E., (1990). Recent papers in larval settlement and metamorphosis: closing the gaps between molecular biology and ecology. *Bulletin of Marine Science*, **46**: 465—483.

Morse, D. E., Tegner, M., Duncan, H., Hooker, N., Trevelyan, G. & Cameron, A., (1980). Induction of settling and metamorphosis of planktonic molluscan larvae III: signaling by metabolites of intact algae is dependent on contact. In: *Chemical Signalling in Vertebrate and Aquatic Animals.* D. Muller-Schwartze and R. M. Silverstein (eds.). Plenum Press, New York. pp. 67—86.

Moss, B., (1948). Studies in the genus *Fucus*. I. On the structure and chemical composition of *F. vesiculosus* from three Scottish habitats. *Annals of Botany (London)*, **12**: 267—279.

Müller, W. A., (1973). Metamorphose-induktion bei Planulalarven. I. Der bakterielle Induktor. *Wilhelm Roux's Archives of Developmental Biology*, **173**: 107—121.

Müller, W. A. & Buchal, G., (1973). Metamorphose-induktion bei Planulalarven. II. Induktion durch monovalente Kationen: Die Bedeutung des Gibbs-Donnan-Verhaltnisses und der Na^+ / K^+ -ATPase. *Wilhelm Roux's Archives of Developmental Biology* ,**174**: 122—135.

Muñoz, J., Cancino, J. H. & Molina, M. X., (1991). Effect of encrusting bryozoans on the physiology of their algal substratum. *Journal of The Marine Biological Association of the United Kingdom*, *71*: 877—882.

Neumann, R., (1979). Bacterial induction of settlement and metamorphosis in the planula larvae of *Cassiopaea andromeda* (Cnidaria: Scyphozoa, Rhizostomeae). *Marine Ecology Progress Series*, **1**: 21—28.

Nybakken, J. & McDonald, G., (1981). Feeding mechanisms of west American nudibranchs feeding on Bryozoa, Cnidaria and Ascidiacea, with special respect to the radula. *Malacologia*, **20**: 439—449.

O'Connor, R. J., Seed, R. & Boaden, P. J. S., (1979). Effects of environment and plant characteristics on the distribution of Bryozoa in a *Fucus serratus* L. community. *Journal of Experimental Marine Biology and Ecology*, **38**: 151—178.

O'Connor, R. J., Seed, R. & Boaden, P. J. S., (1980). Resource space partitioning by the Bryozoa of a *Fucus serratus* L. community. *Journal of Experimental Marine Biology and Ecology*, **45**: 117—137.

Okamura, B., (1987). Seasonal changes in zooid site and feeding activity in epifaunal colonies of *Electra pilosa*. . In: *Bryozoa: Present and Past*. J. R. P. Ross, (ed.). Western Washington University, Bellingham, Washington, USA. pp. 197—203.

Okamura, B. (1988). The influence of neighbours on the feeding of an epifaunal Bryozoa. *Journal of Experimental Marine Biology and Ecology*, **120**: 105—123.

Okamura, B., (1992). Microhabitat variation and patterns of colony growth and feeding in a marine bryozoan. *Ecology*, **73**: 1502—1513.

Osborne, S., (1984). Bryozoan interactions: observations on stolonal overgrowths. *Australian Journal of Marine and Freshwater Research*, **35**: 453—462.

Osman, R.V., (1977). The establishment and development of a marine epifaunal community. *Ecological Monographs*, **47**: 37—63.

Oswald, R. C., Telford, N., Seed, R. & Happey-Wood, C. M., (1984). The effect of encrusting bryozoans on the photosynthetic activity of *Fucus serratus* L. *Estuarine, Coastal and Shelf Science*, **19**: 697—702.

Paine, R.T ., (1974). Intertidal community structure: experimental studies on the relationship between a dominant competitor and its principle predator. *Oecologia (Berlin)*, **15**: 93—120.

Paine, R. T. & Levin, S. A., (1981). Intertidal landscapes: disturbance and the dynamics of pattern. *Ecological Monographs*, **51**: 145—178.

Palumbi, S. R. & Jackson, J. B. C., (1982). Ecology of cryptic coral reef communities. II. Recovery from small disturbance events by encrusting Bryozoa: the influence of "host" species and lesion size. *Journal of Experimental Marine Biology and Ecology*, **64**: 103—115.

Parke, M., (1948). Studies on the British Laminariaceae. I. Growth in *Laminaria saccharina* (L.) Lamour. *Journal of the Marine Biological Association of the United Kingdom*, **27**: 651—709.

Pawlik, J. R., (1990a). Chemical ecology of settlement of benthic marine invertebrates. In: *Marine Secondary Metabolites: Their Functions in Ecological Interactions*. V.J. Paul (ed.). Cornell Chemical Ecology Series.

Pawlik, J. R., (1990b). Natural and artificial induction of metamorphosis of *Phragmatopmoa lapidosa californica* (Polychaeta: Sabellariidae), with a critical look at the effects of bioactive compounds on marine invertebrates. *Bulletin of Marine Science*, **46**: 512—536.

Pechenik, J. A., (1990). Delayed metamorphosis by larvae of benthic marine invertebrates. Does it occur? Is there a price to pay? *Ophelia*, **32**: 63—94.

Pechenik, J. A. & Cerulli, T. R., (1991). Influence of delayed metamorphosis on survival, growth, and reproduction of the marine polychaete *Capitella* sp. I. *Journal of Experimental Marine Biology and Ecology*, **151**: 17—27.

Pechenik, J. A. & Eyster, L. S., (1989). Influence of delayed metamorphosis on the growth and metabolism of young *Crepidula fornicata* (Gastropoda) juveniles. *Biological Bulletin*, **176**: 14—24.

Pechenik, J. A. & Heyman, W.D. (1987). Using KCl to determine site at competence for larvae of the marine gastropod *Crepidula fornicata. Journal of Experimental Marine Biology and Ecology*, **112**: 27—38.

Pennington, J. T. & Hadfield, M.G., (1989). Larvae of a nudibranch mollusc (*Phestilla sibogae*) metamorphose when exposed to common organic solvents. *Biological Bulletin*, **177**: 350 - 355.

Perez, R., (1969). Étude biometrique d'une population de *Laminaria digitata* Lamouroux de l'etage infralittoral profond. *Revue des Travaux de l'Institut des Pêches Maritime*, **33**: 117—135.

Perez, R., (1971). Influence de quelques facteurs physiques sur le development de *Laminaria digitata* (L.) Lamour. *Bulletin de la Societe Phycologique de France*, **16**: 89—105.

Pires, A. & Hadfield, M. G., (1991). Oxidative breakdown of catecholamines and hydrogen peroxide induce partial metamorphosis in the nudibranch *Phestilla sibogae* Bergh (Gastropoda: Opisthobranchia). *Biological Bulletin*, **180**: 310—317.

Quinn, J. F., (1982). Competitive hierarchies in marine benthic communities. *Oecologia (Berlin)*, **54**: 129—135.

Richmond, R. H., (1985). Reversible metamorphosis in coral planula larvae. *Marine Ecology Progress Series*, **22**: 181—185.

Rittschoff, D., Maki, D., Mithchell, R. & Costlow, J. D., (1986). Ion and neuropharmacological studies of barnacle settlement. *Netherlands Journal of Sea Research*, **20**: 269—275.

Rubin, J. A., (1982). The degree of intransitivity and its measurement in an assemblage of encrusting cheilostome Bryozoa. *Journal of Experimental Marine Biology and Ecology*, **60**: 119—128.

Rubin, J. A., (1985). Mortality and avoidance of competitive overgrowth in encrusting Bryozoa. *Marine Ecology Progress Series*, **23**: 291—299.

Rubin, J. A., (1987). Growth and refuge location in continuous, modular organisms: experimental and computer simulation studies. *Oecologia (Berlin)*, **72**: 46—51.

Russ, G. R., (1982). Overgrowth in a marine epifaunal community: competitive hierarchies and competitive networks. *Oecologia (Berlin)*, **53**: 12—19.

Ryland, J. S., (1967). Polyzoa. *Oceanography and Marine Biology An Annual Review*, **5**: 343—369.

Ryland, J. S., (1970). *Bryozoans*. Hutchinson University Library, London. 173 pp.

Ryland, J. S., (1974). Behaviour, settlement and metamorphosis of bryozoan larvae: a review. *Thalassia Jugoslavica*, **10**: 239—262.

Ryland, J. S., (1976). Physiology and ecology of marine bryozoans. *Advances in Marine Biology*, **14**: 285—443.

Ryland, J.S., (1979). Structural and physiological aspects of coloniality in Bryozoa. In: *Biology and Systematics of Colonial Organisms.* G. P. Larwood and B. R. Rosen (eds.). Academic Press, London. pp. 211—242.

Ryland, J. S. & Hayward, P. J., (1977). *British Anascan Bryozoans. Synopses of the British Fauna. No. 10.* Academic Press, London. 188 pp.

Ryland, J. S. & Stebbing, A. R. D., (1971). Settlement and orientated growth in epiphytic and epizoic bryozoans. *Proceedings of the 4th. Marine Biology Symposium*. D. J. Crisp (ed.). Cambridge University Press. pp. 105—123.

Sandberg, P. A., (1977). Ultrastructure, mineralogy, and development of bryozoan skeletons. In: *Biology of Bryozoans.* R. M. Woollacott and R. L. Zimmer (eds.). Academic Press, New York. pp. 143—181.

Scheltema, R. S., (1961). Metamorphosis of the veliger larvae of *Nassarius obsoletus* (Gastropoda) in response to bottom sediment. *Biological Bulletin,* **120**: 92—109.

Scheltema, R. S., (1974). Biological interactions determining larval settlement of marine invertebrates. *Thalassia Jugoslavica*, **10**: 263—296.

Scheltema, R. S., (1986). On dispersal and planktonic larvae of benthic marine invertebrates: an eclectic overview and summary of problems. *Bulletin of Marine Science*, **39**: 290—322.

Schoener, T. W., (1983). Field experiments on interspecific competition. *American Naturalist*, **122**: 240—285.

Schonbeck, M. W. & Norton, T. A., (1978). Factors controlling the lower limits of fucoid algae on the shore. *Journal of Experimental Marine Biology and Ecology*, **31**: 303—313.

Schonbeck, M. W. & Norton, T. A., (1980). Factors controlling the lower limits of fucoid algae on the shore. *Journal of Experimental Marine Biology and Ecology*, **43**: 131—150.

Schultze, K. Janke, K., Krüß, A. & Weidemann, W., (1990). The macrofauna and macroflora associated with *Laminaria digitata* and *L. hyperborea* at the island of Helgoland (German Bight, North Sea). *Helgolander Meeresuntersuchungen*, **44**: 39—51.

Sebens, K. P., (1986). Spatial relationships among encrusting marine organisms in the New England subtidal zone. *Ecological Monographs*, **56**: 73—96.

Sebens, K. P., (1987). The ecology of indeterminate growth in animals. *Annual Review of Ecology and Systematics*, **18**: 371—407.

Seed, R., (1976). Observations on the ecology of *Membranipora* (Bryozoa) and a major predator *Doridella steinbergae* (Nudibranchiata) along the fronds of *Laminaria saccharina* at Friday Harbour, Washington. *Journal of Experimental Marine Biology and Ecology*, **24**: 1—17.

Seed, R., (1985). Ecological pattern in the epifaunal communities of coastal macroalgae. In: *The Ecology of Rocky Coasts*. P. G. Moore and R. Seed (eds.). pp. 22—35.

Seed, R. & Harris, S., (1980). The epifauna of the fronds of *Laminaria digitata* Lamour. in Strangford Lough, Northern Ireland. *Proceedings of the Royal Irish Academy. Section B. Biological, Geological and Chemical Science*, **80**: 91—106.

Seed, R. & O'Connor, R. J., (1981). Community organisation in marine algal epifaunas. *Annual Review of Ecology and Systematics*, **12**: 49—74.

Sieberth, J. McN. & Conover, J. T., (1966). Antifouling in *Sargassum natans* : Recognition of tannin activity. *Proceedings of the Fifth International Seaweed Symposium*, p. 207.

Silén, L., (1966). On the fertilization problem in gymnolaematous Bryozoa. *Ophelia*, **3**: 113—140.

Silén, L., (1972). Fertilization in the Bryozoa. *Ophelia*, **10**: 27—34.

Silén, L., (1987). Colony growth pattern in *Electra pilosa* (Linnaeus) and comparable encrusting cheilostome bryozoans. *Acta Zoologica*, **68**: 17—34.

Slattery, M., (1987). *Settlement and metamorphosis of red abalone (Haliotis rufescens) larvae: a critical examination of mucous, diatoms, and g-aminobutyric acid (GABA) as inductive substrates*. M. A. Thesis, San Jose State University, California.

Sloane, J. F., Ebling, F. J., Kitching, J. A. & Lilly, S. J., (1957). The ecology of the Lough Ine rapids with special reference to water currents. V. The sedentary fauna of the Laminarian algae in the Lough Ine area. *Journal of Animal Ecology*, **26**: 197—211.

Slobodkin, L. B., (1961). *Growth and Regulation of Animal Populations*. Holt, Rinehart and Winston, New York. 184 pp.

Slobodkin, L. B., (1968). How to be a predator. *American Zoologist*, **8**: 43—51.

Slobodkin, L. B., (1974). Prudent predation does not require group selection. *American Naturalist*, **108**: 665—678.

Sokal, R. R. & Rohlf, F. J., (1981). *Biometry. The Principles and Practise of Statistics in Biological Research.* Second edition. W. H. Freeman and Company, New York. 859 pp.

Sousa, W. P., (1979). Experimental investigations of disturbance and ecological successeion in a rocky intertidal algal community. *Ecological Monographs*, **49**: 227—254.

Sousa, W. P., (1984). The role of disturbance in natural communities. *Annual Review of Ecology and Systematics*, **15**: 353—391.

Spindler, K-D. & Müller, W. A., (1972). Induction of metamorphosis by bacteria and by a lithium-pulse in the larvae of *Hydractinia echinata* (Hydrozoa). *Wilhelm Roux's Archives of Developmental Biology*, **169**: 271—280.

Stebbing, A. R. D., (1971). Growth of *Flustra foliacea* (Bryozoa). *Marine Biology (Berlin)*, **9**: 267—273.

Stebbing, A. R. D., (1972). Preferential settlement of a bryozoan and serpulid larvae on the younger parts of *Laminaria* fronds. Journal of the Marine Biological Association of the United Kingdom, **52**: 765—772.

Stebbing, A. R. D., (1973a). Observations on colony overgrowth and spatial competition. In: *Living and Fossil Bryozoa.* G.P. Larwood (ed.). Academic Press, London. pp. 171—183.

Stebbing, A. R. D., (1973b). Competition between the epiphytes of *Fucus serratus* L. *Journal of the Marine Biological Association of the United Kingdom*, **53**: 247—261.

Stricker, S. A., (1988). *Membranipora membranacea* settlement and metamorphosis can be induced by using pieces of algae or artificial seawater (19 mM K^+ ASW). *American Zoologist*, **28**: 71A.

Sundene, O., (1964). The ecology of *Laminaria digitata* in Norway in view of transplant experiments. *Nytt magasin foer Botanikk*, 11: **83**—107.

Tavener-Smith, R. & Williams, A., (1972). The secretion and structure of the skeleton of living and fossil Bryozoa. *Philosophical Transactions of the Royal Society of London. Series B. Biological Sciences*, **264**: 97— 159.

Thompson, T. E., (1958). The natural history, embryology, larval biology and post-larval development of *Adalaria proxima* (Alder and Hancock). *Philosophical Transactions of the Royal Society of London. Series B. Biological Sciences*, **242**: 1—58.

Thompson, T. E., (1959). Feeding in nudibranch larvae. *Journal of the Marine Biological Association of the United Kingdom*, **38**: 239—248.

Thompson, T. E., (1976). *Sea-slugs*. In: *Living Marine Molluscs*. C. M. Yonge and T. E. Thompson (eds.). William Collins Sons and Co. Ltd., London. pp. 121—141.

Thompson, T. E. & Brown, G. H., (1976). *British opisthobranch molluscs. Synopses of the British fauna. No. 8*. Academic Press, London. 204 pp.

Thorpe, J. P., (1979). A model using deterministic equations to describe some possible parameters affecting growth rate and fecundity in Bryozoa. In: *Advances in Bryozoology.* Systematics Association Special Volume No. 13. G. P. Larwood and M. B. Abbott (eds.). Academic Press, London. pp. 113—120.

Todd, C. D., (1979a). Reproductive energetics of two species of dorid nudibranchs with planktotrophic and lecithotrophic larval strategies. *Marine Biology (Berlin),* **53**: 57—68.

Todd, C. D., (1979b). The population ecology of *Onchidoris bilamellata* (L.). (Gastropoda: Nudibranchia). *Journal of Experimental Marine Biology and Ecology,* **41**: 213—255.

Todd, C. D., (1981). The ecology of nudibranch molluscs. *Oceanography and Marine Biology. An Annual Review,* **19**: 141—234.

Todd, C. D., (1983). Reproductive and trophic ecology of nudibranch molluscs. In: *The Mollusca. Vol. VI: Ecology.* W.D. Russell-Hunter (ed.). Academic Press, New York. pp. 225—229.

Todd, C. D., (1987). Reproductive energetics and larval strategies of nudibranch molluscs: the effect of ration level during the spawning period in *Onchidoris muricata* (Müller) and *Adalaria proxima* (Alder and Hancock). *American Malacological Union Bulletin,* **5**: 293—301.

Todd, C. D., (1990). Larval strategies of nudibranch molluscs: similar means to the same end? In: *Ninth International Malacological Congress (Edinburgh, 1986). Symposium Volume I. Evolutionary biology of opisthobranchs.* M. Edwards (ed.). pp. 91—110.

Todd, C. D., Bentley, M. G. & Havenhand, J. N., (1991). Larval metamorphosis of the opisthobranch mollusc *Adalaria proxima* (Gastropoda: Nudibranchia): the effects of choline and elevated potassium ion concentration. *Journal of the Marine Biological Association of the United Kingdom*, **71**: 53—72.

Todd, C. D. & Doyle, R. I. W., (1981). Reproductive strategies of marine benthic invertebrates: a settlement-timing hypothesis. *Marine Ecology Progress Series*, **4**: 75—83.

Todd, C. D. & Havenhand, J. N., (1985). Preliminary observations on the embryonic and larval development of three dorid nudibranchs. *Journal of Molluscan Studies*, 51: 97—99.

Todd, C. D. & Havenhand, J. N., (1988). Physiological ecology of *Adalaria proxima* (Alder et Hancock) and *Onchidoris muricata* (Müller) (Gastropoda: Nudibranchia). III. Energy budgets. *Journal of Experimental Marine Biology and Ecology*, **118**: 191—205.

Todd, C. D. & Havenhand, J. N., (1989). Nudibranch-bryozoan associations: the quantification of ingestion and some observations of partial predation among Doridoidea. *Journal of Molluscan Studies*, **55**: 245—259.

Todd, C. D., Havenhand, J. N. & Thorpe, J. P., (1989). Genetic differentiation, pelagic larval transport and gene flow between local populations of the intertidal mollusc *Adalaria proxima* (Alder and Hancock). *Functional Ecology*, **2**: 441—451.

Todd, C. D. & Lewis, J. R., (1984). Effects of low air temperature on *Laminaria digitata* in south-western Scotland. *Marine Ecology Progress Series* , **16**: 199—201.

Todd, C. D. & Turner, S. J., (1986). Ecology of intertidal and sublittoral cryptic epifaunal assemblages. I. Experiment rationale and the analysis of larval settlement. *Journal of Experimental Marine Biology and Ecology,* **99**: 199—231.

Todd, C. D. & Turner, S. J., (1988). Ecology of intertidal and sublittoral cryptic epifaunal assemblages. II. Non-lethal overgrowth of encrusting bryozoans by colonial ascidians. *Journal of Experimental Marine Biology and Ecology,* **115**: 113—126.

Trapido-Rosenthal, H. G. & Morse, D. E., (1985). L-**a**, **w**- Diamino acids facilitate GABA induction of larval metamorphosis in a gastropod mollusc (*Haliotis rufescens*). *Journal of Comparative Physiology. B. Metabolic and Transport Functions*, **155**: 403—414.

Trapido-Rosenthal, H. G. & Morse, D. E., (1986a). Regulation of receptor-mediated settlement and metamorphosis in larvae of a gastropod mollusc (*Haliotis rufescens*). *Bulletin of Marine Science* **39**: 383— 392

Trapido-Rosenthal, H. G. & Morse, D. E., (1986b). Availability of chemosensory receptors is down-regulated by habituation in larvae of a gastropod mollusc (*Haliotis rufescens*). *Proceedings of the National Academy of Sciences of the United States of America*, **83**: 7658—7662.

Troxler, R. F. & Lester, R., (1967). Biosynthesis of phycocyanobilin. *Biochemistry,* **6**: 3840—3846.

Turner, S. J., (1988). *The ecology of hard-substratum epifaunal assemblages: effects of larval recruitment, competition and grazing.* Ph. D. thesis. University of St. Andrews.

Wahle, C., (1983). Regeneration of injuries among Jamaican gorgonians: the roles of colony physiology and environment. *Biological Bulletin,* **165**: 778—790.

Wahl, M., (1989). Marine epibiosis. I. Fouling and anti-fouling: some basic aspects. *Marine Ecology Progress Series,* **58**: 175—189.

Walters, L. J. & Wethey, D. S., (1986). Surface topography influence competitive hierarchies on marine hard substrata: a field experiment. *Biological Bulletin,* **170**: 441—449.

Walters, L. J. & Wethey, D. S., (1991). Settlement, refuges and adult body form in colonial marine invertebrates: a field experiment. *Biological Bulletin,* **180**: 112—118.

Wass, R. E. & Vail, L. L., (1978). Encrusting Bryozoa exhibit linear growth. *Search,* **9**: 42—44.

Weiner, R. M., Segall, R. R. & Colwell, R. R., (1985). Characterization of a marine bacterium associated with *Crassostrea virginica* (the eastern oyster). *Applied and Environmental Microbiology*, **Jan. '85**: 83—90.

Williams, G. A. & Seed, R., (1992). Interactions between macrofaunal epiphytes and their host algae. In: *Plant-animal interactions in the marine benthos.* D. M. John, S. J. Hawkins and J. H. Price (eds.). Systematics Association Special Volume, No. 46. Clarendon Press, Oxford. pp. 189—211.

Wing, B. L. & Clendinning, K. A., (1971). Kelp surfaces and associated invertebrates. *Beihefte zür Nova Hedwigia*, **32**: 319—341.

Winston, J. E., (1976). Experimental culture of the estuarine ectoproct *Conopeum tenuissium* from Chesapeake Bay. *Biological Bulletin,* **150**: 318—335.

Winston, J. E., (1978). Polypide morphology and feeding behaviour in marine ectoprocts. *Bulletin of Marine Science*, **28**: 1—31.

Wisely, B., (1958). The settling and some experimental reactions of a Bryozoan larva, *Watersipora cucullata* (Busk). *Australian Journal of Marine and Freshwater Research*, **9**: 362—371.

Wood, V. & Seed, R., (1980). The effects of shore level on the epifaunal communities associated with *Fucus serratus* (L.) in the Menai Strait, North Wales. *Cahiers de Biologie Marine*, **21**: 135—154.

Wood, V., (1983). *A population study of the major sedentary faunal associates of Fucus serratus L.* Ph. D. thesis. University College North Wales, Bangor.

Woollacott, R. M. & North, W. J., (1971). Bryozoans of California and North Mexico kelp beds. *Beihefte zür Nova Hedwigia*, **32**: 455—479.

Woollacott, R. M., Pechenik, J, A. & Imbalzano, K. M., (1989). Effects of the duration of larval swimming period on early colony development in *Bugula stolonifera* (Bryozoa: Cheilostomata). *Marine Biology (Berlin)*, **102**: 57—63.

Wynne-Edwards, V.C., (1962). *Animals Dispersion in Relation to Social Behaviour*. Oliver and Boyd, Edinburgh. 653 pp.

Wynne-Edwards, V.C., (1986). *Evolution Through Group Selection*. Blackwell Scientific Publications, Oxford.

Yeaton, R. W., (1981). Invertebrate lectins II. Diversity of specificity, biological synthesis and function in recognition. *Developmental and Comparative Immunology*, **5**: 535—545.

Yodzis, P., (1986). Competition, mortality, and community structure. In: *Community Ecology*. J. Diamond and T. J. Case (eds.). Case, Harper and Row Publishers, New York. pp. 480—491.

Yool, A. J., Grau, S. M., Hadfield M. G., Jensen, R. A., Markell, D. A. & Morse, D. E., (1986). Excess potassium induces larval metamorphosis in four marine invertebrate species. *Biological Bulletin,* **170**: 255—266.

Yoshioka, P. M., (1982). Predator-induced polymorphism in the bryozoan *Membranipora membranacea* (L.). *Journal of Experimental Marine Biology and Ecology,* **61**: 233—242.