The potential for coevolution between the brown alga *Ascophyllum nodosum* (Linn.) LeJolis, the gastropod *Littorina obtusata* and the epiphyte *Polysiphonia lanosa* (Linn.) Tandy

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Abstract

Ascophyllum nodosum is a common brown seaweed on sheltered, North temperate shores. *A. nodosum* is associated with the epiphyte *Polysiphonia lanosa*, which lives almost exclusively on the seaweed . In addition, the snail *Littorina obtusata* is commonly found on the *A. nodosum* and prefers it as a food plant to other fucoids. *A. nodosum* is a dioecious species with a very high reproductive effort and contains high concentrations of secondary metabolites (phlorotannins). These are thought to serve as anti-herbivore defence but are ineffective against *L. obtusata.*

As resources such as nutrients are usually thought to be limited, allocation of resources to one life history function such as reproduction is predicted to result in trade-off with allocation of resources to vegetative growth or anti-herbivore defences. On the basis of the secondary metabolites in *Ascophyllum nodosum* and the preference of the grazer for *A. nodosum* several authors have speculated about the potential for coevolution between *A. nodosum* and *L. obtusata.* To have any potential for coevolution trade-offs between different life-history functions have to be demonstrated.

The aim of this study was to investigate the costs and benefits for the brown seaweed *A. nodosum* arising from its association with *L. obtusata* and *P. lanosa* and to examine possible trade-offs between life history traits in *A. nodosum.*

During the study it was observed that reproduction and growth could proceed simoultaneously, indicating a lack of a trade-off between vegetative growth and reproduction. Using individual year classes, no obvious trade-offs between reproductive effort and vegetative growth on one hand and phlorotannin production and vegetative/ reproductive growth on the other hand were apparent. In fact in female plants positive relationships rather than trade-offs were often found between phlorotannin concentrations in *A. nodosum* and reproductive output. But both grazer and epiphyte affected tissue loss in *A. nodosum* indirectly. *P. lanosa* greatly increased drag and thereby the risk of frond breakage in *A. nodosum,* leading to a considerable loss of both reproductive and vegetative tissue Grazing by *Littorina obtusata* can have a similar affect by weakening branches.

However, this study has demonstrated a capacity of *A. nodosum* for clonal regrowth of axes from the perennating holdfast and has shown that growth of new shoots is greatly affected by light conditions. On sheltered shores *Ascophyllum nodosum* is very abundant and self-shading by older axes prevents any substantial growth of new axes. Removal of plant biomass as the result of grazing and epiphytism is therefore an essential part of the population ecology of *Ascophyllum nodosum* as it reduces self-shading and allows replacement of older axes that are often severely damaged and epiphytised. Such an indirect beneficial effect of grazers and epiphytes on their host plant has not previously been demonstrated. Reduced costs of reproduction should reduce the potential for reciprocal evolutionary interactions between host and grazer/ epiphyte. However, the clonal growth pattern of *A. nodosum* might mean that the success of sexual reproduction alone could be an unsuitable measure of the ‘fitness’ of the organism.

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General Introduction

All life histories are a combination of different traits, e.g. size at birth, growth rates, age and/ or size at maturity and longevity. In terrestrial systems, for which the main concepts of life history theory were first developed (Begon and Mortimer 1986) a large number of different life histories exist.

Various authors have attempted to categorize different life history types and associate them with different habitat types. One of the broadest distinctions is made between semelparous and iteroparous species. Semelparous species produce all of their offspring in a single reproductive event and within a relatively short period of time. Iteroparous species on the other hand produce offspring in several separate events, maintaining themselves in a condition that favours survival to reproduce again (Bell 1980). Iteroparity will therefore be favoured in conditions where the chances of reproductive success are low, while semelparity should be selected for in the opposite situation where the chances of successful reproduction at a given time are high (DeWreede and Klinger 1988).

One of the earliest comprehensive classification systems for the evolution of life-history types is the system of r- and K- selection of MacArthur and Pianka (1966) who attempted to link life history traits to certain types of environment. According to these authors, k-selected organisms are usually associated with a relatively stable environment and predictable seasons. K-selected species should be common in environments in which density dependent mortality is high (Gadgil and Solbrig 1972). In such an environment the population should be at or near its carrying capacity and intraspecific competition would be intense (Parry 1981). Grime (1977) in a similar system distinguished three life-history types according to how hazardous the environment is. He made a distinction between disturbance (destruction of plant biomass by grazing for example) and stress (conditions that restrict reproduction), and distinguished competitive, stress-tolerant and ruderal plants, with ruderals being defined as plants adapted to withstand severe disturbances such as drought or in marine environments, wave action. Both theories make similar predictions about the most advantageous life-history types in different environments.

According to their schemes, stable environments favour a delayed onset of reproduction, but several reproductive events and a decreased reproductive effort, i.e. more investment into vegetative growth (rather than reproductive growth) early on in life. This enables the plant to become and remain established. Rather than many small offspring, a smaller number of large offspring will then be selected for. Larger offspring might be able to persist longer, survive initially unfavourable conditions and outgrow smaller competitors. On the other hand, in more unpredictable environments with high, density independent mortality, small adult size, early onset of reproduction, and the production of large numbers of small offspring, and possibly semelparity will be favoured (Pianka 1970). Unpredictable conditions are thought to be associated with early successional habitats e.g. shortly after the formation of gaps in a canopy (Sousa 1985). It has to be emphasized that these concepts can only meaningfully be applied in the comparison of different species, for example an organism can be judged to be r or k selected only in comparison with individuals from another population or species.

There is some evidence of species conforming relatively well to these schemes. In a general sense it was found by Harper *et al.* (1970) that progressively less resources are allocated to reproduction from annual herbs to perennial herbs to trees. More specifically, Abrahamson and Gadgil (1973) investigated changes in life history traits in several species of goldenrod (*Solidago*) growing in different types of habitats. They found that in two of the species biomass allocation to reproductive tissue was higher in early successional communities, than for example in woodlands, demonstrating that even in the same species a range of positions can be occupied along the r-k continuum. Similar results were obtained by Gadgil and Solbrig (1972) with respect to seed formation in the dandelion *Taraxacum*.

Although these concepts were developed for terrestrial plants, a large number of different life history types also exist among algae and some authors have attempted to apply classifications such as the r/ K dichotomy or Grime's classification to marine plants, including seaweeds (Clayton 1990). The life history of individuals in a given species is favoured by natural selection if it increases their survival chances in a given type of environment. In the case of seaweeds one might distinguish, on a large scale, between intertidal and subtidal habitats or between intertidal habitats of different exposure to waves. In the intertidal zone seaweeds are exposed to air as well as water. They have to withstand drag forces when submerged and desiccation, wind and freshwater inputs when emerged (Dring 1982, d'Antonio 1985). Physical disturbances generally become more severe with increasing exposure (Sousa 1985). If the r/ K dichotomy holds, one would expect more r selected species with increasing exposure and higher up on the shore. According to the theory, these species would also show a tendency to be semelparous rather than iteroparous. This seems to hold for some species. The iteroparous fucoid species given in Table 1 all decrease in abundance with increasing exposure (Lewis 1964), and in those plants surviving on exposed shores reproductive efforts have been found to increase from sheltered sites to sites of intermediate exposure, e.g. *Ascophyllum nodosum* (L.) Le Jolis (Cousens 1982) which is consistent with the findings of Abrahamson and Gadgil (1973) for terrestrial plants. The only fucoid seaweed, which decreases in abundance with decreasing exposure, is the semelparous species *Himanthalia elongata* (L.) S Gray (Brenchley *et al.* 1995).

Most fucoids common on sheltered shores, for example *Ascophyllum nodosum*, and *Fucus serratus* (L.), show characteristics of iteroparous species (Table 1). Many kelps and fucoid seaweeds, show a delayed onset of reproduction and are relatively long-lived compared with other seaweeds, showing traits associated with k-selected species. These species are often forming dense stands in well established communities and might therefore be regarded as late successional species. *Laminaria setchellii* Silva can apparently live for up to sixteen years (Klinger and DeWreede 1988). Most fucoid seaweeds also live for several years. *Fucus* spp have a maximum life span of 4 years and *Pelvetia canaliculata* (L.) can survive for up to 5 years. By far the most long-lived species among fucoids is *Ascophyllum nodosum*, which can probably live in excess of 50 years (Baardseth 1970). However, while none of these species start reproducing in their first year of life and are relatively long lived, they also often have a very high reproductive effort and produce large numbers of offspring, which is inconsistent with the r/k concept (Table 1). Between the species shown in Table 1 there is no clear trend in the relationship between fecundity and longevity, although the semelparous species have very high reproductive effort as predicted (Brenchley *et al.* 1995). Insufficient information was available to relate fecundity or longevity to the onset of reproduction.

Table 1: Reproductive characteristics of some mebers of the Fucales and Laminariales

Species	Reproductive period	Habitat	Longevity	Onset of reproduction (years)	Reproductive effort
Cystoseira osmundacea	Seasonal	intertidal	Annual loss of axes (perrenating holdfast remains)	2?	Increases with age (80-90%)[1]
Fucus vesiculosus	Peak: May	Intertidal	4	2?	62±15% (UK)[2] 11-22 (Sweden)[3]
Fucus serratus	Seasonal (fertile in winter), iteroparous	Intertidal	4	2?	Year 1 38%[4] Year 2 50.5%
Himanthalia elongata	Semelparous	Intertidal	2	2	>98%[4]
Ascophyllum nodosum	March-May	Intertidal	20+ years	Several years	33-74% depending on age[5]
Sargassum muticum (Spain)	May-September	Intertidal	Annual loss of axes perennial hodfast	2?	max. 20%[6]
Pelvetia canliculata	Seasonal, iteroparous, hermaphrodite	intertidal	5	2?	
Kelps					
Pterygophora californica	Seasonal	subtidal	App 8-13 years	Year 2	[7] 44% (sporophylls)
Macrocystis pyrifera	Continuous (but two peaks)	Subtidal	< one year		
Phyllariopsis purpurascens	September	subtidal	Annual		13%±4-27±6%[8]
Laminaria longicruris	Continuous (peak in autumn)	Subtidal	2		1-37%[9]

[1]Schiel (1985), [2]Mathieson and Guo (1992), [3]Serrao *et al.* (1999), [4]Brenchley *et al* (1996), [5]Åberg (1996) [6]Arenas and Fernández (1998), [7]Reed *et al.* (1996), [8]Flores-Moya (1997), [9]Patten and Yarish, 1993

Of the species that can be classed as iteroparous, one of the longest lived species, *Ascophyllum nodosum*, also has one of the highest reproductive efforts (Åberg 1996). It is noteworthy in this context that for some of the iteroparous species listed, e.g. *Ascophyllum nodosum* and *Sargassum muticum* (Yendo) Fensholt, the life span given does not imply mortality of the whole plant, but of plant parts (fronds, blades) attached to a perrenating holdfast. Therefore, while losses of vegetative and reproductive biomass from these holdfasts can be severe, the plant remains attached and the holdfast forms a basis for future growth and reproduction (McCourt 1985, Schiel 1985). This should be taken into account when examining the life strategies of plants with respect to optimal reproductive outputs and the relationships between reproductive effort, longevity and onset of reproduction and vegetative growth (Rocheleau and Houle 2001).

Whether plants are marine or terrestrial, the capacity for asexual reproduction and clonal growth should also be taken into account when studying their life-histories. The latter might be regarded as a ‘cheaper alternative’ to sexual reproduction and could be particularly favourable where offspring mortality is very high (Bazzaz *et al.* 1987). It is well known, for instance, that plants towards the geographic limits of their distribution reduce their reproductive output (Patten and Yarish 1993) or shift from sexual reproduction to asexual reproduction or vegetative spread (Lee 1980, Jacobsen *et al.* 1991, Lobban and Harrison 1994). Pre-empting space in this way, increases the chances of remaining established, even if sexual reproduction does not occur. One might therefore expect asexual reproduction and clonal growth to become increasingly important as environmental conditions become more stressful, as for example towards the geographic limit of the distribution of a species. The overall fitness of an organism might then be the result of a balance of processes: sexual and asexual reproduction or clonal growth (Bellingham and Sparrow 2000). However, while the possible evolutionary consequences and the adaptive significance of different life histories has been widely debated for terrestrial plants, the value of such strategies for seaweeds have received less attention (Clayton 1990, Clayton and Ashburner 1994). What all these strategies have in common is the ability to maximize the fitness of an organism, i.e. the proportion of surviving offspring this individual contributes to the next generation, compared with other individuals in the population.

Costs of reproduction

Whatever the apparent strategy, the resources available for an organism to fulfill all the life history functions such as growth, reproduction and defence, are usually finite. Allocation of resources cannot be made equally to all functions the organism must perform and costs are therefore incurred (Lovett Doust and Lovett Doust 1988).

The idea that resources are allocated strategically to conflicting traits was first developed by Cody (1966), but see also Lawlor and Maynard-Smith (1976), Bell (1980) and Tuomi *et al.* (1983). This approach to the study of life-history traits, which tries to find the combination of resource allocation that optimizes fitness, has been termed optimality analysis (Roff 1992). If resource limitation occurs, then one would predict trade-offs between functions, i.e. one aspect of the life history causing an increase in reproductive value would be offset by an associated change in a trait causing a decrease in reproductive value. The optimal strategy, achieving the highest fitness, will be the one that finds the best compromise between two or more costly traits, rather than a strategy, which maximizes reproductive output *per se* (Stearns 1977, 1992). If, for example, reproduction and vegetative growth require the same resource at the same time and that resource is limiting, then an increase in vegetative growth would have to be achieved at the expense of reproduction.

Trade-offs as the result of reproductive costs can be manifested in a number of ways . Stearns (1992) identified 45 different trade-offs between traits related to reproduction and growth. Probably the best-documented trade-off is that between reproduction and vegetative growth, although it was mainly shown in animals rather than plant species (Warner 1984). However, some examples for trade-offs in plants do exist (Harper 1977). Eis *et al.* (1965) for instance found that in fir trees vegetative growth was inversely related to cone production. El-Kassaby and Barclay (1992) described a similar result between vegetative growth and cone production. Henriksson and Ruohomaeki (2000) made similar observations about trade-offs between vegetative growth and reproduction for Mountain birch (*Betula pubescens czerepanovii*). It has also been argued that vegetative spread might cause a trade-off with sexual reproduction (Abrahamson 1975, Bazzaz *et al.* 1987).

In algae, trade-offs between vegetative and reproductive growth have been examined occasionally. In some species relationships were indeed found, which suggested a trade-off. In *Dumontia incrassata* (Lamouroux) the female plants start producing reproductive branches only after vegetative growth has stopped (Dunn 1917). Similarly in *Cystoseira osmundacea* (Tunr.) C. Ag. vegetative and reproductive growth are clearly separated. While the greatest elongation of the vegetative frond occurs between March and June, fronds are reproductive between June and August and in September the fronds are shed (Schiel 1985) i.e. in general it does not seem to be possible to maintain both vegetative and reproductive growth at the same time. Other trade-offs were also reported for marine algae e.g. between reproduction and survival. In the green alga *Halimeda* for example, sexual reproduction is followed by the death of the plant (Hillis-Colinveaux 1980).

However there are also many examples of algal species, that do not seem to show any trade-offs. In the red algae *Gigartina stellata* (Stackh.) Batters and *Codium fragile* (Suringar) Hariot , maximum growth and maximum reproduction occur simultaneously (Burns and Mathieson 1972, Fralick and Mathieson 1973). In *Iridaea cordata* (Turner) blade growth continues throughout the reproductive period (Hansen and Doyle 1976). Pfister (1992) failed to find any conclusive evidence for a reproductive cost in the kelp *Alaria nana* Shrader while Ang (1992) found no evidence of cost of reproduction in terms of longevity or mortality risk in *Fucus distichus* (L.). A study by Reed (1987) also found that removal of vegetative tissue from the giant kelp *Macrocystis pyrifera* (L.) C. Ag. did not lead to an increased production of reproductive tissue, which is inconsistent with a trade-off between reproduction and growth (Harper 1977).

Some authors have in fact argued that, because the reproductive structures of seaweed are usually at least in part photosynthetic, the cost of reproduction should be much reduced or even absent in seaweeds and indeed some terrestrial plants (Kain 1963, Giesel 1976, McLachlan and Bidwell 1978, Bazzaz and Carlson 1979, Reed *et al.* 1988). This might be true for species with rather undifferentiated reproductive parts such as some kelps and ectocarpoids. However, it was argued by Schiel (1985), that reproductive cost estimates only including the cost of the production of the gametes, for example those by Vernet and Harper (1980) are likely to be gross underestimates. In species that devote large proportions of their biomass to the formation of receptacles and other reproductive fronds (Mathieson *et al.* 1976, Bäck *et al.* 1991), which are often seasonal and are shed after the reproductive period, reproductive costs could be increased.

Sex specific costs

So far costs have only been discussed for individuals irrespective of their sex. But one might expect there to be different trade-offs between males and females of a particular species due to the higher cost of producing female than male gametes. It is generally accepted that the advantage of sexual over asexual reproduction is that it allows a genetic exchange and recombination to take place, which maintains a high genetic diversity and therefore allows faster adaptation of an organism to changing environmental conditions (Maynard-Smith 1971, Taylor 1979). However, for an individual with an already high fitness, there will be a cost associated with sexual reproduction, as only 50 % of this individual's genes will be passed on to its offspring. Asexual reproduction on the other hand will lead to a small but stable gene pool. Some authors have therefore argued that sexual reproduction should be selected for in populations in heterogeneous and variable environments (Giesel 1976), while a tendency towards asexual reproduction might be expected in populations living in very predictable environments (Clayton 1982). In most seaweeds, particularly intertidal species, variability is the prominent feature of their environment and therefore sexual reproduction should be favoured.

It is commonly found that in dioecious plants females allocate more resources to reproduction than do males (Wallace and Rundel 1979, Bazzaz *et al.* 1987). Therefore, one might expect greater trade-offs between reproduction and other life history functions in females than in males (Lloyd and Webb 1977). For example, females have been found to have a greater age at reproduction than males in the lily *Chamaelium luteum* (Meagher and Antonovics 1982) and show periods of reduced growth following reproduction (Popp and Reinartz 1988, Maze and Whalley 1990, Rocheleau and Goule 2001). However, there are instances where costs seem to be absent. Delph (1995) also did not find any trade-offs between growth and reproduction between males and females of the flowering plant *Silene latifolia*, although such a trade-off was found within males and females investigated separately. They concluded that trade-offs did probably exist but were masked by the fact that in the plants they investigated carbon acquisition patterns varied between males and females (see below).

Problems relating to the measurement of reproductive costs

While theoretically the concept of trade-offs between life-history traits is simple and allows the formulation of clear hypotheses, in practice there are a number of difficulties with the measurement of reproductive costs, and several factors, which if not taken into account, can confound the outcome (Partridge 1990).

In general terms it is possible that physiological functions are not known adequately and therefore erroneous trade-offs between life-history traits are identified. Furthermore, identifying the resource that is actually limiting the traits for which possible trade-offs are investigated is likely to be a problem as might be finding the appropriate way of measuring it. If the two traits are limited by different resources e.g. energy vs. mineral nutrients, trade-off (Abrahamson and Caswell 1982). It is also quite possible that two traits vary in their sensitivity to resource limitation. This might lead to trade-offs only being identified during times of nutrient shortages (Stearns 1992, Reznick *et al.* 2000).

Potential problems in the detection of trade-offs could also be caused by differences in the acquisition of resources which might obscure allocation patterns (van Noordwijk and de Jong 1986). One might for instance observe the same reproductive effort in two individuals of a species, but if one of them were more efficient at acquiring energy, then it would have allocated proportionally less to reproduction than the less efficient one. Related to this, differences in condition of the two organisms can lead to positive rather than negative relationships because they might respond differently to crowding (Begon *et al.* 1986). All the factors discussed above can confound any studies of reproductive costs. If not taken into account, the absence of trade-offs might be falsely identified as the absence of costs.

Reciprocal evolutionary relationships: host herbivore interactions

The importance of the environment for the evolution of life history strategies has been stated in the preceding sections. However, so far only abiotic factors, affecting the predictability or stability of habitats have been explored, but organisms also encounter and interact with large numbers of other plant and animals. Such interactions might occur through intra and interspecific competition, mutualism or predation. Just as environmental conditions might affect the adaptive value of a life history trait, so can the risk of being eaten. The grazer has the potential to impose a cost by reducing both reproductive and vegetative production of its host plant. In the case of epiphytes, this might occur by reducing light and nutrient availability or increasing drag. A large body of literature exists on the relationships between host plants and grazers (e.g. Lawton and McNeill 1979, Lubchenco and Gaines 1981, Faeth 1988, Futuyma and Keese 1992; Hougen-Eitzman and Rausher 1994). Again, the effects of life histories on the type of interactions likely to evolve have mainly been considered for terrestrial systems.

When faced with an enemy, the host plant has two options: it can either tolerate them or defend against them. The latter will reduce the adverse effects of grazers or epiphytes, but might incur some extra costs for the production of the defences be they structural or chemical. Such defences might be an additional factor with which reproduction has to compete so there might be trade-offs between reproduction and the ability to produce defences (Feeny 1976, Bazzaz *et al.* 1987, Hay and Fenical 1988, Pavia 1999). Again, resources are expected to be allocated to defences in a way that maximizes the organism's fitness.

Terrestrial and aquatic plants produce an array of chemicals (secondary metabolites), which are known to deter grazers. Although several theories have been advanced to explain the distribution patterns of secondary metabolites in host plants, relatively few studies have investigated trade-offs between production of these chemicals and other essential processes such as reproduction. Coley *et al.* (1985) found that there was an inverse relationship between the growth rates of the plants he investigated and the amount of secondary metabolites, in this case polyphenols. Such effects have also been found in marine plants (Pavia *et al.* 1999).

Most of the theories involving plant defences have concentrated on the relationship between secondary metabolite levels and the ambient nutrient levels in which the plants grow. High nutrient levels apparently selected for fast growth rates and low secondary metabolite levels, whereas low nutrient conditions gave slow growth and high defence levels (Appleton and vanStaden 1989, Glyphis and Puttick 1989). However, other authors have argued that the production of carbon based secondary metabolites such as polyphenols is merely the result of nutrient limitation, i.e. they build up at times when conditions for growth are unfavourable (Briggs and Schultz 1990, Denslow *et al.* 1990). Since possible trade-offs would be dependent on local nutrient conditions, the absence of a trade-off cannot be inferred unless rates of nutrient acquisition are known (Herms and Mattson 1992). In addition, it has to be taken into account that secondary metabolites might serve other functions than just defence such as protection against UV-light (Les and Sheridan 1990) and structural support (Haslam 1988).

If defensive chemicals are indeed costly to produce, several predictions can be made about their allocation patterns to plant tissues (combined in what has become known as optimal defence theory). Firstly, optimal allocation to defence should be a balance between the risk of attack and the cost of producing the defensive chemical (Rhoades 1979). This first point was explored by Feeny (1976) and Rhoades and Cates (1976) who stated that the type and quantity of defence allocation should depend on the ease with which they are detected by herbivores. Secondly, one might expect allocation of defences to the tissues most at risk of herbivore attack and thirdly, defences should be decreased when herbivores are absent and increased when the plant is under attack. The last condition has been observed in a number of terrestrial and marine plants, in which increased chemical defences are induced as a result of herbivore attack (Peckol *et al.* 1996, Van Alstyne *et al.* 1999).

Some authors have speculated that a cost resulting from the actions of grazers or epiphytes, which is high enough to elicit a defensive response by the host plant could lead to reciprocal interactions ending in a coevolutionary relationship. This process was defined by Janzen (1980) as 'An evolutionary change in a trait of the individuals in a population in response to a trait of the individuals of a second population, followed by an evolutionary response by the second population to the change in the first'

Janzen (1980) stated that for this process to occur, trade-offs between vegetative growth and reproduction on one hand and defence on the other, need to be established for the host plant. The possible occurrence of this phenomenon has so far mainly been investigated in terrestrial systems in which a large number of plant-herbivore associations with a high degree of feeding specialization exist. These studies often involve insects and the plants with which they are associated (Benson *et al.* 1976, Edmunds and Alstad 1978, Futuyma 1983). Many species or groups of species of terrestrial insects only feed on one or a small number of plants and according to Bernays and Graham (1988) approximately 90% of herbivorous insects are estimated to feed on three or fewer families of plants. A high degree of specialization is thought by many authors to be a prerequisite for coevolution, since only if the association between herbivore and plant is intimate and long-lived, will there be sufficient selection pressure to cause changes to become genetically fixed in a population (Ehrlich and Raven 1964, Spencer 1988). While selection pressures should be sustained long enough to allow time for a coevolutionary relationship to develop, this does not necessary imply that only one host plant and one herbivore species are involved. Plants are frequently subject to attack by an array of herbivores. Coevolution between a plant and several herbivores is termed diffuse coevolution (Fox 1981, Gould 1988). As there are a large number of highly specialized plant insect associations in terrestrial systems, but low numbers of specialized associations in marine environments, few studies have been carried out in the latter (Hay *et al.* 1989, Hughes and Gliddon 1991). One example of a relatively specialised association is that between the grazer *Littorina obtusata,* the brown seaweed *Ascophyllum nodosum* and its epiphyte *Polysiphonia lanosa* (L.) Tandy.

The organisms used in this study

The host plant:

Ascophyllum nodosum is a very common iteroparous brown seaweed of Northern temperate Atlantic shores. It often forms dense canopies on sheltered shores, but density declines with increasing exposure to waves (Cousens 1985). The life history of *Ascophyllum nodosum* only follows to a limited extent the predictions of the r/k scheme. In keeping with the classification of MacArthur and Pianka (1966) *Ascophyllum* is long lived and has a delayed onset of reproduction. It is also a weak colonizer, i.e. juvenile mortality is extremely high (Åberg 1996). Reproduction is seasonal with fronds being fertile between March and May with a peak in late March to April. The reproductive structures (receptacles) are produced on lateral pits on the fronds (Terry and Moss 1980). Receptacles are initiated in the summer, mature throughout the autumn and release the gametes in spring. Thereafter, the spent receptacles are shed and new receptacles are initiated. The lateral pits from which receptacles arise also produce vegetative laterals. However, in contrast to what would be expected from life history theory, it has a very high reproductive effort (Åberg 1996). As juvenile mortality is so high, it relies mainly on vegetative propagation from a perrenating holdfast, which may survive fifty years or more (Keser *et al.* 1981, Cousens 1985).

A. nodosum also has phlorotannins in its tissues. Although their concentrations can vary seasonally (Ragan and Jensen 1978) and geographically (Targett *et al.* 1992) they are generally thought high compared with other seaweeds such as laminarians (Van Alstyne *et al.* 1999). On some Manx shores *Ascophyllum's* phlorotannin levels were found to be comparable to those of other fucoids such as *Fucus vesiculosus* by Manley (1989). A prerequisite for reciprocal evolutionary interactions would be to demonstrate costs of phlorotannin production in terms of reduced vegetative or reproductive growth. This has been attempted for *Ascophyllum nodosum* (Pavia *et al.* 1999), but costs could not be established unequivocally, and seemed to be correlated with ambient nitrogen levels.

The grazers:

The phlorotannins in *A. nodosum* have been found to be an effective deterrent against the periwinkle *Littorina littorea,* but are ineffective against *Littorina obtusata.* In fact *L. obtusata* seems to prefer *A. nodosum* over other fucoids (Watson and Norton 1985, 1987). This apparent specialization of *L. obtusata* for its host plant has led some authors to speculate on the potential for coevolution in this association (Manley 1989). Establishing the deterrent effect of a compound on some grazers is another prerequisite for a possible coevolutionary relationship between the host and the associated grazers. However, in addition to *Littorina obtusata, Ascophyllum nodosum* is host to a small number of other grazers, which are characteristically associated with it (Johnson and Scheibling 1987). The isopod *Idotea granulosa* for example is associated with *Ascophyllum nodosum*, but in contrast to *L. obtusata* it is a more generalist feeder (Salemaa 1987, Karez *et al.* 2000). Several amphipod species can also occur on *A. nodosum*, although their feeding preferences have not been investigated in detail except for a study carried out by Pavia *et al.* (1999) using *Gammarus locusta*. However, work on other amphipods has shown that they can respond to the chemistry of their food plants and are often associated with chemically well-defended seaweed species, which are avoided by fish (Hay *et al.* 1990, Duffy and Hay 1991).

Polysiphonia lanosa

This filamentous red alga is apparently an obligate epiphyte on its host plant, although it has occasionally been found on other fucoids (pers. obs.). It is one of the few epiphytes commonly associated with *Ascophyllum nodosum* and the only one to occur on the host all year round. Like *A. nodosum* it reproduces both sexually and by vegetative spread (Kaczmarska and Dowe 1997). *Polysiphonia lanosa* is dioecious and has the triphasic life-cycle common in red algae (Lee 1980). Gametophytes are established in the autumn from tetraspores, produced near the branch tips of the *Polysiphonia* plants. After the release of the tetraspores the branches that produced them usually become necrotic. Tetrapores germinate on the host plant and form a rhizoid that quickly penetrates the host tissue (Rawlence and Taylor 1970). After the new plant has established, it can spread further by the formation of secondary rhizoids (Lining and Garbary 1992). The spermatia produced by the male plants fertilize the female gametes in carpogonia in the female plant. The cystocarp formed after fusion of the gametes then leads to the production of a large number of diploid spores, which can again penetrate the host (South and Whittick 1987).

The distribution *P. lanosa* within the host population is usually very patchy. While most host fronds might only carry light epiphyte loads, some branches can be almost completely covered (Lobban and Baxter 1983). The incidence of infestation has also been found to vary with wave exposure, with higher epiphyte loads occurring on more exposed shores (Levin and Mathieson 1991). On the host plant the distribution of the epiphyte is non-random, with settlement often occurring on wound sites or the lateral pits (Pearson and Evans 1990).

The epiphyte could potentially adversely affect the host plant in a number of ways, all of which could directly or indirectly lead to a reduction host fitness. For instance the epiphyte is likely to increase the drag experienced by the host (D'Antonio 1985, Ruesink 1998). Dense cover of the epiphyte could also decrease light and nutrient availability to the host plant. All these effects have previously been observed in other species of aquatic and (in some cases) terrestrial plants (Sand-Jensen *et al.* 1985). Such costs resulting from the presence of the epiphyte could exert selection pressures on the host plant to evolve adaptations reducing the incidence of epiphytisation. This might present an additional use for the chemical defences of *Ascophyllum nodosum*.

Because of the apparent specialization of the herbivore and epiphyte for its food plant and the high phlorotannin levels in the plant, this system should be a good candidate for a study into the potential for coevolution in a marine system. The aim of the present study is to detect possible mechanisms via which epiphyte and grazers can affect the reproductive costs of *Ascophyllum nodosum* and exert a selection pressure that could lead to reciprocal evolutionary interactions. An attempt will be made to quantify the magnitude of the effects and to examine how factors interact. While it is appreciated that due to the longevity of the organisms involved only phenotypic correlations will be investigated, it is hoped that costs and factors that might affect the closeness of the association, can still be examined.

Chapter 1: Population Ecology and strategies of *Ascophyllum nodosum*

1.1 Introduction

Ascophyllum nodosum, one of the most common seaweeds on North temperate shores, has adopted a life strategy somewhat different from its main competitors. These competitors often rely heavily on juvenile recruitment resulting from settled zygotes (Amsler *et al.* 1992, Creed 1995). Although fertilization success is usually low and juvenile mortality is very high, it is the surviving zygotes, which form the basis of the next generation of the population (Brawley 1992, Brawley and Johnson 1992). In contrast, while *A. nodosum* has a massive reproductive output (an estimated 2.9×10^7 newly settled germlings per m^2; Åberg and Cervin 1997), most of this output is lost soon after settlement e.g. due to wave action (Vadas *et al.* 1990) or grazing (Barker and Chapman 1990) and juveniles are usually rare. Therefore, the maintenance of a stable standing crop relies on clonal propagation by regrowth from the perennating holdfasts of established plants. Life strategies relying to a large extent on vegetative spread have also been described for other intertidal algae (Sousa 1985, Braga 1990).

However, the few gametes, that are successfully fertilized, manage to settle on the substratum and survive to a reproductive age, stand to have a very high fitness. This is also due to the vegetative (or clonal) growth strategy of *Ascophyllum nodosum*. The surviving germling eventually forms a holdfast from which a large number of axes can grow. The holdfast can have a considerable longevity (Baardseth 1970).

Each of the main axes will produce lateral branches arising from lateral pits. Some of these laterals will produce new vegetative biomass whereas others become reproductive. Each holdfast originating from a single zygote can therefore generate a very large reproductive output giving the zygote a very high fitness. However, as the canopy of mature stands becomes denser, light penetration through the canopy declines rapidly (Cousens 1985). Several previous studies have shown that reduced light levels can affect length growth as well as branching levels and intensity (Evans 1992, Schmitt and Wulff 1993, Huber and Stuefer 1997). These adverse effects are likely to last possibly until some canopy fronds are lost (Lazo and Chapman 1998).

There are already a number of studies investigating the growth of different life stages e.g. germlings (Vadas *et al.* 1990) and different size classes of *Ascophyllum nodosum* (Åberg 1992, Åberg 1992, Lazo *et al.* 1994). While these studies are undoubtedly important, the mere fact that so few recruits resulting from sexual reproduction survive, means that survival of vegetative shoots arising from existing holdfasts also needs to be investigated. In light of the high mortality of germlings and juveniles, the growth rates and mortality of these newly arising shoots might be more important determinants of population structure and stability. Therefore, competition occurring between these shoots might be at least as important as that which would be expected to occur between zygotes and germlings recently settled on the shore.

The only study to date, which has investigated the effects of different environmental conditions on shoot dynamics, was carried out by Indergaard, *et al.* (1986) testing different spray culture regimes. However, in their study only growth rates in cultured shoots were measured. As they did not make *in situ* measurements, they could not investigate population parameters such as shoot mortality.

The aim of this study is therefore to follow the growth and survival of adult plants and particularly those of vegetative shoots simultaneously and to examine factors such as grazing that might influence shoot development and mortality. The survival and morphological development of primary shoots was monitored both underneath a canopy of older fronds and where the canopy had been removed. The study addressed three hypotheses:

1. If light levels are the main determinant of shoot growth patterns, removal of the overlying canopy plants will lead to accelerated growth rates and higher morphological complexity of previously shaded shoots compared with shoots remaining under a canopy
2. Shoots underneath the canopy, although shaded, are also protected from desiccation resulting from exposure to full sunlight. Sudden exposure will therefore increase mortality in previously shaded shoots.
3. Removal of the canopy plants will increase exposure of the primary shoots to grazers

Answering these questions should help to assess whether canopy removal might be beneficial or even necessary to facilitate vegetative proliferation and regrowth of new fronds from established holdfasts.

1.2 Materials and Methods

1.2.1 Growth and survival of canopy plants

The growth study at Fort Island, at the Southern tip of the Isle of Man (54°05'N, 4°37'W) was set up in August 1999. The study was carried out on three different shore levels as the differences in axis lengths at different shore levels suggested possible differences in growth rates. The shore levels represented the upper and lower limits and the centre of the distribution of *Ascophyllum nodosum* (approximately 1.5, 3 and 4m above Lowest Astronomical Tide (Young 1987, Åberg and Pavia 1997)), hereafter called high, mid and low zone respectively. At each level 50 fronds were selected randomly. As most main axes were broken, laterals arising from the main axes were used for the study. Only fronds from the top of the canopy were chosen. These fronds were marked with thin strips of orange fluorescent tape (Colas Ltd), which was tied around the base of the lateral branch (between the point of attachment of the lateral to the main axis and the first gas bladder of the lateral). Branches were only chosen if they had an intact meristem at the tip and no recent grazing damage. Lateral branches used varied in length and lacked the common epiphyte *Polysiphonia lanosa*. Total length of the branch was measured initially and then monthly for a year. The only exception was February 2000 when no measurements could be taken for the low zone fronds.

Large numbers of branches were lost within the first two months of the study or incurred damage resulting in the loss of the apical meristem, so that further growth measurements could not be carried out. The term 'loss' only refers to the loss of the labelled lateral (or part of it) not the entire frond or plant. In October 1999 additional laterals were labelled to replace those lost or damaged, resulting again in 50 labelled laterals per shore level (Table 1.1). Subsequently a number of shoots of newly and previously labelled shoots were lost or damaged. Since the aim of the study was to investigate not only growth but also loss rates, shoots were not relabelled, but instead the total and partial loss of axes was recorded, although this reduced the number of replicates on each shore level.

Cousens (1985), in a similar study, found that breakage seemed to be the result of decay of frond tissue underneath the labels rather than breakage. This was judged to be a small source of error in the present study for a number of reasons. Several labels were found on the shore, still attached to part of the frond without any apparent decay on the frond at the point of contact with the label. A considerable number of labelled fronds experienced breaks at various points along the axes not in contact with the label, i.e. branch breakage was a very common event. On the high and low transects whole groups of holdfasts became dislodged. Shoots were inspected several times during the course of the study. Only in a very small number of cases was any decay found although often not restricted to the vicinity of the label.

A preliminary study had also been carried out in spring 1999 where several plants had been examined for the degree of damage to their fronds. Whole plants were collected haphazardly from the same shore levels at which the main study was to be carried out and in addition fronds were removed from an exposed site. For this study all laterals and main axes on a plant were counted and the number of intact and broken laterals was recorded.

In addition to the growth measurements for the canopy plant growth study, counts of the numbers of new vegetative and reproductive laterals produced on each labelled lateral branch were also carried out. Numbers were recorded for the whole of the lateral from the point, where it had arisen from the main axis to the tip. Counts were carried out in March and April 2000 during the period of maturation of the receptacles and formation of new gasbladders. Three types of laterals were distinguished (Figure 1.1):

1. laterals which had been initiated in the previous year (1999) and were now maturing to form receptacles
2. laterals which had been initiated in the previous year (1999) but remained vegetative and formed gasbladders (hereafter called vegetative laterals)
3. laterals which appeared in either of the two months (hereafter called lateral initials).

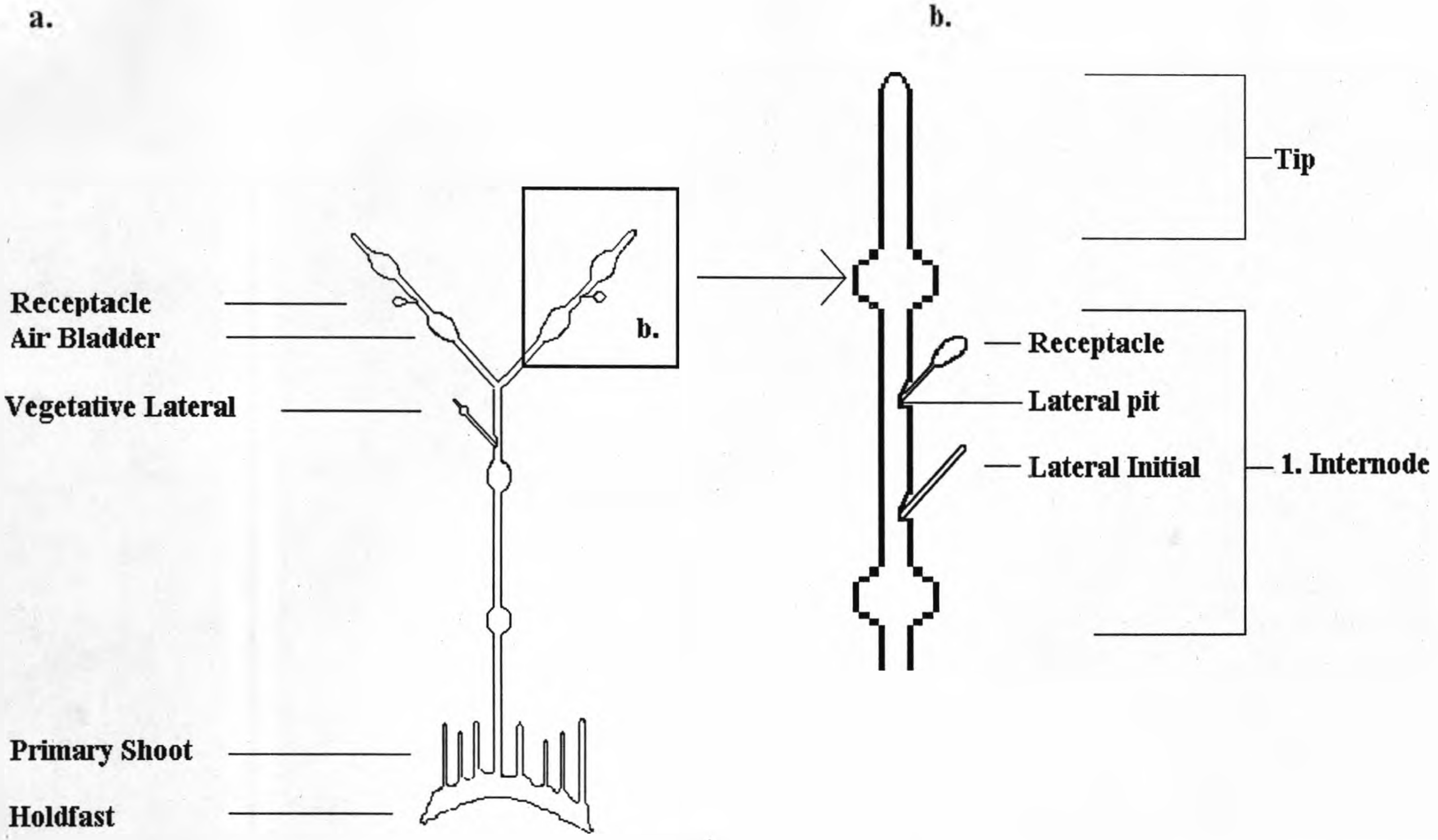


Fig 1.1 Schematic drawing of *Ascophyllum nodosum* showing all the morphological characters used in this study including vegetative laterals, lateral initials, receptacles and internodes; **a.** whole frond, **b.** tip and first internode

1.2.2 Primary shoot growth study

The study was set up on the sheltered shore at Fort Island in August 1999 and terminated in March 2001. Ten plots, 1m^2 in size, were chosen in the center of the *Ascophyllum* zone, from which the canopy plants were removed to reveal the holdfasts and the primary axes arising from them (hereafter called exposed shoots). Ten further plots were chosen in the vicinity of the exposed shoots. In these plots the canopy plants were left intact, so that the primary shoots underneath remained shaded as controls (hereafter called shaded shoots). The plots were spaced at least 2m apart to make sure that each represented different genetic individuals (Åberg 1989) and were independent replicates. In each plot 10 shoots were labelled with thin strips of orange fluorescent tape. Only shoots without grazing damage or signs of vesicle or lateral formation were chosen. All were between 3.2 and 5.9 cm long. A two-way ANOVA showed no significant differences between the initial length of shoots between shaded and exposed treatments or bewteen plots (Table 1.1). It has to be kept in mind though that while the shoots in the study had a uniform size structure this does not necessarily mean that they were of the same age.

Table 1.1: Preliminary two-way ANOVA testing differences in shoot lengths between plots and treatments (shaded vs exposed) in the primary shoot growth study carried out between August 1999 and March 2001

Source of Variation	df	MS	*F*	p-value
Light condition	1	2.906	0.440	0.508
Replicate	9	0.519	0.786	0.630
Interaction	9	0.194	0.294	0.976
Error	179	0.661		

Length measurements (to the nearest mm) were made monthly between August 1999 and September 2000 so that 12 monthly growth rates could be calculated. Growth rates are presented as absolute increases in shoot length per month. Such frequent intervals were chosen to allow the monitoring not only of growth but also of loss rates, the onset of grazing damage and epiphytisation. Measurements included the lengths of the main axis, newly arising laterals and the number and length of new dichotomies. On some fronds several orders of dichotomies were observed (Figure 1.2) and length measurements were then made separately for each. But only the length of the main axis and the forks (but not any newly arising laterals) present on each shoot were used for the calculation of dry weights. Grazing damage, both abrasions and more severe tissue loss, was also recorded as present or absent.

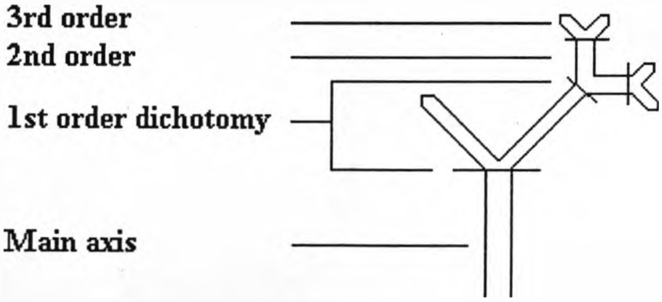


Fig 1.2 Schematic drawing of the three levels of dichotomies analysed in the primary shoot growth study

On a last visit to the plots in late March 2001, all the experimental shoots were removed and taken back to the laboratory and for the last time the length of shoots, dichotomies and laterals were measured. The tissue was then dried at 60°C to constant weight. Dry weights were used to construct a calibration curve for the relationship between shoot weight and length, from which dry weights of shoot lengths measured earlier in the study could be estimated. The laterals formed on the shoots by the end of the study (the current year's growth) had a slightly smaller diameter than tissue constituting the forks, therefore dry weight estimates for laterals are a slight overestimate. But since the length of laterals was generally small compared with all other tissues, this will not have affected the outcome of any of the statistical tests and the same predictive equation was therefore used to estimate all dry weights.

Originally it had been planned to carry out the study low in the *Ascophyllum* zone where there is the maximum density of the epiphyte *Polysiphonia lanosa*. However, this was not feasible as the measurements were time consuming and the low shore sites not accessible for very long. Therefore the 'primary shoot' study was set up in mid zone. A preliminary comparison of densities of shoots per holdfast and their length was carried out using 20 randomly chosen holdfasts in the center and lower limit of the *Ascophyllum* zone to check that conditions were comparable. The mid shore counts were made within the areas which were to form the plots in the main study. Two counts were made per plot. Only plants where individual holdfasts could be clearly distinguished were used.

1.2.3 Statistical Analysis

Length-frequency distributions for the canopy plants were compared using the Kolmogorov-Smirnov two-sample test (Sokal and Rohlf 1995). This procedure tests for differences in population means as well as, the shape of two distributions. Development of size structure in the young shoots was examined by calculating the skewness and Gini-coefficient for the sample population, which gives an indication of the degree of inequality of the size structure of the population, with a value of 1 indicating the same size in all individuals. The Gini coefficient was calculated with a Pascal program, using the algorithm described in Bendel *et al.* (1989).

Morphometrics data such as total plant length and length of last internode were analyzed using one-way or two-way Analysis of Variance. Where several morphometric measurements were made on the same shoots, these were not independent. Random numbers were therefore used to choose one measurement from each shoot. The analysis was carried out on the new series of measurements constructed in this way. Prior to the analyses, data were tested for homogeneity of variances using Cochran's C-test. Changes in shoot length and growth rate were examined using repeated measurements ANOVA. Where necessary, data were log transformed (calculated as log (x+1)). Post-hoc comparisons were carried out using a Student-Newman-Keuls critical range test. All data were analyzed using the STATISTICA package (Release 5.5).

1.3 **Results**

1.3.1 Adult Ascophyllum nodosum growth study

In high zone plants two peaks in growth rates could be identified. One occurred just after the start of the study in October 1999. Growth rates then declined to 0.2-0.6 cm per month between November and January. From February onwards they steadily increased to a second peak in June 2000. In the mid zone plants only the May peak occurred (Figure 1.3).

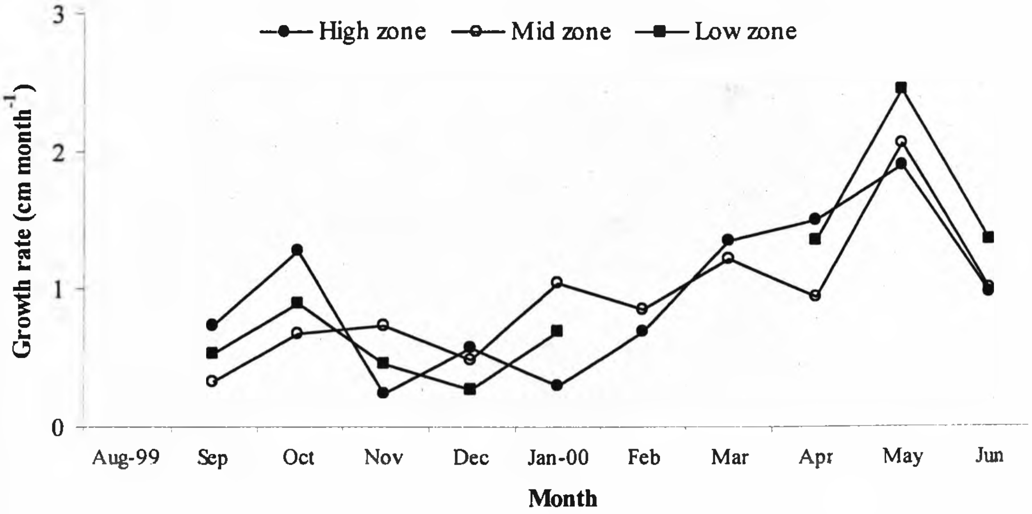


Fig 1.3 Monthly changes in the growth rate of laterals of *Ascophyllum nodosum* from three shore levels on a sheltered shore on Fort Island: the upper limit of distribution of *A. nodosum* (closed circles), the centre (open circles) and the lower limit of its distribution (closed squares).

1.3.1.1 Losses in the adult growth study

The results of this study have to be treated with caution as the high axis and branch mortality considerably reduced the number of replicates. Losses included both complete loss of labelled laterals and breakage above the label. These losses were highest in the low zone laterals. At the end of the study in August 2000 8 % of the initial branches remained undamaged and 5 % were still present but had lost the meristem, giving a total of 13 % of the initial laterals remaining at the end of the study. Survival was highest in the mid shore samples with a total of just over 26% (including damaged and undamaged laterals) srviving (Table 1.2).

Table 1.2: Number laterals labelled and relabelled on the three shore levels and the numbers lost and damaged during the course of the study at Fort Island. Figures in parentheses are percentages of laterals of the total number of labelled laterals surviving

Shore level	Labelled initially	Relabelled October 99	Damaged at end of study, (%of total)	Intact at end of study, (%of total)
High	50	22	9 (11.68)	7 (9.72)
Mid	50	17	9 (13.43)	9 (13.43)
Low	50	30	4 (5.00)	10 (8.00)

The preliminary study in 1999, which had specifically examined the degree of damage of laterals in whole plants, had also indicated that in excess of 50 % of laterals on a branch could be broken. The degree of damage was most variable in plants collected from an exposed shore at Scarlett Point with between 31-75% of tips meristems being damaged. At the sheltered site the degree of damage seemed to vary with shore level, being lowest in the lowest zone, where between 20-50% of the laterals were found to have lost their apical meristems compared with 51 –71% in laterals from high zone plants (Figure 1.4).

The breakage or detachment of plants in the adult-growth study did not critically depend on the length (and therefore the age) of the plant. Figure 1.5a-c indicates that complete loss occurred approximately proportionally in all size groups, i.e. size classes with the largest number of individuals lost most. This was confirmed by the Kolmogorov-Smirnov two sample tests, which were carried out separately for each shore level. The results were not significantly different for any of the shore levels (Table 1.3).

Table 1.3: Kolmogorov-Smirnov two sample test for the comparison of length-frequency distributions of adult *Ascophyllum* lateral branches labeled in August 1999 and length frequency distributions of lateral branches becoming dislodged during the course of the study (August 1999-August 2000). Means are given ± 1 standard deviation

Shore level	Max negative difference	Max. positive difference	p-level	Mean 1	Mean 2
High	-0.066	0.03	>0.10	21.68 ±6.90	22.56 ±6.16
Mid	-0.151	0.184	>0.10	25.62±6.19	24.01±5.60
Low	-0.019	0.085	>0.10	26.63±6.57	25.83±6.63

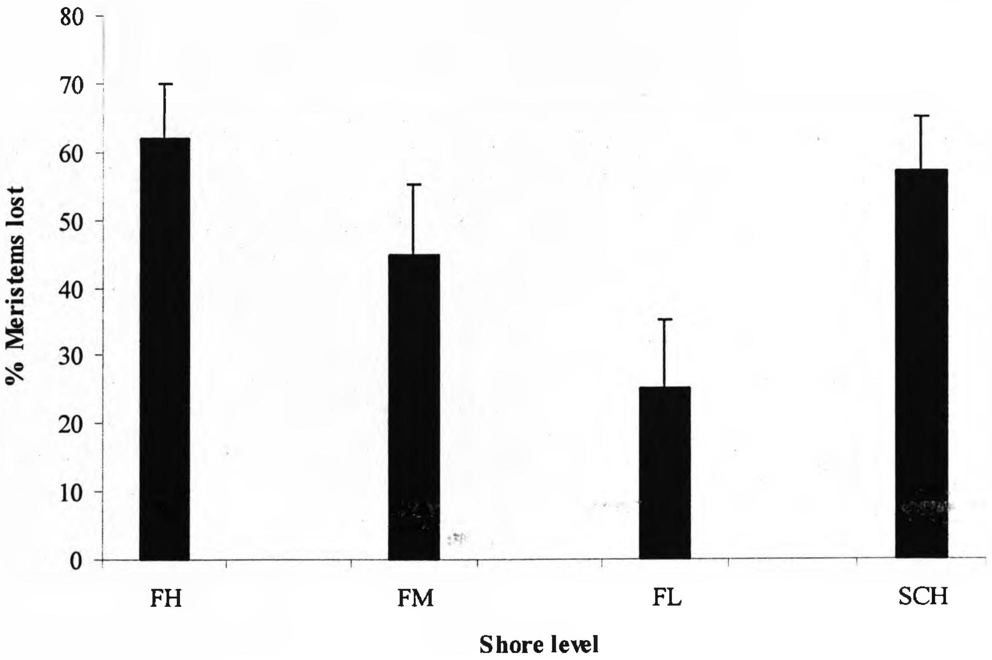


Fig 1.4 Results of a preliminary study investigating the degree of lateral damage on fronds of *A. nodosum*. Damage was expressed as the percentage of laterals, which had lost their growing meristems (FH=Fort Island high zone, FM=Fort Island Mid zone, FL=Fort Island low zone, SCH=Scarlett Point high zone); n=6 for Fort Island sites and 12 for Scarlett. Error bars=1 standard error

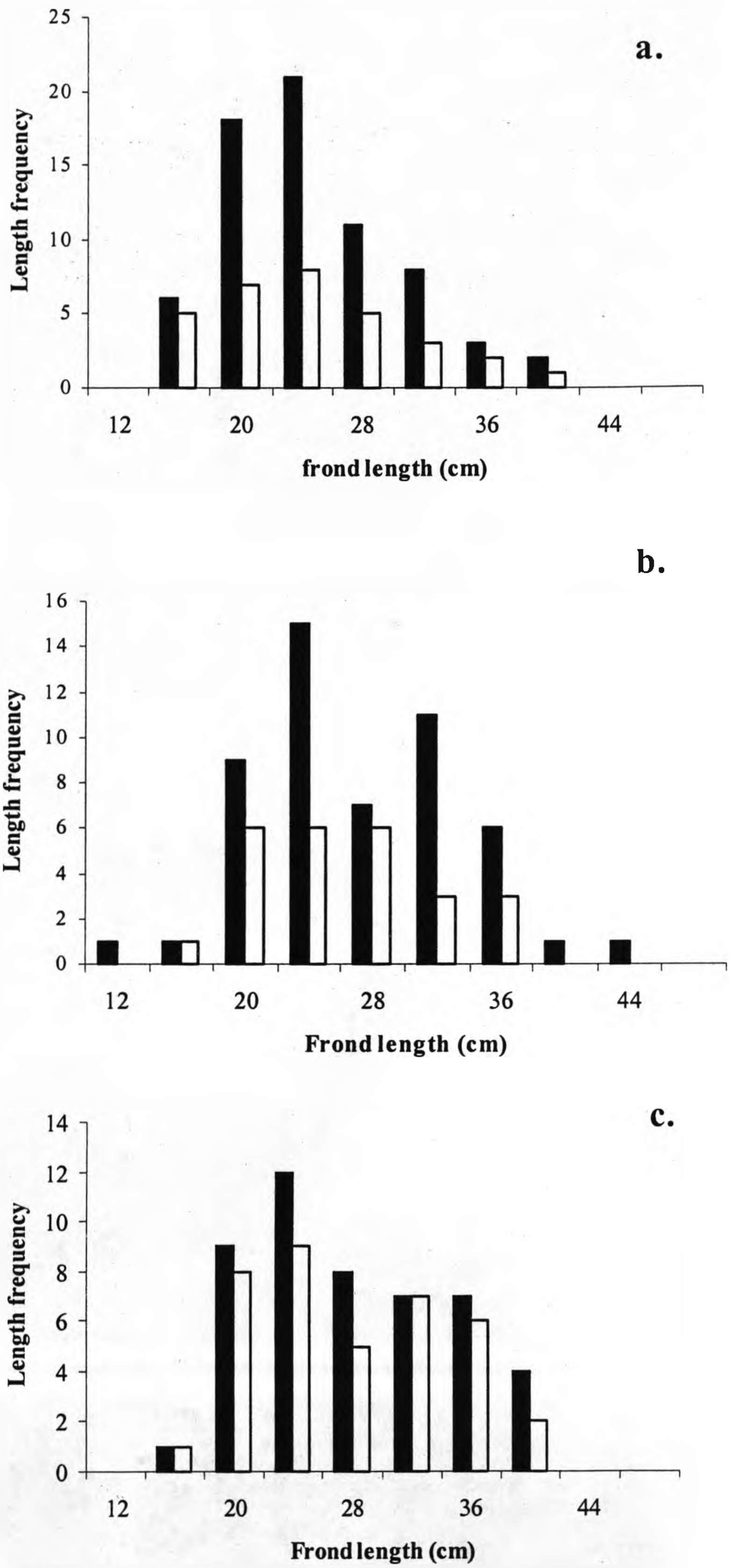


Fig 1.5 Initial length of lateral axes used for the adult growth study (solid bars) and the length of axes lost during the course of the study (open bars); **a.** high zone, **b.** mid zone, **c.** low zone

1.3.1.2 Morphological changes

At all shore levels some development of receptacles and vegetative laterals occurred on the labelled branches. For high zone plants there was a clear difference in the type of lateral found during the course of the study. Branches produced either predominantly vegetative or reproductive laterals. In some cases no laterals stemming from the previous year's growth (i.e. spring/ summer 1999) were observed at all. Instead, new laterals were produced in spring 2000 (Figure 1.6a). In contrast, in the low zone some labelled laterals produced no new lateral growth at all. The growth patterns were also generally different in the mid and low zone. A larger proportion of branches did not reproduce using receptacles initiated in 1999 but had formed a large number of lateral initials at the time of observation in April 2000. The total number of laterals produced also decreased from the high to the low zone (Figure 1.6b-c).

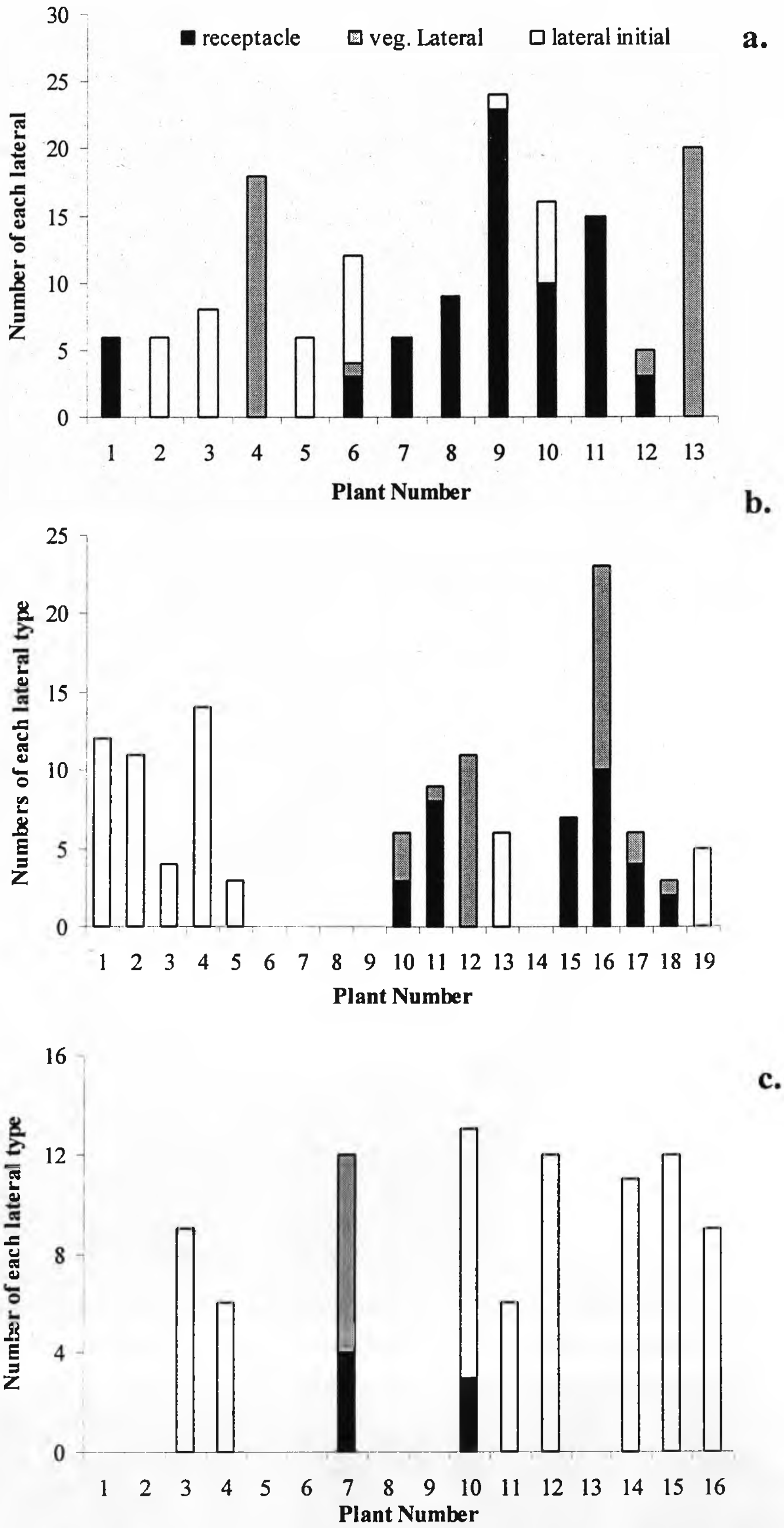


Fig 1.6 Formation of new laterals and differentiation into vegetative and reproductive laterals in surviving laterals in the adult shoot study in April 2000; **a.** high zone, **b.** mid zone, **c.** low zone

1.3.2 *Primary shoot growth study*

1.3.2.1 Growth rates

In the preliminary study no statistically significant difference occurred in the ratio of unbranched vs. older shoots per holdfast in controls on either mid zone or low zone (t-test: df=20, T=-0.671, p=0.509 two tailed). Shoot lengths were also comparable. Therefore, results of the experiment can be examined with respect to the situation on the low shore.

Shoots from which the canopy had been removed showed accelerated growth rates with respect to the controls within a month of the start of the experiment. The shoots from which the canopy had been removed, exhibited growth rates similar and in some cases higher than for the fastest growing adult axes on the mid shore level in Fort Island (Figure 1.7 a-b). A two-way repeated measures ANOVA showed that the differences in growth rate between shaded and exposed shoots were statistically significant ($p<0.001$, Table 1.4). The SNK test also revealed that up until April 2000 significantly different growth rates were not found between consecutive measurement dates, thereafter all comparisons were statistically significant.

In the exposed shoots, and to a lesser extent in the shaded shoots, growth rates also varied seasonally. The exposed shoots showed distinct peaks in growth rate in October 1999 and May 2000. After a slight decrease in June growth rates increased steadily until September (Figure 1.7a). It has to be kept in mind, that during the last few measurements the main axes had already produced dichotomies and therefore these growth rates include several growing tips not just one. Lower growth rates in the shaded shoots therefore also reflect the low incidence of the formation of dichotomies.

Table 1.4: Repeated measures ANOVA with two dependent factors for the differences in the mean shoot length in ten plots of shaded and exposed shoots for 15 monthly measurements carried out between August 1999 and March 2001

Source of variation	df	MS(Effect)	df	MS(Error)	*F*	p-value
Light Condition (L)	14	227.431	126	2.074	109.677	<0.0001
Inter Plot variation (P)	1	827.361	9	5.175	159.870	<0.0001
Interaction	14	27.819	126	1.601	17.410	<0.0001

SNK Procedure Aug 99=Oct 99=Nov 99=Jan 00=Feb00=Mar 00=April 00 >(May 2000-Mar 2001)

Loss rates in the exposed and the shaded shoots were the same during the course of the study (Figure 1.8). The average number of shoots per plot decreased from 10 to 5.9 in the exposed plots and 5.7 in the shaded plots. Only a small further loss of shoots had occurred by the time of the last visit in March 2001, indicating a sharp decrease in the risk of detachment or breakage with increasing size of the shoot

The number of shoots damaged by grazing was very low until March 2000, then increased sharply in both exposed and shaded shoots. However, while it levelled out in shaded shoots, grazing damage continued to increase in exposed shoots so that by the end of the study it was considerably higher than in shaded shoots (Figure 1.9).

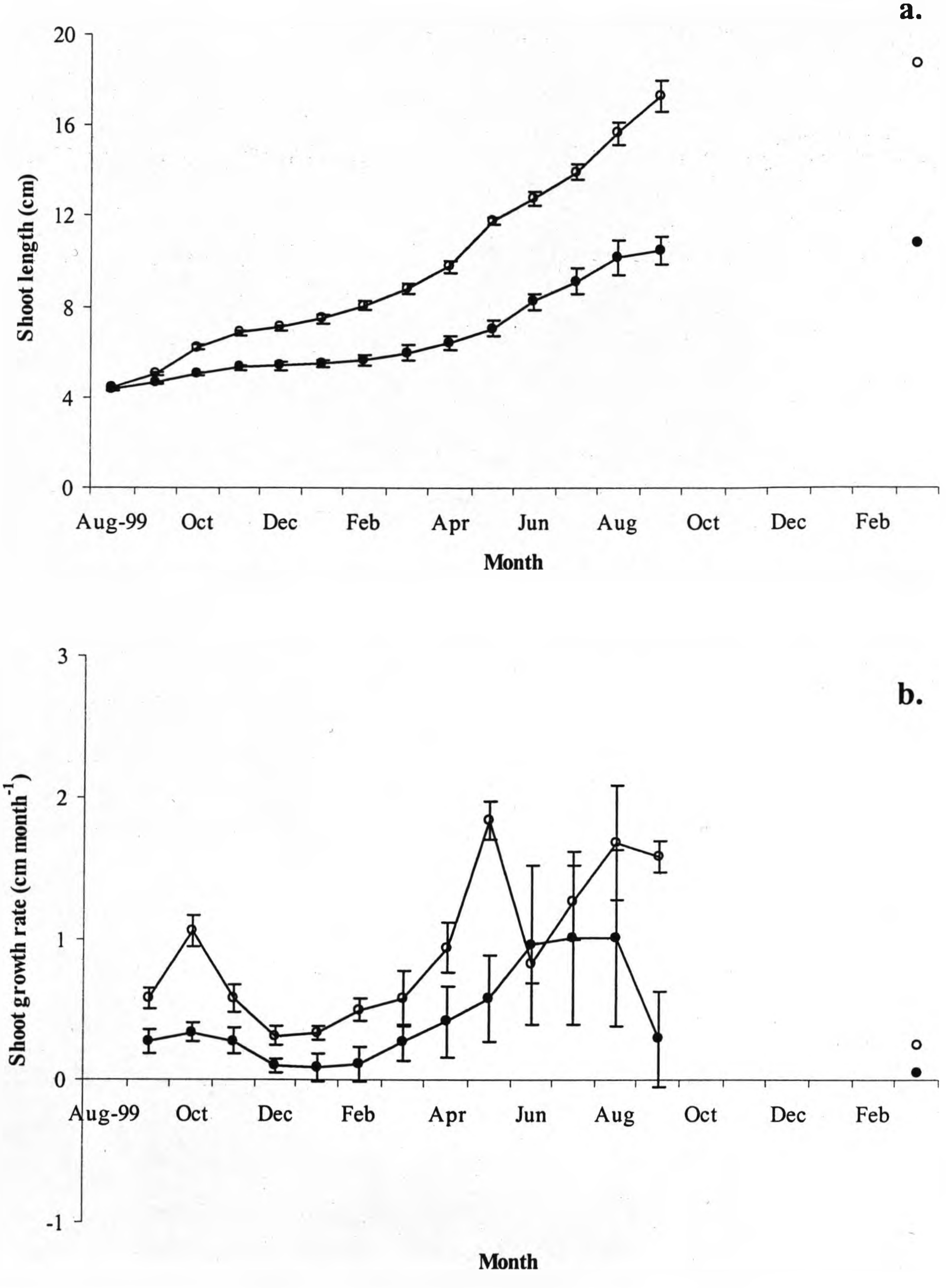


Fig 1.7 Growth of primary shoots at Fort Island between August 1999 and March 2001. Data shown are for exposed shoots (open circles) and shaded shoots (closed circles); **a.** absolute length changes, **b.** monthly growth rates. Data presented are the means from ten plots. Error bars are ± one standard error.

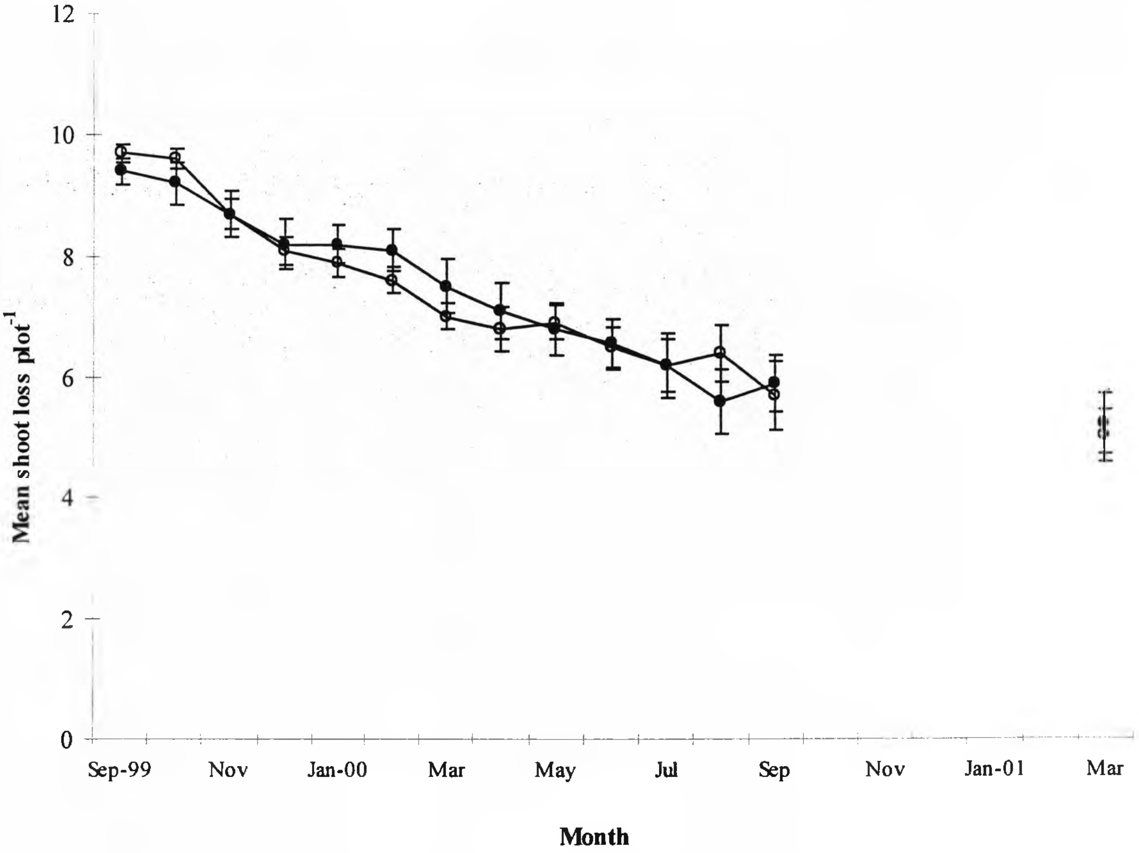


Fig 1.8 Monthly loss rates of primary shoots from the exposed (closed circles) and shaded (open circles) primary shoots studied at Fort Island August 1999 – March 2001); n= 10 plots, error bars ± 1 standard error

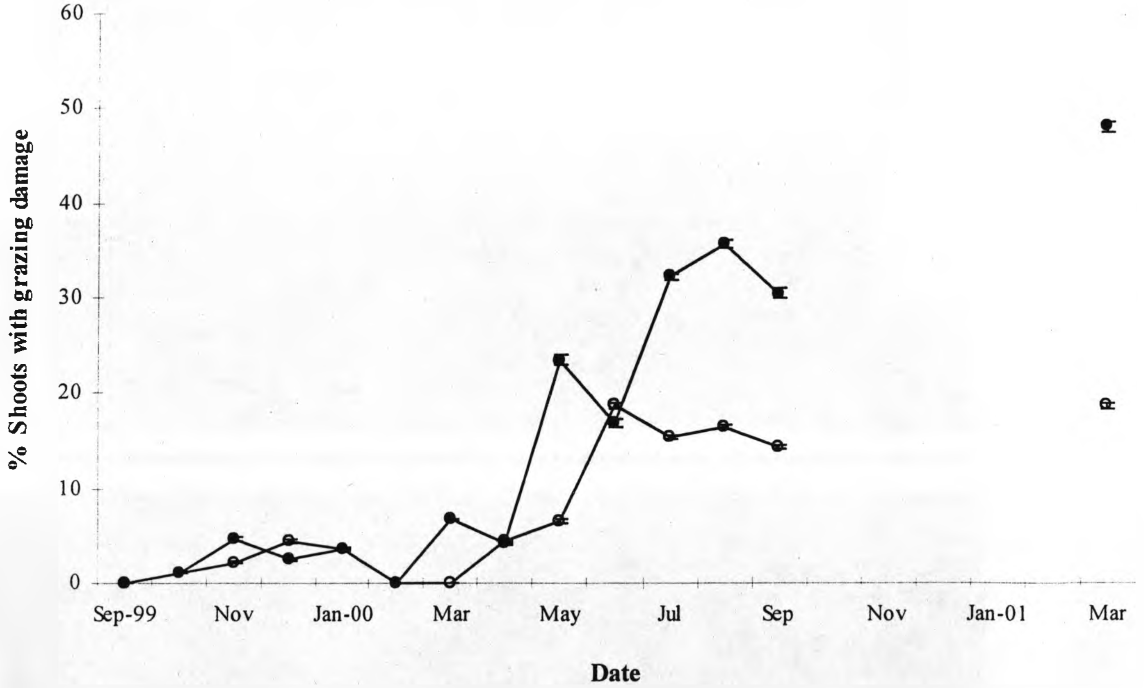


Fig 1.9 Changes in the number of primary shoots from exposed (closed circles) and shaded (open circles) treatments, showing signs of grazing damage, n=10 plots, error bars are ± 1 standard error

1.3.2.2 Morphological development

Within each treatment, formation of morphological characteristics such a lateral branches (Figure 1.10a) and dichotomies (Figure 1.10b) followed a set sequence. Dichotomies appeared first, followed by lateral initials. In the exposed shoots, gas bladders were not formed until spring 2001 and none were seen in the shaded shoots. Marked differences occurred in the timing of changes in morphological complexity between the two treatments. In exposed shoots, formation of dichotomies was first seen in October 1999, but remained at very low frequencies until March 2000. There was then a period of rapid increase between March and June. Laterals were absent until May 2000 and then increased for a very short period but from June onwards remained fairly static. In shaded shoots dichotomy formation also started in October and then showed a short period of increased formation of dichotomies between May and July 2000. Lateral formation was observed on only one plant (Figure 1.10. a-b), but it occurred at a similar time as in the exposed shoots the previous year. Air bladder formation, which usually occurs in March and April, was only observed in March 2001 in both exposed and shaded shoots (Table 1.5).

Table 1.5: Development of gas bladders on exposed and shaded shoots in the Primary shoot study at Fort Island, August 1999-March 2001.

Treatment	April 2000	March 2001	% of remaining shoots
Exposed	0	19	36.52
Shaded	0	5	10.0

The length and dry weight of each of the morphological features was also much reduced in shaded shoots (Figure 1.11 and 1.12). Differences in dry weight of morphological features were confirmed by a two way ANOVA (Table 1.6).

Table 1.6: Two way ANOVA for the estimated dry weights of main axis, forks and laterals of *Ascophyllum nodosum* in the primary shoot study in Fort Island. Data from April 2000 and late March 2001 were compared, as during this period new laterals and air bladders are often initiated.

Source of variation	df	MS	*F*	p-value
Exposed vs shaded	1	0.206	21.79	<0.0001
Morphology	2	0.161	17.017	<0.0001
Interaction	2	0.001	0.159	0.853
Error	90	0.009		

There was also considerable variation in the increase in morphological development between the 10 plots (Figure 1.12).

A more detailed analysis of the formation of dichotomies on exposed and shaded shoots also revealed a slightly higher degree of morphological complexity of exposed shoots, with 3 orders of dichotomies appearing on some exposed shoots (Figure 1.13a-b). On the shaded shoots a maximum of 2 orders of dichotomies was observed. A regression analysis carried out at the end of the study to examine the relationship between the dry weight of the main axis and that of either laterals or dichotomies, revealed only one significant relationship between total dichotomy dry weight and main axis dry weight in shaded shoots ($F_{1,50}$=6.66, p=0.001, Figure 1.14a-d).

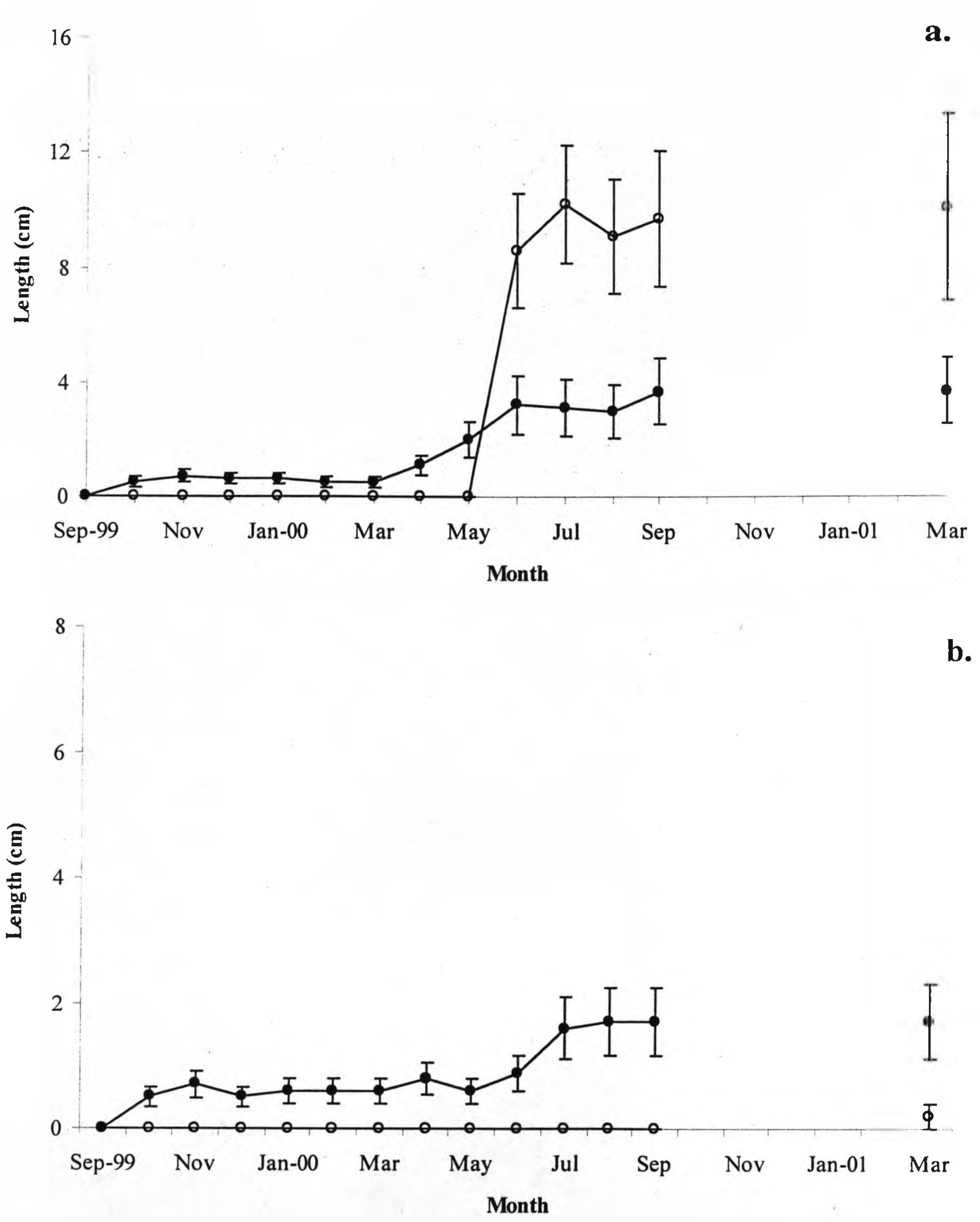


Fig 1.10 Average number of laterals per plot (open circles) and average number of dichotomies (closed circles) in the primary shoot study in Fort Island, carried out between August 1999 and March 2001, **a**. exposed shoots, **b**. shaded shoots; n=10 plots, Error bars are ± one standard error

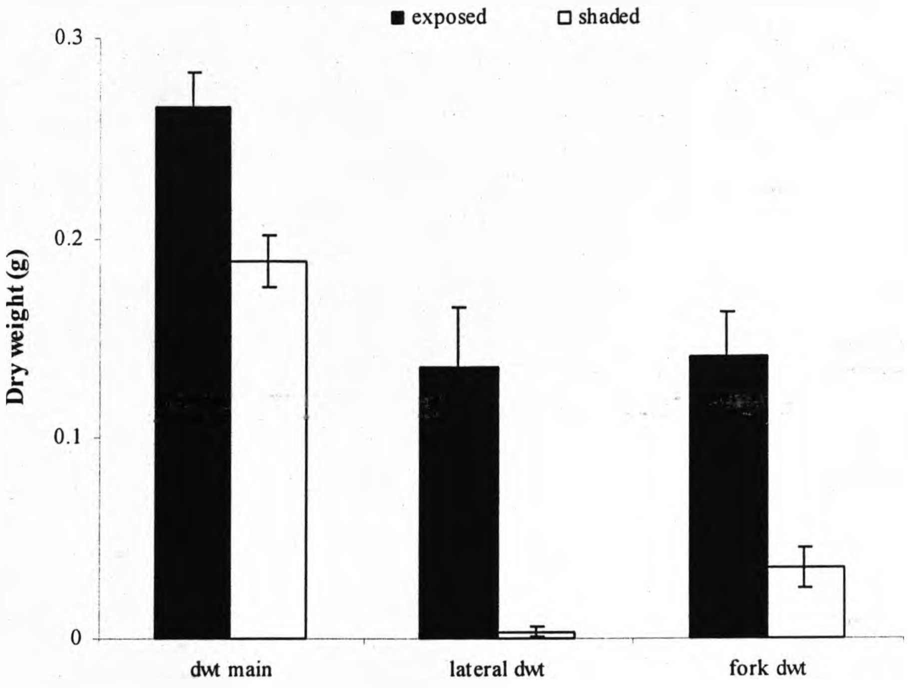


Fig 1.11 The mean dry weight of main axes, laterals and forks in the primary shoots study at Fort Island. Measurements were taken in April 2001; n=53 (exposed shoots), n=50 (shaded shoots); Error bars are ± 1 standard error

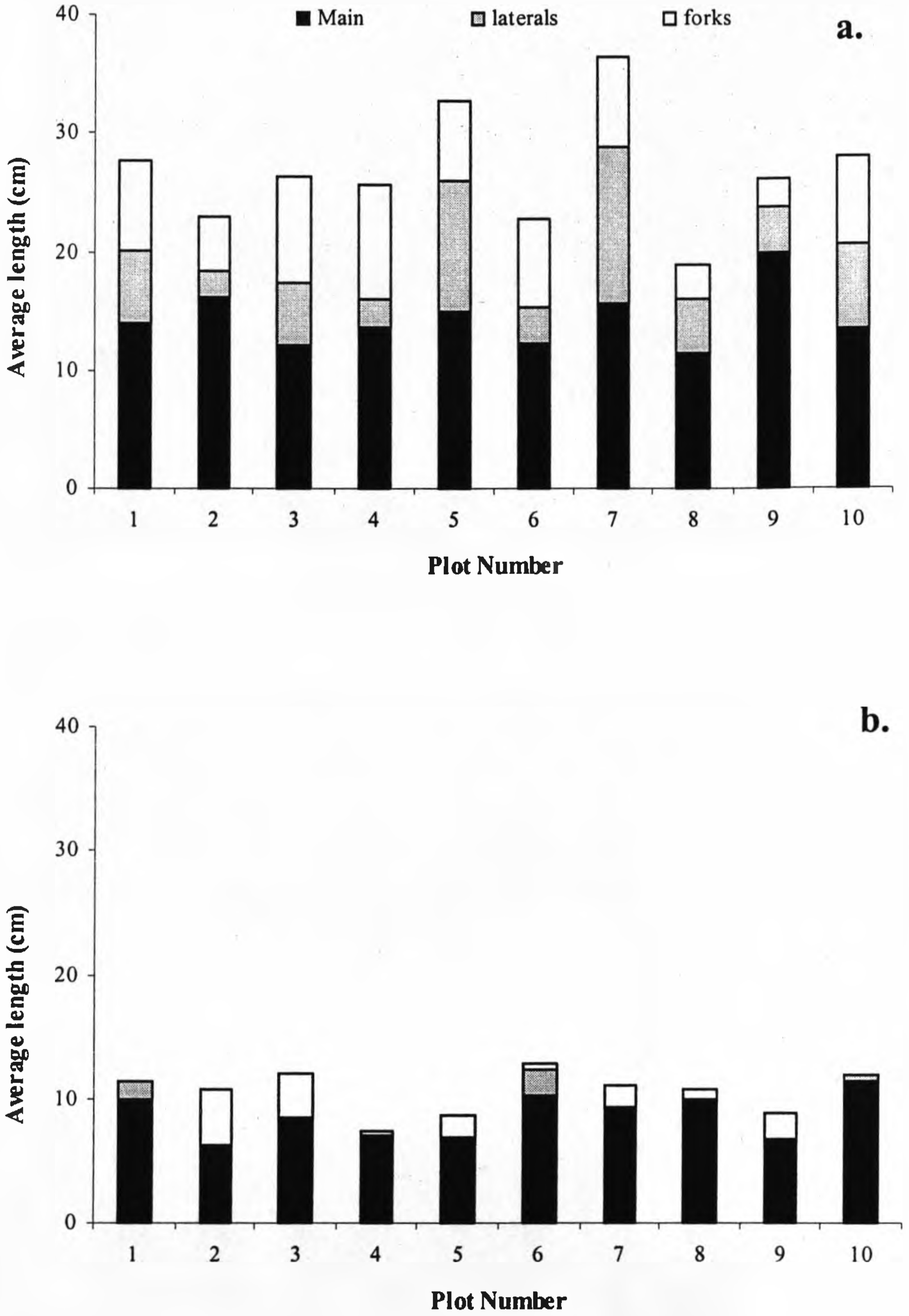


Fig 1.12 The average length of main axes, laterals and dichotomies of primary shoots in the ten plots set up for **a.** the exposed treatment, **b.** the shaded treatments. Measurements were taken in March 2001.

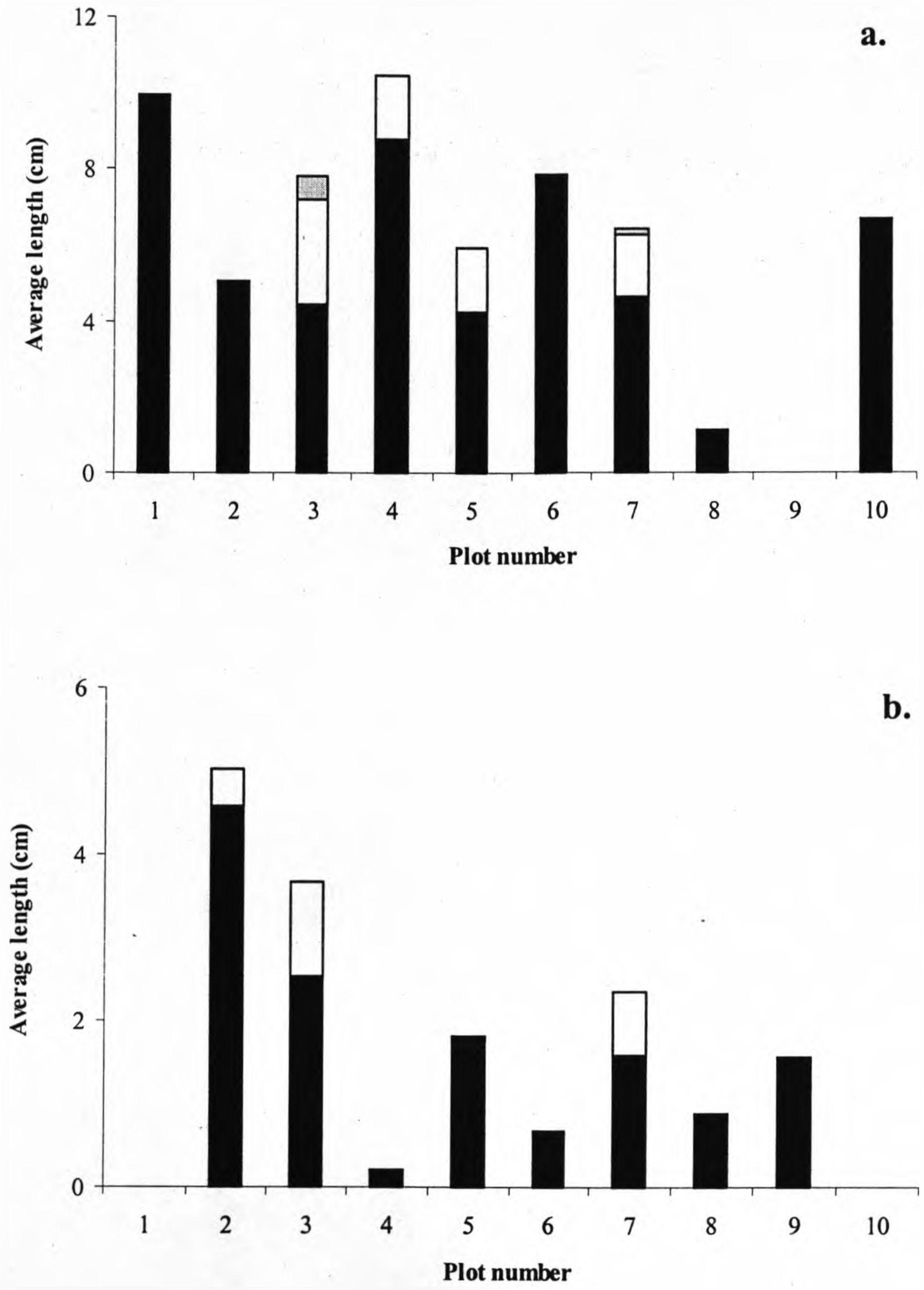


Fig 1.13 The state of development of dichotomies on **a**. exposed and **b**. shaded shoots. Three orders of dichotomies were distinguished: 1st order (black portion of the bar), 2nd order (white) and 3rd order (grey). Measurements were made in March 2001.

1.3.2.3 Changes in population structure

Considerable differences in the degree of skewness of the length of shoots occurred between shaded and exposed shoots. In both treatments skewness varied relatively little between August 1999 and May 2000, but was generally slightly higher in shaded shoots. In May, positive skewness in exposed shoots began to increase, while it decreased in shaded shoots (Figure 1.15a). From June onwards skewness was negative in shaded shoots. At the end of the study, in March 2001 the difference in the degree of skewness in the two populations had decreased again. The Gini coefficient was very similar for both shaded and exposed shoots. Size inequality increased slowly but steadily throughout the study (Figure 1.15b). The size frequency distributions for both, shaded and exposed shoots showed little development of size structure until March 2000 in the exposed shoots and May 2000 in the shaded shoots (Figures 1.16 and 1.17). By April 2001 both, had approached a normally distributed size structure.

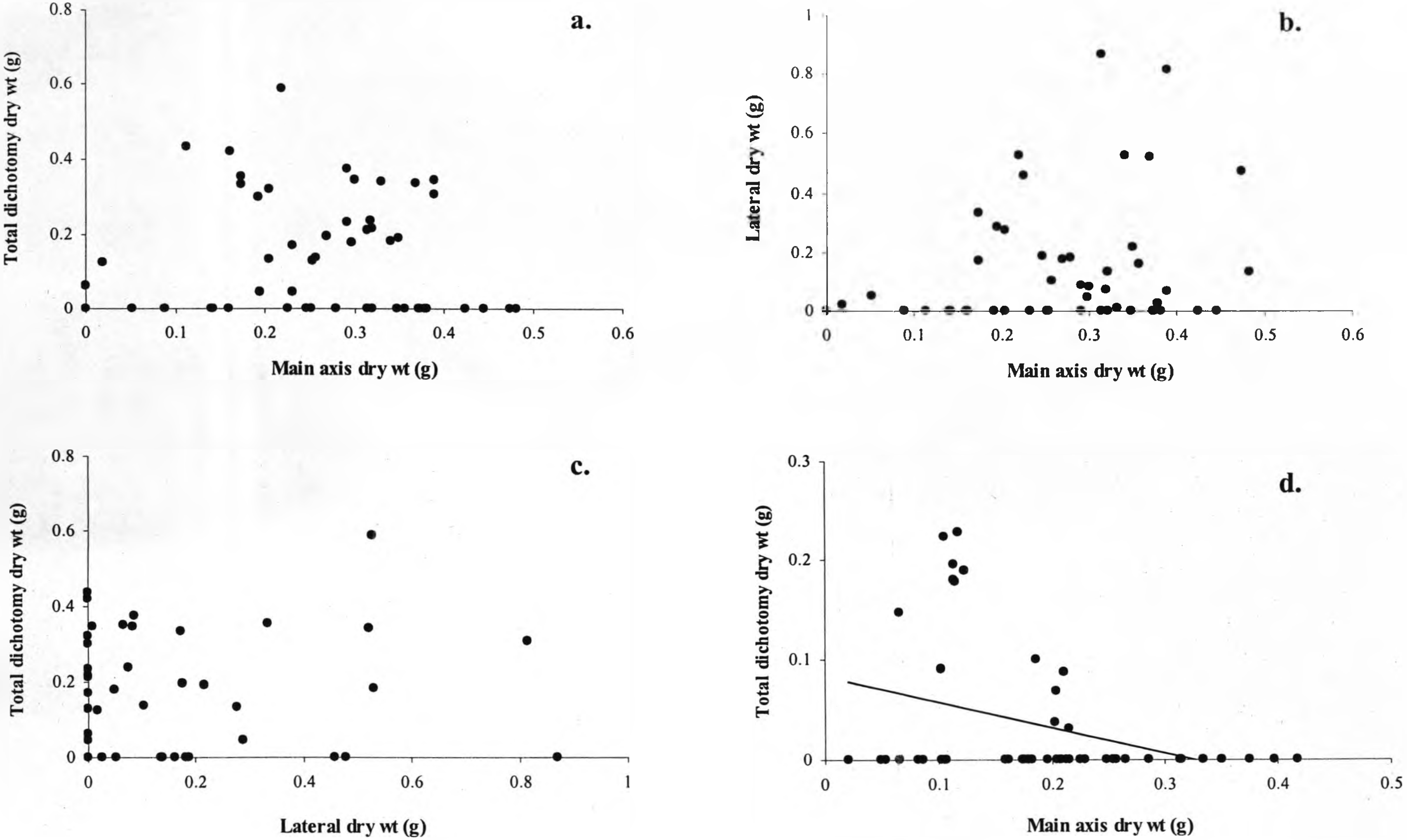


Fig 1.14 Regression analysis for the relationship between the dry weight of the main axis and **a.** dichotomy dry weight, **b.** dry weight of lateral branches formed during the course of the study, **c.** the relationship between dichotomy length and lateral dry weight in the exposed shoots, **d.** relationship between main axis dry weight and dichotomy dry weight in the shaded shoots ($F_{1,50}=6.66$, p=0.001)

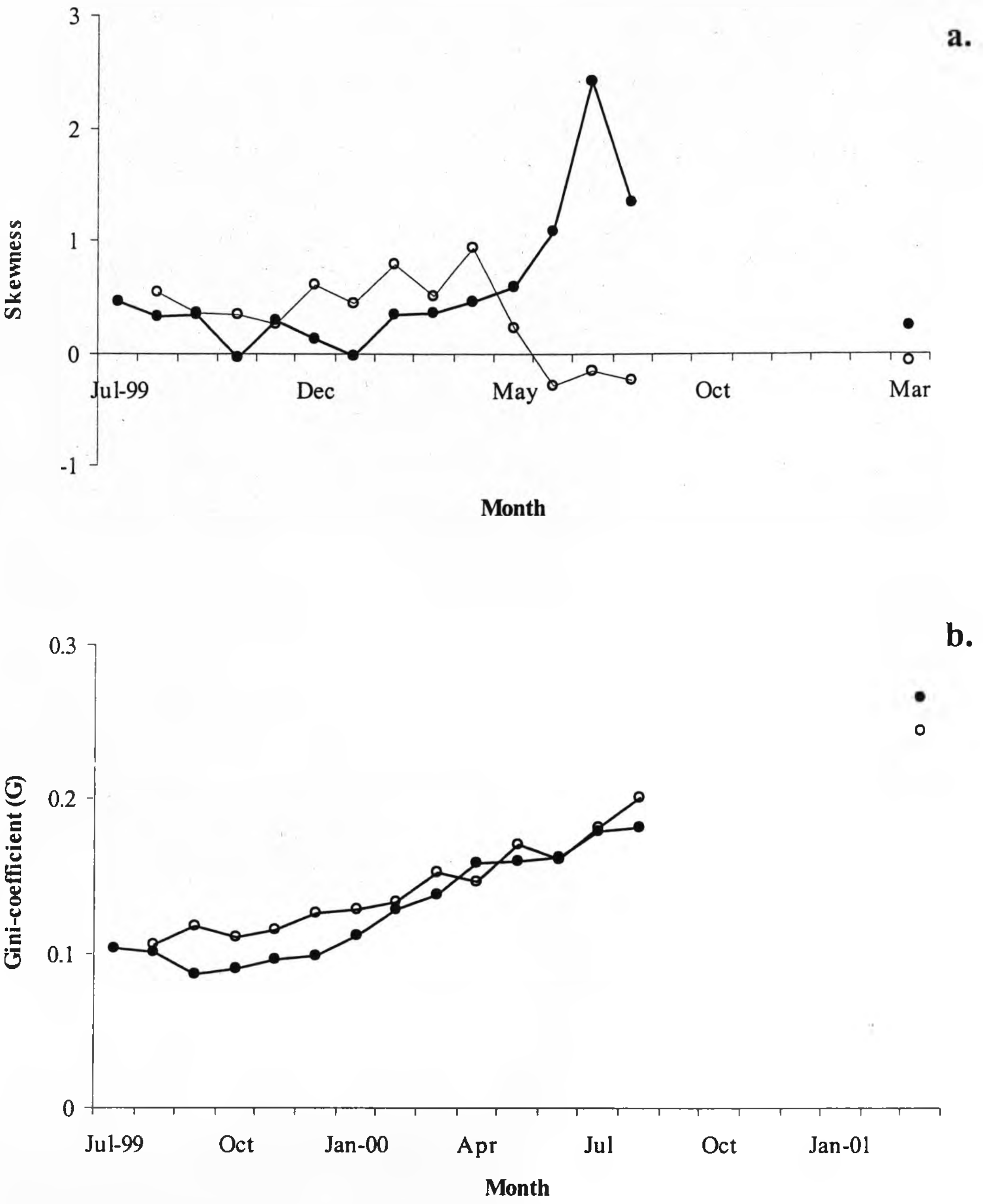


Fig 1.15 Changes in **a.** Skewness and **b.** Gini coefficient of the primary shoot population between July 1999 and March 2001; exposed shoots = closed circles, shaded shoots = open circles

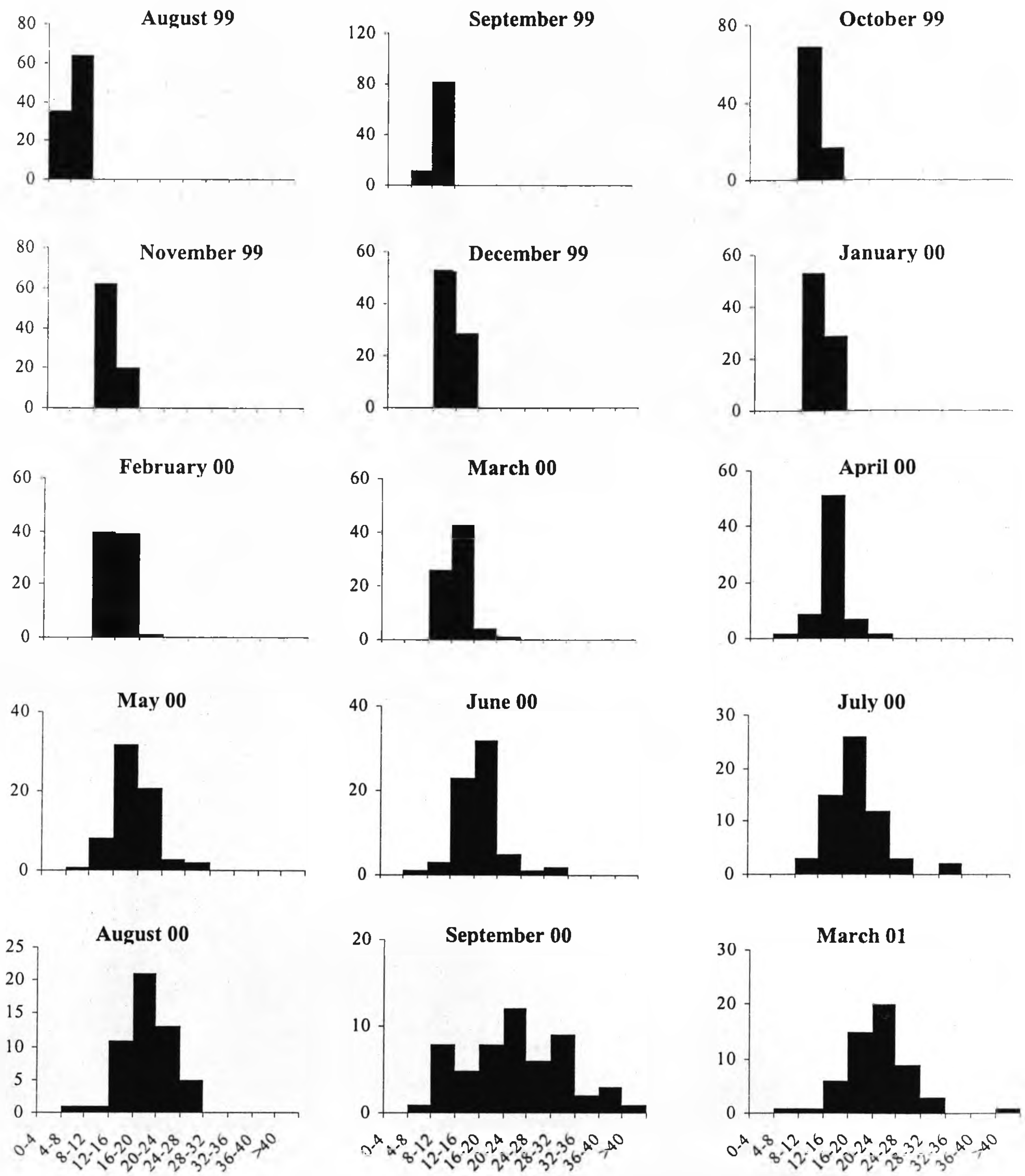


Fig 1.16 Size frequency distributions for exposed shoots in the primary shoot growth study carried out between August 1999 and March 2001 at Fort Island . Length categories are shown on the x-axis and numbers per length category on the y-axis

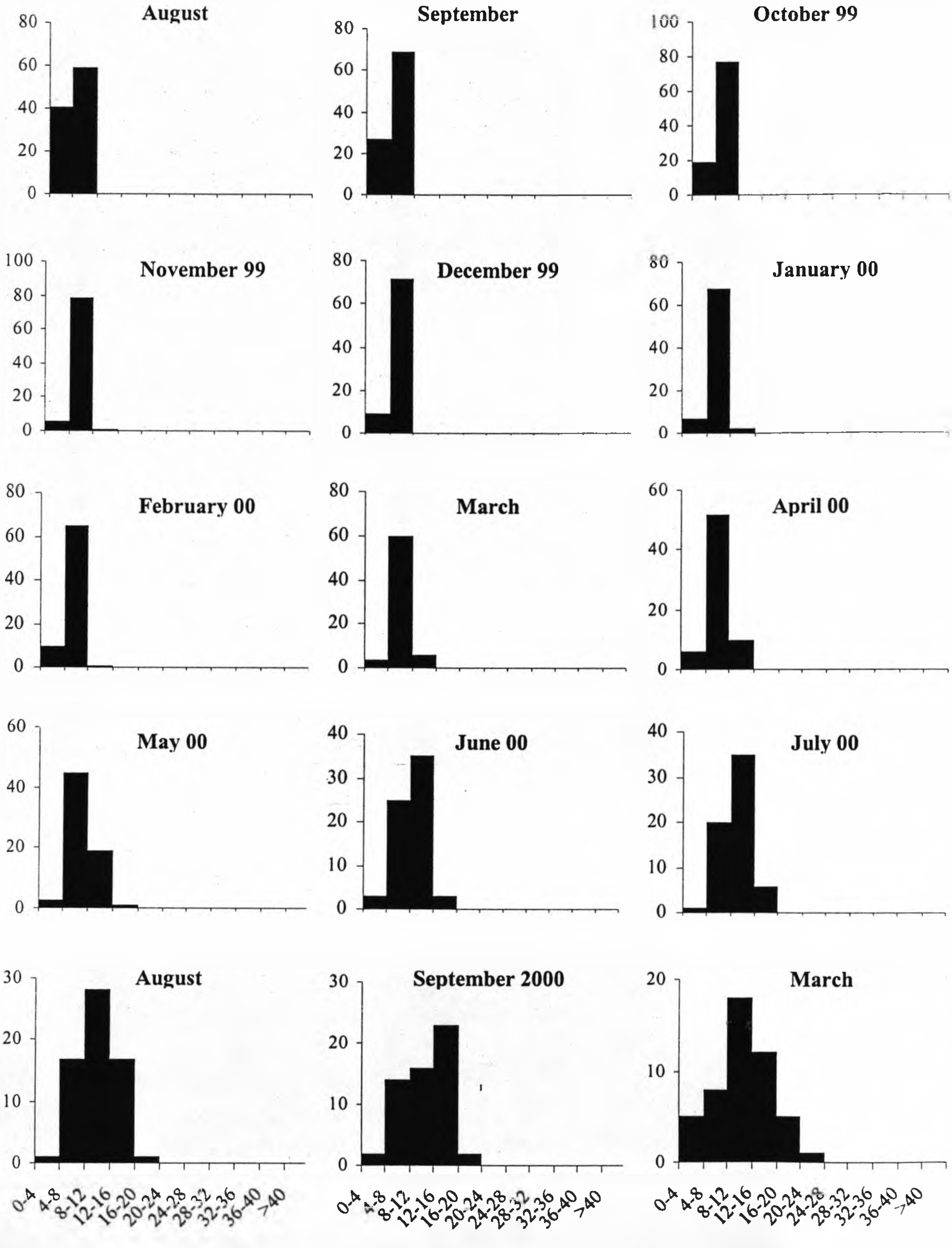


Fig 1.17 Size frequency distributions for shaded primary shoots in the study carried out between August 1999 and March 2001. Shoot length categories are given on the x-axis and numbers per category on the y-axis.

1.4 Discussion

Similar growth rates were found in adult laterals and primary shoots exposed by the removal of the overlying canopy, while those of naturally shaded primary shoots were much reduced. This indicates that light conditions are an important factor in determining growth rates (Dring 1974, van der Toorn and Mook 1982, Ekstam 1995). The growth rates for laterals found in the present study are in agreement with those found by Stengel and Dring (1997) for a population of *Ascophyllum* in Northern Ireland. Stengel and Dring (1997) also found differences in plant morphology such as frond width and air bladder volume at different shore levels. Similar vertical changes in morphology were also found in the present study with respect to the production of laterals and receptacles. They too might have been a functional response to the lower light levels experienced. Terry and Moss (1980) showed that in low light conditions *Ascophyllum* produced predominantly reproductive rather than vegetative laterals. Mathieson *et al.* (1976) also stated that the onset of reproduction of *A. nodosum* in a population in new England was controlled by seasonal differences in light and temperature. As submergence times increase with decreasing shore levels, total light availability to the fronds will decrease as well, which could have influenced the production of receptacles.

Morphological changes of clonal plants in different light regimes have been widely reported particularly in terrestrial plants (Hutchings and Slade 1988, Humphrey and Swaine 1997, Blundell and Peart 2001). Various hypotheses have been put forward to explain different patterns of morphological development between shaded plants and those exposed to sunlight (Goulet *et al.* 2000). Reduced branching in shade conditions is commonly thought to present a foraging behaviour by the plant, enabling it to reach favourable conditions by allocating limited resources to increasing growth in height rather than branch formation (De Kroons and Hutchings 1995, Van Kleunen *et al.* 2000). However, this concept is usually applied to plants that can produce vegetative upright shoots as well as horizontal stolons or rhizoids, and it is often these stolons, that are thought to cause placement of new growth in favourable patches e.g in spots where the canopy has been opened up giving better light conditions (Williams 1971, Sutherland and Stillman 1988). In the case of *Ascophyllum,* however, such horizontal growth is absent and the location of new favourable patches from an existing patch would depend on outgrowth from an existing holdfast and eventual fragmentation of the holdfast to produce a separate unit (Åberg 1989). This process is very slow and cannot therefore be used by the plant to respond rapidly to changing environmental conditions. Foraging behaviour in *Ascophyllum*, if existent, is therefore likely to be dependent on vertical growth of new shoots from the holdfast. A common change in clonal plants gaining access to full light after a period of shading is the rapid development of lateral branches from stolons and rhizomes (Slade and Hutchings 1987, De Kroon and Hutchings 1995). Such proliferation was observed for the growing shoots in the present study, indicating that they can respond relatively quickly to changes in environmental conditions. This shows that in this species 'locating favourable patches is proceeding vertically, i.e. by growing upward to join the canopy rather than horizontally, by locating favourable patches of substratum as in many terrestrial clonal plants.

While the development and the degree of morphological complexity varied greatly between treatments in the primary growth study, hypothesis 2 clearly had to be rejected as there was no noticeable difference in the mortality suffered by shaded and exposed shoots. But hypothesis 3 had to be accepted as exposed shoots were considerably more damaged by grazing. The mortality of both shaded and exposed shoots was also considerably lower than that expected for sexual recruits (Åberg and Cervin 1997). Such low annual mortality for vegetative shoots has also been reported in previous studies of angiosperms (Tamm 1972, Grime 1979, Ekstam 1995). The even smaller mortality rate between September 1999 and April 2001 indicated that mortality at this early stage in the growth of a primary shoot seems to decrease with age despite increasing grazing damage, although more long term studies would be desirable to draw any firm conclusions with respect to whether low mortality rates are in fact maintained for prolonged periods of time. This is extremely important, since these shoots are essential for the replacement of canopy fronds in the absence of sexual recruits. Together with the shoot's ability to accelerate growth after exposure to full light conditions, this emphasizes their role as future biomass producers.

The importance of vegetative growth in *Ascophyllum* poses the question of why there is such a massive reproductive effort in this species. Such a strategy might at first sight appear to be very wasteful. However, in a clonal plant sexual reproductive success might be less critical. Moreover, although reproduction is usually thought to incur a cost in terms of reduced growth, for example, it has been argued by some authors working on a number of seaweeds that reproduction might not incur a cost (Hansen and Doyle 1976, Pfister 1992). In addition, the present study has shown that *A. nodosum* could sustain both vegetative growth and receptacle production in spring. If there is little cost to reproduction, there might be no factor constraining it.

While considerable differences in morphological development have been demonstrated in this study, it has to be stressed that while growth was much reduced in the shaded shoots, they were by no means dormant. This means they represent a source of new fronds, which might eventually join the adult canopy even in the absence of canopy gaps. However, this could take a very long time and would only be advantageous if the resulting new fronds survived long enough to reach sexual maturity as some new recruits will eventually be needed to replace lost plants and to maintain some genetic variability.

Since increased light levels are required for the enhanced growth of the primary shoots it would appear that some losses of canopy fronds are actually beneficial. In this study, high losses of tissue were found in mature plants. These were rarely whole plants, however, but usually parts of fronds and lateral branches. Although the losses could be considerable in terms of biomass, the fact that the base of the plant remained attached to the substratum, ensured continued replacement of axes. Several previous studies indicated very slow growth rates (e.g. Cousens 1985). Since *Ascophyllum nodosum* can be extremely abundant on sheltered shores and forms relatively stable populations, it might be assumed that little loss of biomass takes place. However, in the present study it was shown, that although losses of biomass can be severe, they could play a beneficial role in the turnover the population, namely the replacement of old, damaged or epiphytised fronds by new shoots from the holdfast.

Chapter 2: Seasonal population dynamics of the grazers associated with *Ascophyllum nodosum*

2.1 Introduction

A major source of biomass losses from plant populations is damage caused by grazers. Consequently, herbivore grazing pressure is often seen as a major determinant of 'defensive' chemicals in plants. For grazing to produce a selection pressure determining the phlorotannin concentrations in seaweeds it should be very intense. It is also often thought to result from specialized associations between species (Futuyma 1983, Futuyma and Keese 1992).

In the case of *Ascophyllum nodosum*, *Littorina obtusata* has been identified as a major grazer of the seaweed. It feeds on its host plant preferentially, although in single diet experiments it will also consume other fucoids such as *Fucus vesiculosus* (Barker and Chapman 1990). Its distribution is, not surprisingly, concentrated in the *A. nodosum* belt (Watson and Norton 1987). However, it is not the only grazer associated with the seaweed. The isopod, *Idotea granulosa*, has a very wide distribution on sheltered shores (Salemaa 1987), but when among seaweeds, it feeds on *A. nodosum* (Pavia *et al.* 1999). Amphipod species are also associated with *A. nodosum* (Johnson and Scheibling 1987). While the seasonal population dynamics and feeding habits of *L. obtusata* have been investigated in several studies (Goodwin and Fish 1977, Goodwin 1978, Watson and Norton 1987), less information is available on the distribution and dynamics of isopods and amphipods within the *A. nodosum* belt. The amphipods of the *Ascophyllum* zone for example *Eulimnogammarus obtusatus* and *Hyale prevostii* have been especially neglected, although they and other small grazers are considered by some authors to be likely to exert selection pressures on the 'host' plant with respect to its defensive chemistry (Hay *et al.* 1987, Hay *et al.* 1987). Feeding experiments carried out with amphipods have shown potentially very different effects on the algal assemblage they browse. In some cases they were found to feed mainly on the epiphytes present on macroalgae (Brawley and Adey 1981, Brawley and Xiugeng 1987) and were therefore considered to be beneficial to the host plant by reducing the adverse effects of the epiphyte, while in other cases the host plant itself was consumed (Poore 1994).

Depending on their feeding habit and abundance, the amphipods and other grazers associated with seaweed can affect the fitness of the host plant in very different ways. One of the few investigations of the feeding preferences of amphipods associated with *Ascophyllum nodosum* was carried out by Pavia *et al.* (1999). Their study revealed that *Gammarus locusta* fed on both host tissue and epiphytes present. However at his site, *Polysiphonia lanosa*, the red epiphyte commonly associated with *A. nodosum* on the Isle of Man, was absent.

This chapter is therefore divided into two parts. Firstly, it aims to establish the vertical and seasonal patterns of distribution and abundance and the degree of overlap between the grazers associated with *Ascophyllum nodosum* and *Polysiphonia lanosa* on Fort Island and Scarlett Point. It can then be assessed whether a selection pressure exerted by any of the grazers is likely to be distinct from that of other grazers, particularly *Littorina obtusata*, and whether selection pressures are continuous throughout the year (Faeth 1988). Secondly the chapter will investigate feeding preferences of the most abundant species associated with *A. nodosum* and *P. lanosa.*

2.2 Materials and Methods

2.2.1 Shore Surveys: Sample Collection and Analysis

Three transects were established at the sheltered site at Fort Island (54°05'N, 4°37'W) and two at the exposed site at Scarlett Point (54°4'N, 4°39'W), both located at the southern tip of the Isle of Man. On the latter shore were established along two distinct ledges. On Fort Island samples were taken along three horizontal transects representing the upper and lower limits of the distribution of *Ascophyllum nodosum* as well as half way in-between as in study in the previous chapter (approximately 1.5, 3 and 4m above lowest astronomical tide). As in the previous chapter the different shore levels are referred to as high, mid and low zone respectively. The need for several transect lines arose after preliminary vertical surveys in Fort Island, which revealed different vertical distributions of some of the grazers, particularly amphipods, and changes in the abundance of individual species.

Samples were taken bimonthly at random points along the transects, from August 1999 to July 2000 at Fort Island and from October 1999 to July 2000 at Scarlett Point. On each occasion 10 samples were taken from each transect at the sheltered site and 8 samples from each transect at the exposed site, resulting in a total number of 180 samples at Fort Island and 80 at Scarlett Point. Fewer replicates were taken at Scarlett Point because of the low abundance of the seaweed there. Plants were removed from the substratum at the holdfast. They were placed in polythene bags until the bags were half full. This was equivalent to between 1 and 1.5 kg seaweed per sample. Samples were frozen immediately after transport to the lab, which aided extraction of the organisms. For grazer extraction the samples were defrosted in filtered seawater. When the seaweed had defrosted completely it was removed and the water was filtered through a mesh (approximately 1 mm mesh size) to retain smaller organisms such as amphipods and juvenile gastropods. Each seaweed sample was washed repeatedly in filtered seawater until no further animals could be extracted. All fauna was stored in 10% Formaldehyde (in seawater) in 30 ml Sterilin containers. As the aim was to assess the feeding impact of the fauna, small species like ostracods and harpacticoid copepods were excluded from the analysis, as these were more likely to feed on detritus and smaller organisms like diatoms and ciliates (Alongi and Tenore 1985, Miliou and Moraitou-Apostolopoulou 1991). It was appreciated that this meant that in terms of abundance a major part of the fauna associated with *A. nodosum* and *P. lanosa* was therefore ignored. Before discarding the seaweed samples the wet weight of *A. nodosum* and the epiphyte were measured (to the nearest 0.1 g), as well as the overall length of the host plant (to the nearest cm). The length was measured from the base of the holdfast to the tip of the longest frond arising from that holdfast.

2.2.2 Comments on species identification

On sheltered shores adult *Littorina obtusata* and *Littorina fabalis* which is synonymous with *Litorina mariae* (Reid 1996) tend to be very different in size and distribution. They could usually be distinguished in the field on account of their shell morphology and colour. Occasional dissections were carried out in the laboratory to confirm the species on the basis of their internal anatomy as recommended by Reid (1996). At the exposed site the two species were very similar in size and there was a greater overlap in their vertical distribution. Most individuals were therefore dissected. While adults of *L. obtusata* and *L. fabalis* could be clearly distinguished, this was not the case for juveniles. The abundance data for *L. obtusata* and *L. fabalis* in Figure 2.3 are therefore only given for adults. This was done for both the sheltered and the exposed sites, although on the basis of shell morphology alone, based on the available literature (Graham 1988, Hayward and Ryland 1995, Reid 1996) the juveniles were considered to be more likely to be *Littorina obtusata*. The two groups, adults and juveniles, were retained for the multivariate analysis. Names and authorities of all species in this study were used according to the species directory of the fauna and flora of the British Isles (Howson and Picton 1997) unless otherwise stated.

2.2.3 Measurement of size frequency distributions

Size frequency distributions were measured for *Littorina obtusata* and *Idotea granulosa*. For *Idotea granulosa* the length of the animal was measured from between the bases of the first antennae to the tip of the telson. For *L. obtusata* the width of the shell (measured at a right angle to the spire, at the widest part of the shell) was measured following the method of Goodwin and Fish (1977). For adults, measurements were carried out using calipers, while for juveniles an image analysis system was used (Scion Image Analysis System). For each shore level and sampling date, all individuals in randomly chosen replicates were measured until 100 measurements had been made or all undamaged individuals had been measured, the latter being more often the case for *I. granulosa*.

2.2.4 *Feeding experiments*

2.2.4.1 Preliminary Study

Before the start of the experiment a preliminary study was carried out to investigate the accuracy with which repeated weight measurements could be made on the same *Polysiphonia lanosa* clumps. Ten clumps were soaked in seawater until fully hydrated and then blotted and weighed. They were re-immersed and stored for 4 days in a CT room at 10°C. On the fourth day they were weighed again. In these trials a high degree of plant fragmentation often occurred in the treatments. These fragments were removed from the containers using fine forceps and weighed with the remaining plant material. Differences in clump total wet-weight (including small fragments) between day 1 and 4 were compared using a paired sample t-test. The differences between consecutive measurements were insignificant (df: 9, p=0.91).

2.2.4.2 Main study

For all experiments, animals were collected from the sheltered shore on Fort Island several days prior to the start of the experiments and kept in small aquarium tanks in a CT room at 10°C to acclimatise them. They were not starved but fed on Ascophyllum nodosum prior to the start of the experiment. Plant material was also collected well in advance and soaked in filtered seawater to ensure that it was fully hydrated. During the experiment animals were kept in 150 ml Sterilin containers under low light conditions (approximately 5 μE m^{-2} s^{-1}) to avoid plant growth (Karez 2000). This low light intensity also mimics the light conditions found under a dense canopy of fronds on many sheltered shores.

Both single diet and multiple-choice experiments were carried out. The experiments were conducted in November and December 1999 and January 2000 when the receptacles were still immature. Earlier experiments with mature receptacles failed because these receptacles deteriorated very rapidly in culture. They could not be blotted for weighing without also removing large amounts of the mucilage they contained. This would have led to considerable overestimates of grazing rates.

Both series of experiments were carried out with *Littorina obtusata*, *Idotea granulosa* and *Eulimnogammarus obtusatus* (with the exception of single-diet *Polysiphonia lanosa* experiments, which were only carried out with the two last species). The shore survey data available at the time the feeding experiments were carried out, showed these species to be the most abundant at the sheltered site at Fort Island. Groups of 15 animals were fed on either vegetative tissue of *Ascophyllum nodosum*, receptacle tissue or *Polysiphonia lanosa*, with the exception of the single diet experiment with *P. lanosa* where only 8 individuals of *E. obtusatus* were available.

It had originally been intended to measure wet weights of the algae presented to the grazers after 10 and 20 days. However in the multiple-choice experiments mortality of littorinids was very high after day 10. The experiment was repeated but the same problem occurred. No such problems occurred in the single-diet experiments. Only the measurement after 10 days was therefore included in the analysis, although data for surviving animals after 20 days are presented. From the data after 10 days, feeding rates as g per day per organism were calculated. Prior to weighing, each plant fragment was blotted between tissue paper until no more moisture could be seen on the paper.

In the single diet experiments approximately 0.10±0.02 g of either receptacles or vegetative tissue of *A. nodosum* or a clump of *P. lanosa* of similar wet weight was presented to the three grazer groups. For each test the vegetative tissue was removed from the second youngest year class of *Ascophyllum* fronds. Receptacles were removed from internodes of the same age but from different fronds. Faecal pellets were regularly removed from the containers and new food was added if all the initial material was consumed. This was necessary mainly for *I. granulosa.* Owing to space constraints, single diet experiments involving the food item *Polysiphonia lanosa* could not be carried out at the same time for *Idotea granulosa* and *Eulimnogammarus obtusatus*, therefore separate controls had to be run and the experiments were analysed separately.

In the multiple choice experiments approximately 0.1±0.01 g of each of the food types presented in the single diet experiment was presented simultaneously to each grazer.

2.2.5 Statistical Analysis

As a preliminary measure, Shannon diversity indices were calculated for each month and shore level for the sheltered site at Fort Island (eqn. 1). Diversity indices were not calculated for the exposed site because of the identification problems with juvenile *Littorina obtusata/ fabalis.*

$$H_1 = \sum \frac{n}{N} \log_2 \frac{n}{N} \qquad (1)$$

n = numbers of individuals per species, N = total number of individuals

For the dominant species changes in density between shore levels and sampling dates TWO-Way Analysis of Variance was used. Prior to the analysis, the data were examined for homogeneity of variance using Cochran's C-test. Where variances were heterogeneous, data were log transformed (calculated as log (x+1)) prior to the analysis.

Correspondence analysis

The data for the two types of community, shore level and sampling dates were further analysed using ordination techniques: As a preliminary measure a Detrended Correspondence analysis was carried out on the species-site data to determine the environmental gradient length. A short environmental gradient means that detection of unimodal species abundance curves would be unlikely and linear techniques such as Principal Component analysis or Redundancy analysis should be used. A gradient length of more than two is usually considered indicative of unimodal species abundance curves. In this study the gradient length for the first axis was longer than two and therefore a Canonical correspondence analysis was carried out (Table 2.1) for the species-environment data (Ter Braak 1986).

Table 2.1: Eigenvalues and gradient lengths for the first four axes in the preliminary detrended correspondence analysis for the species data for the three shore levels at Fort Island and two shore levels at Scarlett Point, indicating the length of the gradient of each axis

Axis	1	2	3	4
Eigenvalues	0.491	0.150	0.049	0.029
Gradient length	2.625	1.492	1.414	1.442
Cumulative % species variance	31.0	40.5	43.7	43.5

Exposure, shore level, epiphytism and sampling date were used as environmental factors. All variables were treated as nominal variables. In the analysis environmental gradients are presented as arrows, with long arrows being more related to the species patterns in the ordination than short arrows (the absolute length of the arrows is not important, only the relative length of one arrow compared to another). The significance of each environmental variable was tested with Monte Carlo permutation tests.

This analysis was carried out on the means of the log-transformed values from ten replicates at each shore level on each sampling date. There are a variety of multivariate ordination techniques for the analysis of shore community data. For a number of reasons correspondence analysis was deemed the most appropriate for the present study. Firstly, in contrast to many other techniques, it ordinates species and samples simultaneously (Hill 1973), it is also less affected by high beta diversity, i.e. large species differences between samples (James and McCulloch 1990, Gauch *et al.* 1977). Multivariate analyses were carried out using STATISTICA (Version 5.5) for classification analysis and CANOCO (Version 3) for the correspondence analysis.

The multiple choice feeding experiments presented statistical problems caused by a lack of independence of the data (Chapman 1990, Roa 1992, Lockwood 1998) but as the grazers often encounter these foods simultaneously in the field, it was therefore deemed a more realistic test of feeding preferences. Pavia *et al.* (1999) encountered similar problems but overcame them by using data on weight changes of only one randomly chosen tissue type per container and carried out a Two-way ANOVA. It has to be taken into account though, that such a procedure reduces the number of replicates. The same approach was used in the present study.

After randomly selecting the data for analysis using random number tables differences in feeding rates between grazers on different diets were analysed using a Two-way Analysis of Variance. Prior to the analysis, data were examined for heterogeneity of variances using Cochran's C test and where necessary were log transformed (calculated as log x+1) for feeding rates expressed as grams of tissue consumed per day.

2.3 Results

2.3.1 Epiphyte distribution

The epiphyte *Polysiphonia lanosa* was clearly zoned. At Fort Island it occurred almost exclusively in the low zone samples, occasionally in mid zone samples and in only one of the high zone samples at Fort Island. At Scarlett Point *P. lanosa* was very abundant in both transects, but epiphyte loads were significantly higher in the low zone transect. The biomass of *Polysiphonia lanosa* (per kg of host tissue) was considerably lower at Fort Island than at Scarlett Point. No differences were found between sampling dates (Table 2.2, Figure 2.1 a-b).

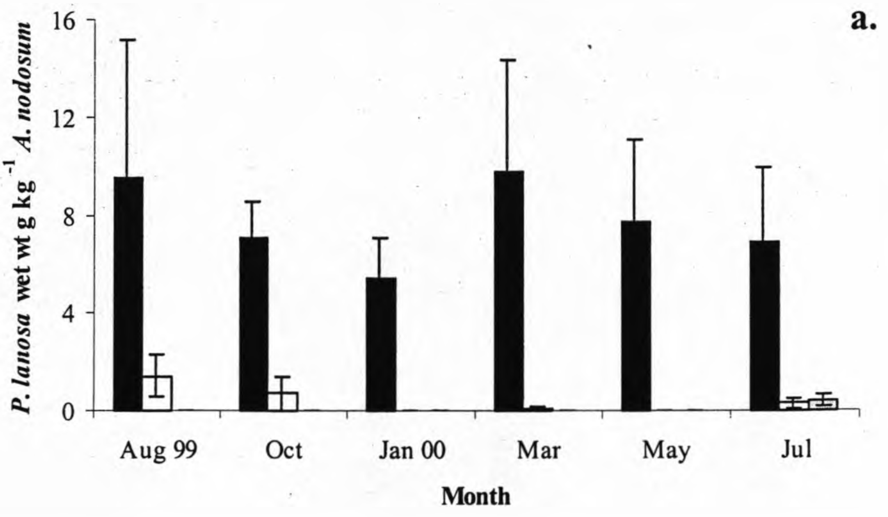
Table 2.2: ANOVA results for the seasonal changes in *P. lanosa* abundance on its host *Ascophyllum nodosum* at Fort Island and Scarlett Point. At Fort Island a one-way analysis was performed for the low zone samples. At Scarlett Point a two-way analysis was carried out incorporating sampling date and shore level using a GLM model.

a. Fort Island

Source of Variation	df	MS	*F*	p-value
Date	5	67.559	0.516	0.763
Error	54	130.964		

b. Scarlett Point

Source of Variation	df	MS	*F*	p-value
Intercept	1	352.137	188.2	<0.0001
Shore level	1	32.903	17.59	<0.0001
Date	4	3.505	1.873	0.128
Error	55			



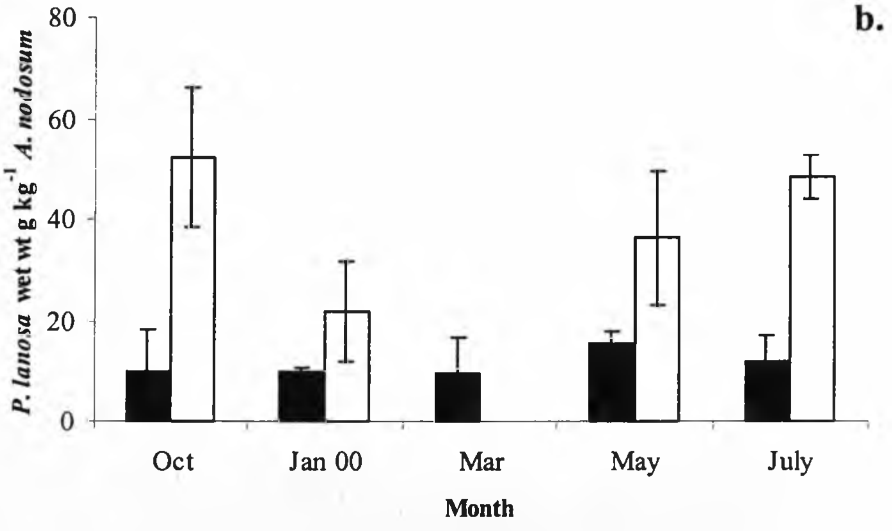


Fig 4.1 The seasonal changes in distribution of the epiphyte *Polysiphonia lanosa* on its host plant *Ascophyllum nodosum* **a.** three shore at Fort Island and; black bars= high zone, grey bars=mid zone, white bars=low zone **b.** two shore levels at Scarlett Point; black bars=high zone, white bars=low zone. Data are means of 10 samples for Fort Island and 8 samples for Scarlett Point. Error bars are ± 1 standard error

2.3.2 *Grazer Community Analysis: Relationships between common grazers*

2.3.2.1 Diversity indices

The total number of species present at the sheltered site (all ten replicates combined) ranged from 7 in the high zone transect in January to 14 in the low zone transect in May. No clear patterns emerged with respect to the number of species present. The number of species per sample was generally low in all transects and at all sampling dates compared with previous studies and varied only slightly seasonally (Figure 2.2 a).

The number of individuals showed more distinct patterns. The fewest organisms were found in January on both high and low zone transects but in March on the Mid zone transect. The greatest seasonal changes were found at the low zone transect where the numbers of organisms declined from an average of 170 in August to 24 in October (Figure 2.2 b). Two-way ANOVAS carried out for species numbers, number of individuals and species diversity revealed significant differences with shore level and sampling date for each of the three parameters (Table 2.3 a-c). There was also a significant interaction term in all three analyses, indicating that seasonal patterns of species numbers, number of individuals and species diversity differed between shore levels. In January the zone with the highest diversity was the low zone, whereas in October and May the mid zone samples were most diverse.

Table 2.3: Two-way ANOVA for the effect of shore level and sampling date on the number of species, number of individuals and diversity of the community

a. Number of species per sample

Source of Variation	df	MS	*F*	p-value
Shore level	2	12.885	6.301	0.002
Month	5	6.983	5.381	0.006
Interaction	10	7.237	3.550	0.0003
Error	154	2.038		

b. Number of individuals

Source of Variation	df	MS	*F*	p-value
Shore level	2	36326	15.106	<0.0001
Month	5	35604	14.806	<0.0001
Interaction	10	14244	5.923	<0.0001
Error	154	2404		

c. Diversity

Source of Variation	df	MS	*F*	p-value
Shore level	2	3.403	16.589	<0.0001
Month	5	1.166	5.684	<0.0001
Interaction	10	1.846	9.000	<0.0001
Error	154	0.205		

2.3.2.2 Changes in species composition with shore level and exposure

While the number of species did not vary greatly between sites, species composition varied considerably with shore level and exposure, and between samples, with several species only occurring in one or a few samples (Figure 2.3). Amphipoda in particular showed distinct distributions from high to low zone. At Fort Island *Eulimnogammarus obtusatus* was most abundant on the high zone, *Amphithoe rubricata* at the low zone transect with *Apherusa bispinosa* occurring at all shore levels in small numbers.

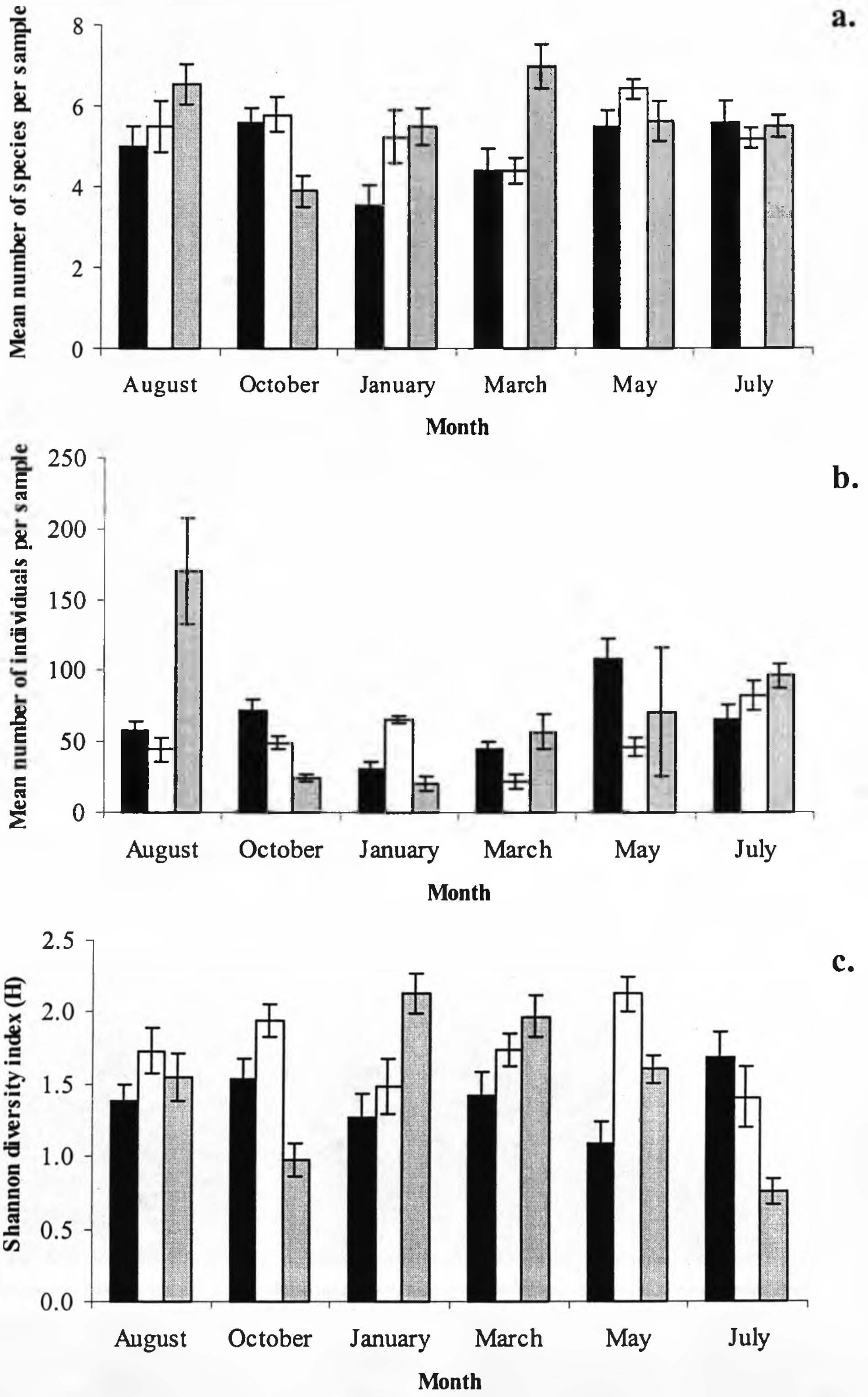


Fig 2.2 Bimonthly changes in **a.** the numbers of species, **b**. number of individuals and **c.** the Shannon diversity index in the samples for high shore (black bars), mid shore (open bars) and low shore (grey bars) at Fort Island. Data presented are the means of 10 samples. Error bars are ±1 standard error.

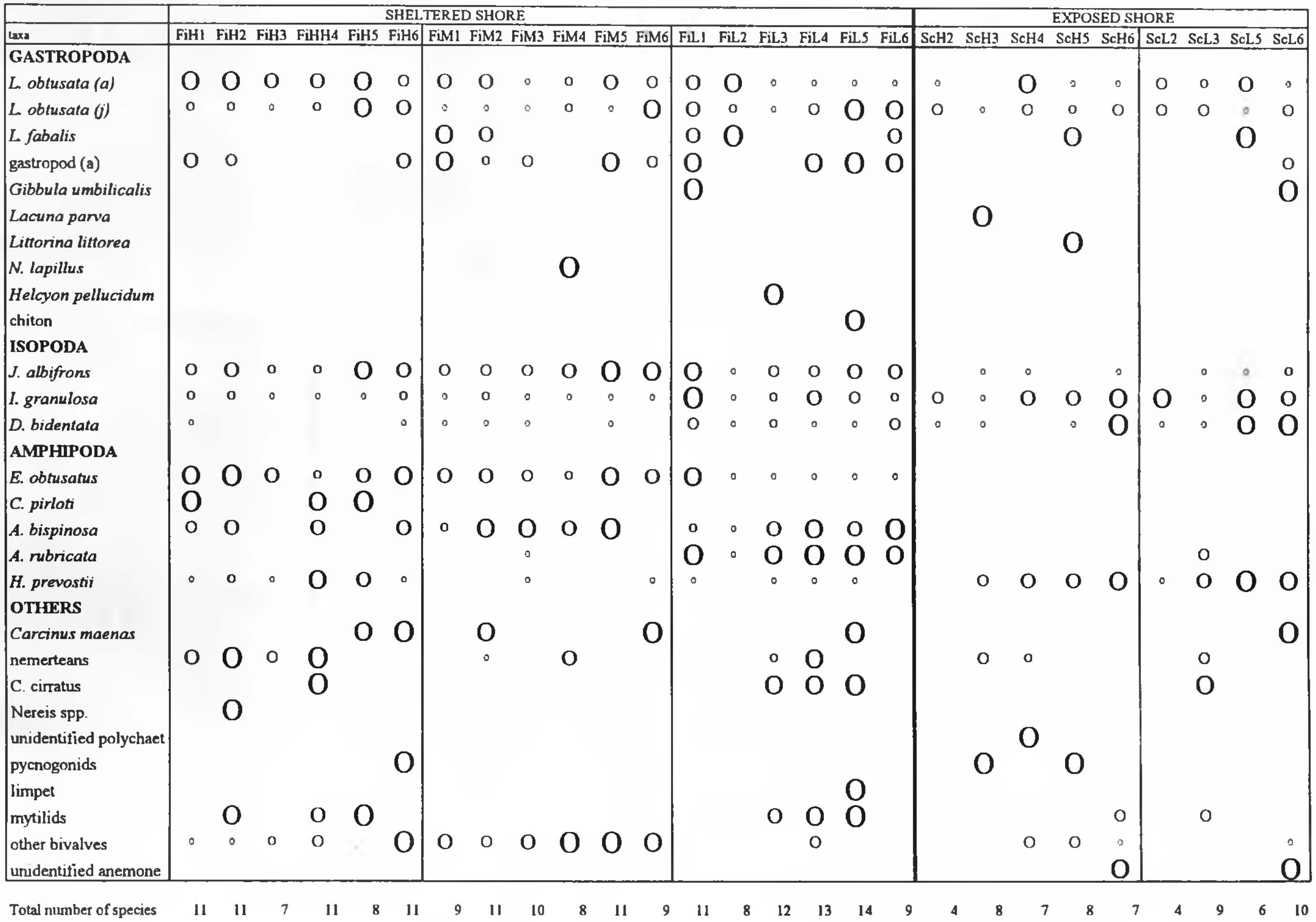


Fig 2.3 Species matrix showing the seasonal changes in the relative distribution of invertebrate species at the three shore levels at Fort Island and two shore levels at Scarlett Point between October 1999 and July 2000. (FIH= Fort Island high shore, FIM=Fort Island mid shore, FIL=Fort Island low shore) 1=August, 2=October, 3=January, 4=March, 5=May, 7=July)
For each species the size of the symbols represents the abundance of the species in a sample. The largest symbols indicate the sample of highest abundance. Comparisons of abundance are only possible between samples within a species but not between the same samples for different species, I.e. the same sized symbol can denote different abundances for different species

Two of these species were absent from the transects at Scarlett Point; the exception was *Amphithoe rubricata*, two individuals of which were found at the low zone transect at Scarlett in January. At Scarlett Point only one amphipod (*Hyale prevostii*) and two isopod species (*Dynamene bidentata* and *Idotea granulosa*) occurred in appreciable numbers (Figure 2.3).

Littorina obtusata and *Idotea granulosa* occurred on both shores, at all shore levels and sampling dates, albeit with different abundances (Figure 2.4). Two-way Analysis of Variance carried out for the dominant species revealed significant differences between shore level and season for *L. obtusata* (mature individuals only) and *E. obtusatus*. For *I. granulosa* only the differences between between sampling dates were significant. For all three species there was also a significant interaction term between sampling date and shore level, indicating that the changes in abundance across shore levels differed across months (Table 2.4).

The post-hoc tests showed that for *Eulimnogammarus obusatus* there were no significant differences between high and mid zone level, but densities were significantly lower at the low zone transect. For *Littorina obtusata* and *Idotea granulosa* the post-hoc procedure revealed significant differences between all shore levels.

Table 2.4: Two-way ANOVA for the comparison of grazer densities at different shore levels and sampling dates at Fort Island

a. *Littorina obtusata*

Source of Variation	df	MS	*F*	p-value
Shore level	2	15.815	47.447	<0.0001
Month	5	4.657	13.972	<0.0001
Interaction	10	1.832	5.497	<0.0001
Error	156	0.333		

SNK Procedure: Shore level: High> Low>Mid
Month: Aug=Oct, Mar=May, all other comparisons were statistically significant

b. *Idotea granulosa*

Source of Variation	df	MS	F	p-value
Shore level	2	0.240	0.722	0.487
Month	5	1.565	4.706	0.0005
Interaction	10	2.386	7.174	<0.0001
Error	162	0.333		

SNK Procedure: Month Aug> Oct-July, Oct=Jan=Mar=May=July

c. *Eulimnogammarus obtusatus*

Source of Variation	df	MS	*F*	p-value
Shore level	2	50.97	66.675	<0.0001
Month	5	9.197	12.100	<0.0001
Interaction	10	2.932	3.835	<0.0001
Error	157	0.765		

SNK Procedure: Shore Level: High=Mid > Low
Month: Aug=Oct, May=July, all other comparisons were significant

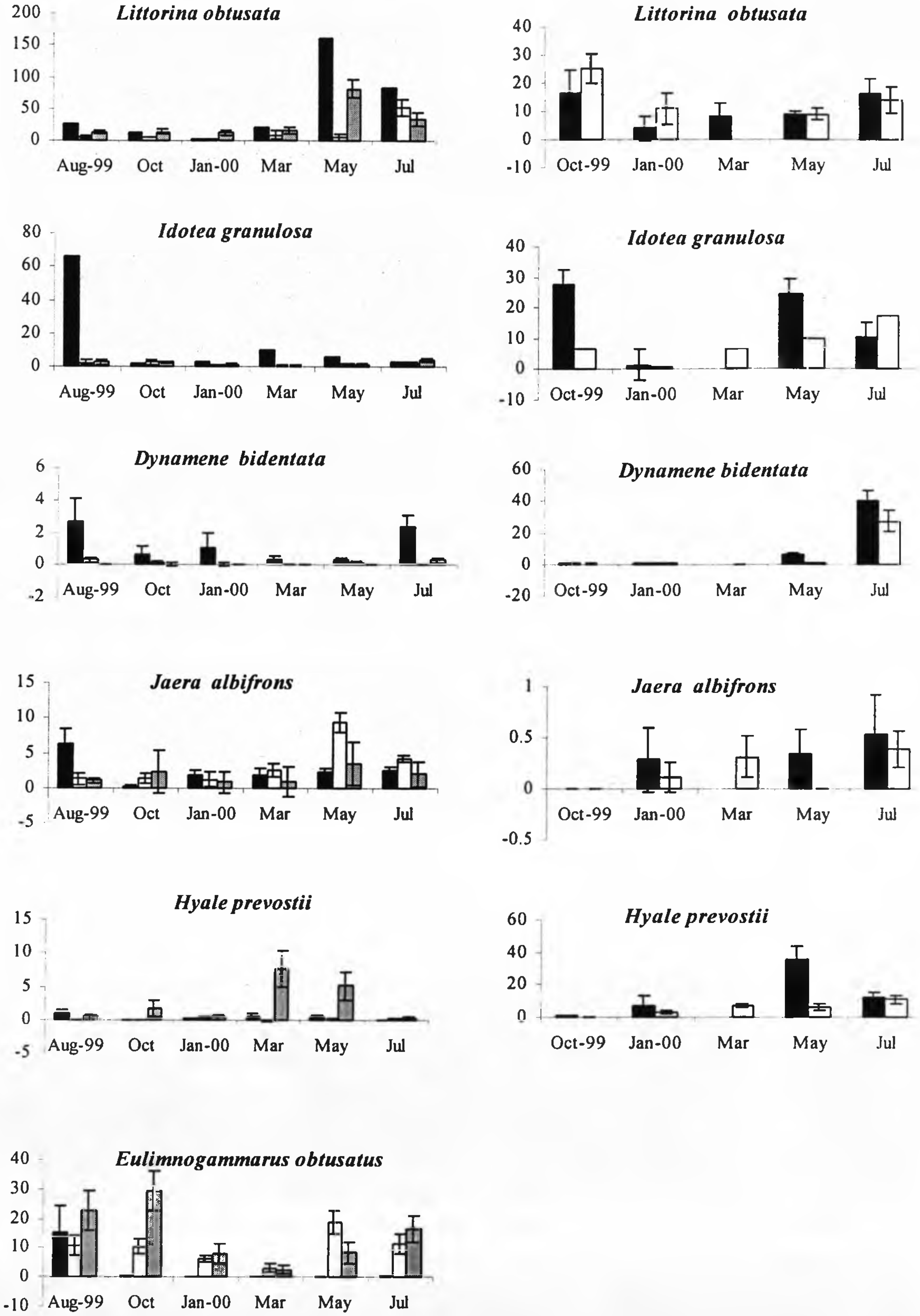


Fig 2.4 Seasonal changes in vertical distribution patterns of the most abundant species found in Fort Island and Scarlett Point: For Fort Island results are shown for High zone (Black bars), Mid zone (open bars) and Low zone. For Scarlett Point the data are presented for High zone (black bars) and low zone samples (open bars). Data presented are means of ten samples (Fort Island) and 8 samples (Scarlett). Error bars are ±1 standard error.

At Scarlett Point the identification of the grazers *Littorina obtusata* and *Littorina fabalis* proved to be problematic. Dissection of specimens had revealed the presence of large numbers of juveniles. The few mature individuals present were with few exceptions identified as *Littorina obtusata.* Many of the juveniles were thought to be *Littorina obtusata* judging from their external anatomy although the size frequency distributions were consistent with those expected for *Littorina fabalis*. For exposed shores at Fort Island there is clearly a need for a more detailed investigation into the external morphology of the two species as it can change from shore to shore (Williams 1990; Williams 1992. Reid pers. com.).

2.3.2.3 Seasonal changes

Of the isopod species only *Idotea granulosa* showed a distinct seasonal pattern at Fort Island with the appearance of large numbers of juveniles in August 1999. *Jaera albifrons* and *Dynamene bidentata*, occurred in very low numbers throughout the year. At the exposed site the patterns were very different. *D. bidentata* showed distinct peaks of abundance in July at the low zone transect and in May and July at the high zone transect while *I. granulosa* was abundant in all but the January and March samples. *Hyale prevostii*, the only amphipod to occur regularly at both the sheltered and exposed site, showed distinct seasonal patterns in both sites, although the maximum abundance occurred at different times and shore levels at Scarlett and Fort Island (Figure 2.4). *Idotea granulosa* was often associated with the epiphyte *Polysiphonia lanosa* in the low zone samples at Fort Island and at Scarlett Point (Figure 2.5). But significant positive correlations were found on only 3 occasions (Table 2.5a). The density of *Littorina obtusata* only showed a significant positive correlation with *P. lanosa* density in March

2000 (Table 2.5b, Figure 2.6). For the exposed shore there was a significant positive relationship for *L. obtusata* in both May and July at the low zone level. For *I. granulosa* this relationship was positive only in May.

Table 2.5: Correlation analysis using Pearson's product-moment correlation coefficient for the relationship between the density of two grazers, *Idotea granulosa* and *Littorina obtusata* with the wet weight of *Polysiphonia lanosa*. Data presented are presented for all sampling months at Fort Island and for May and July at the two transects at Scarlett Point

a. ***Idotea granulosa***

Fort Island Low shore

Month	r	p-value
August 1999	0.9777	<0.0001
October	0.5068	0.112
January 2000	0.4838	0.157
March	0.7792	0.008
May	0.3530	0.317
July	0.6231	0.073

Scarlett Point: High shore

Month	r	p-value
May	0.0368	0.931
July	0.2757	0.509

Scarlett: Low shore

Month	r	p-value
May	0.8965	0.002
July	0.8579	0.063

b. *Littorina obtusata*

Fort Island

Month	r	p-value
August 1999	0.1895	0.600
October	0.4343	0.210
January 2000	0.4838	0.157
March	0.9272	<0.0001
May	0.0903	0.804
July	0.4798	0.191

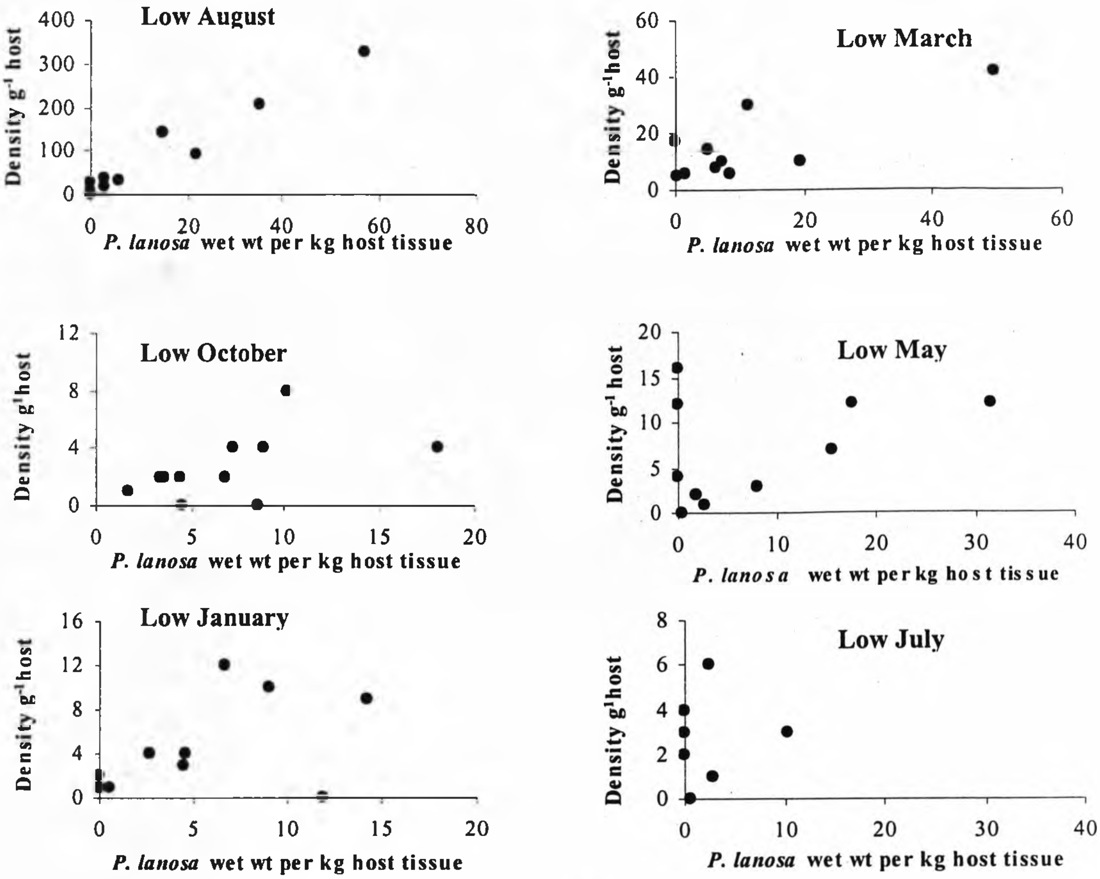
Scarlett Point: High shore

Month	r	p-value
May	0.0589	0.890
July	-0.1262	0.766

Scarlett Point: Low shore

Month	r	p-value
May	0.7872	0.020
July	0.8996	0.038

Fort Island



Scarlett Point

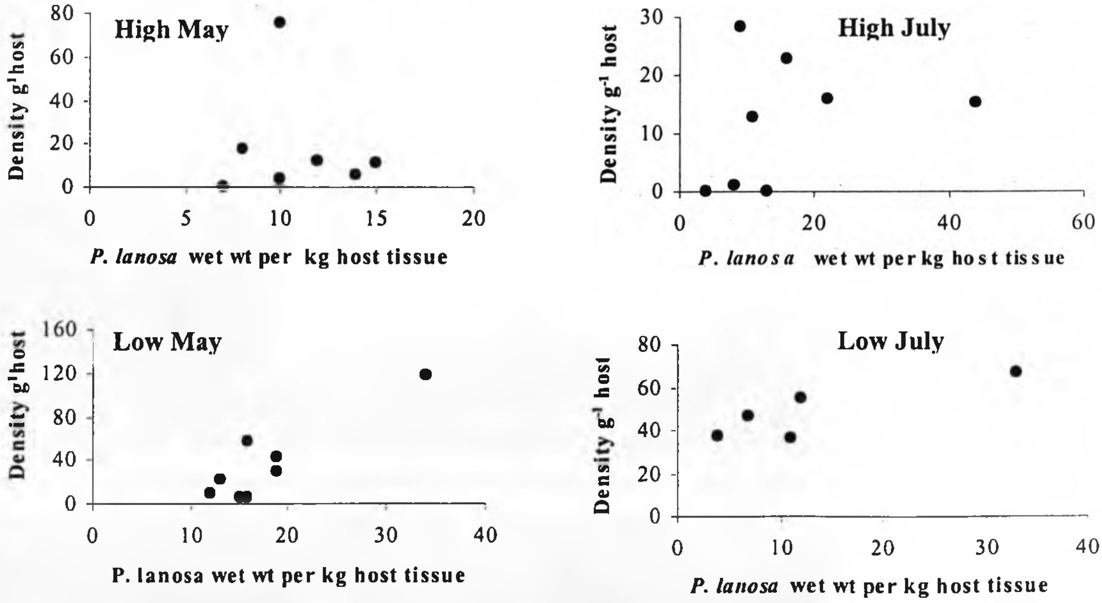


Fig 2.5 The relationship between the density of *Idotea granulosa* per kg host tissue and the wet weight of *Polysiphonia lanosa* for the six sampling dates at the sheltered site (Low zone) and the May and July data at the exposed site (high and low zone).

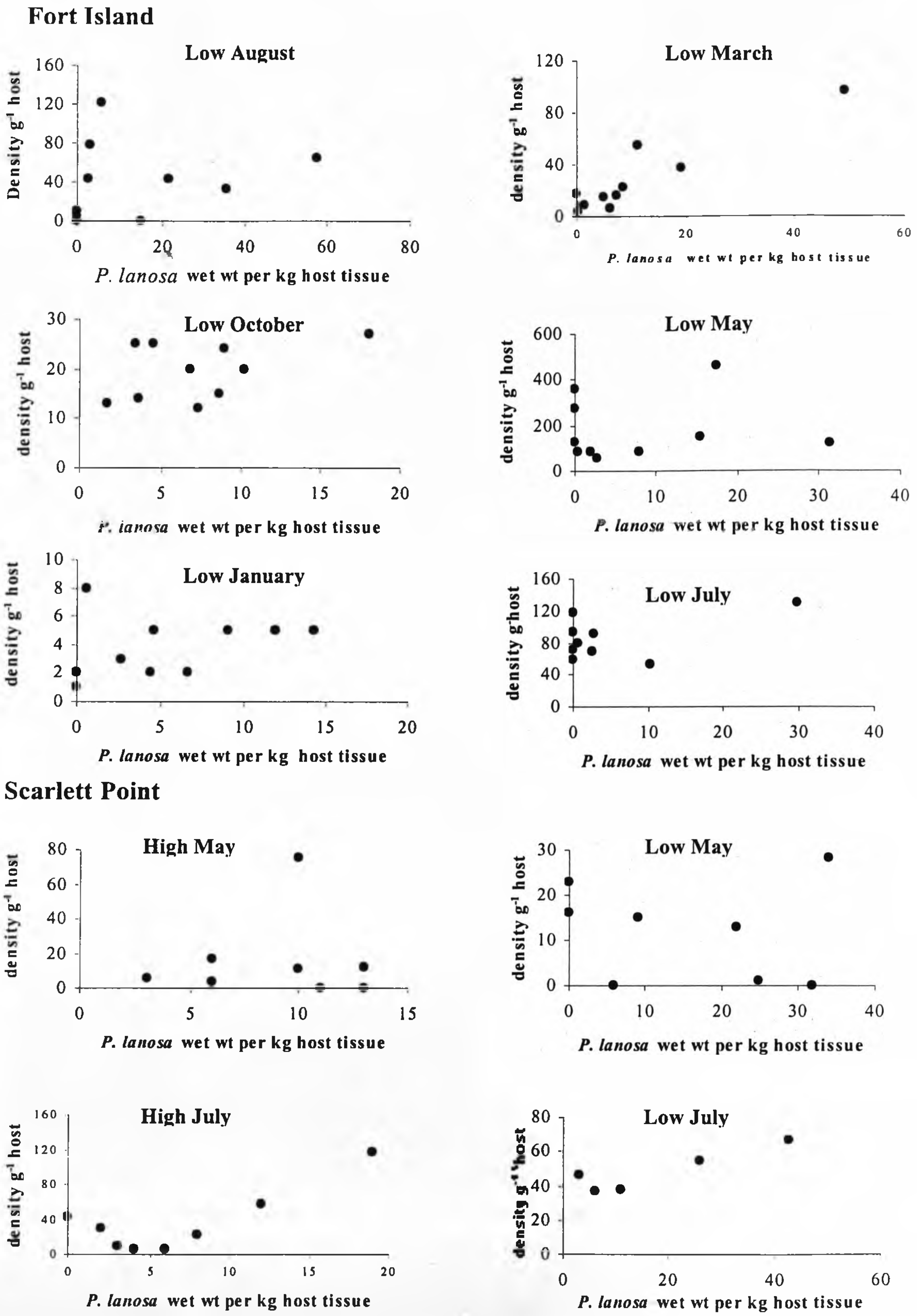


Fig 2.6 Relationship between the density of *Littorina obtusata* per kg host tissue and the wet weight of *Polysiphonia lanosa* for the six sampling dates at the sheltered site (Low zone) and the May and July data at the exposed site (High and low zone).

2.3.2.4 Size frequency distributions: Littorina obtusata and Idotea granulosa

The size-frequency distributions of *Littorina obtusata* were usually bimodal (Figure 2.7). One peak occurred at a shell width of 0.5-0.6 cm and a second peak at 1.5-1.6 cm. The magnitude of these peaks was very stable seasonally and at the three shore levels. However in May and July 2000 there were single peaks with large numbers of newly hatched snails (<than 0.3 cm in shell width) appearing in the high zone and low zone samples. Although reproduction peaked at this time, small numbers of newly hatched individuals were found throughout the year (Figure 2.7).

At the exposed site, size-frequency distributions were more uniform, the size range of individuals was narrower and individuals were generally considerably smaller in all samples. Individuals with a shell width of more than 0.9-1.0 cm were very rare. At the high zone transect, this larger size class was only found in January (one individual) and in March (7 individuals). In all samples from the exposed site the majority of individuals was smaller than 0.7 cm (Figure 2.8). This means that throughout the year this population consisted almost entirely of juveniles.

In contrast to *Littorina obtusata* the size-frequency distributions for *Idotea granulosa* were usually unimodal (Figure 2.9). From March onwards the distribution was skewed towards smaller individuals. Juveniles of this species were particularly abundant at the low zone site. At Scarlett Point smaller individuals also dominated the population in May and July (Figure 2.10).

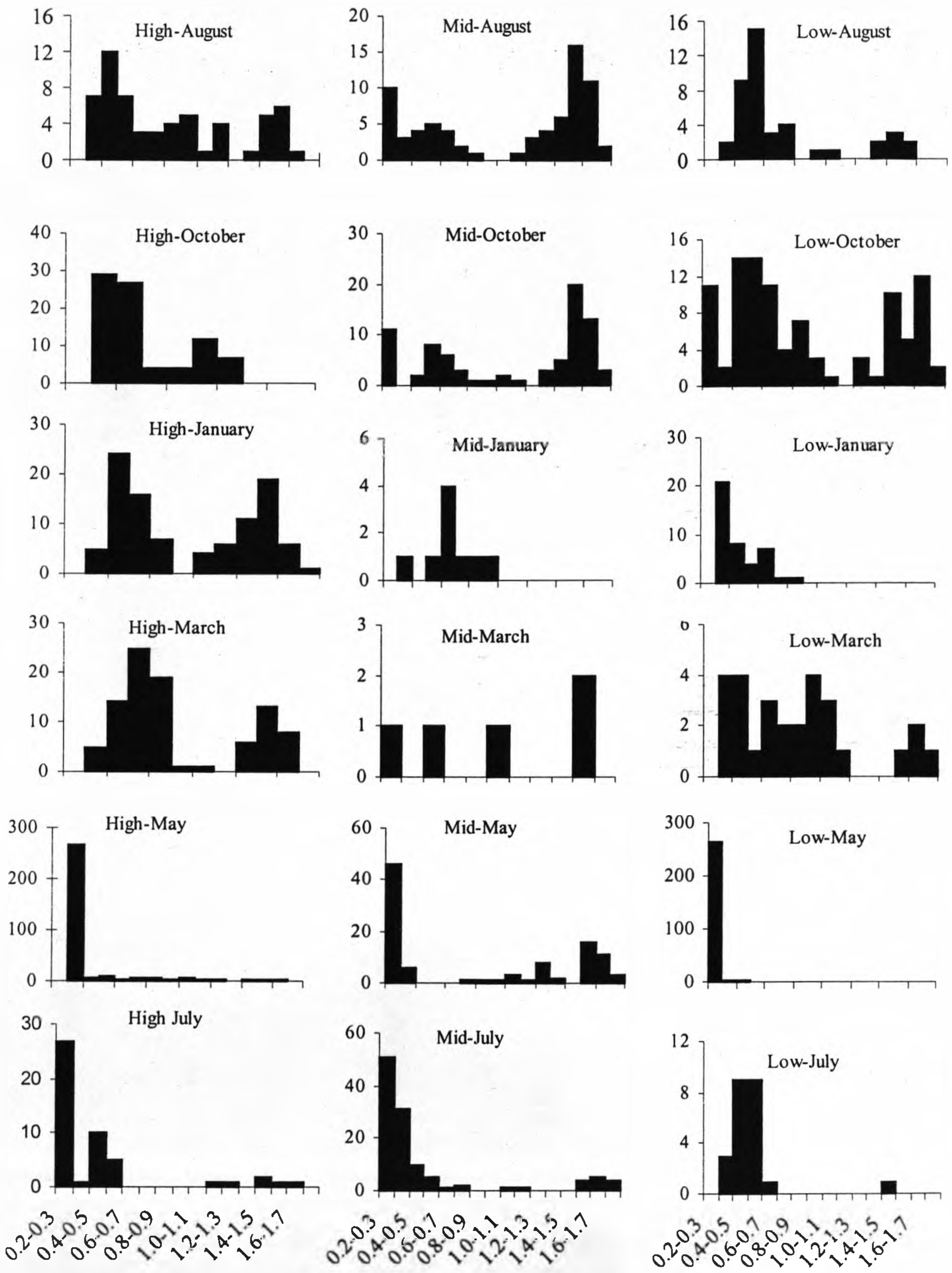


Fig. 2.7 Size frequency distributions of *Littorina obtusata* at three shore levels at Fort Island between August 1999 and July 2000. Categories for shell width are given on the x-axis and numbers of measurements per category on the y-axis.

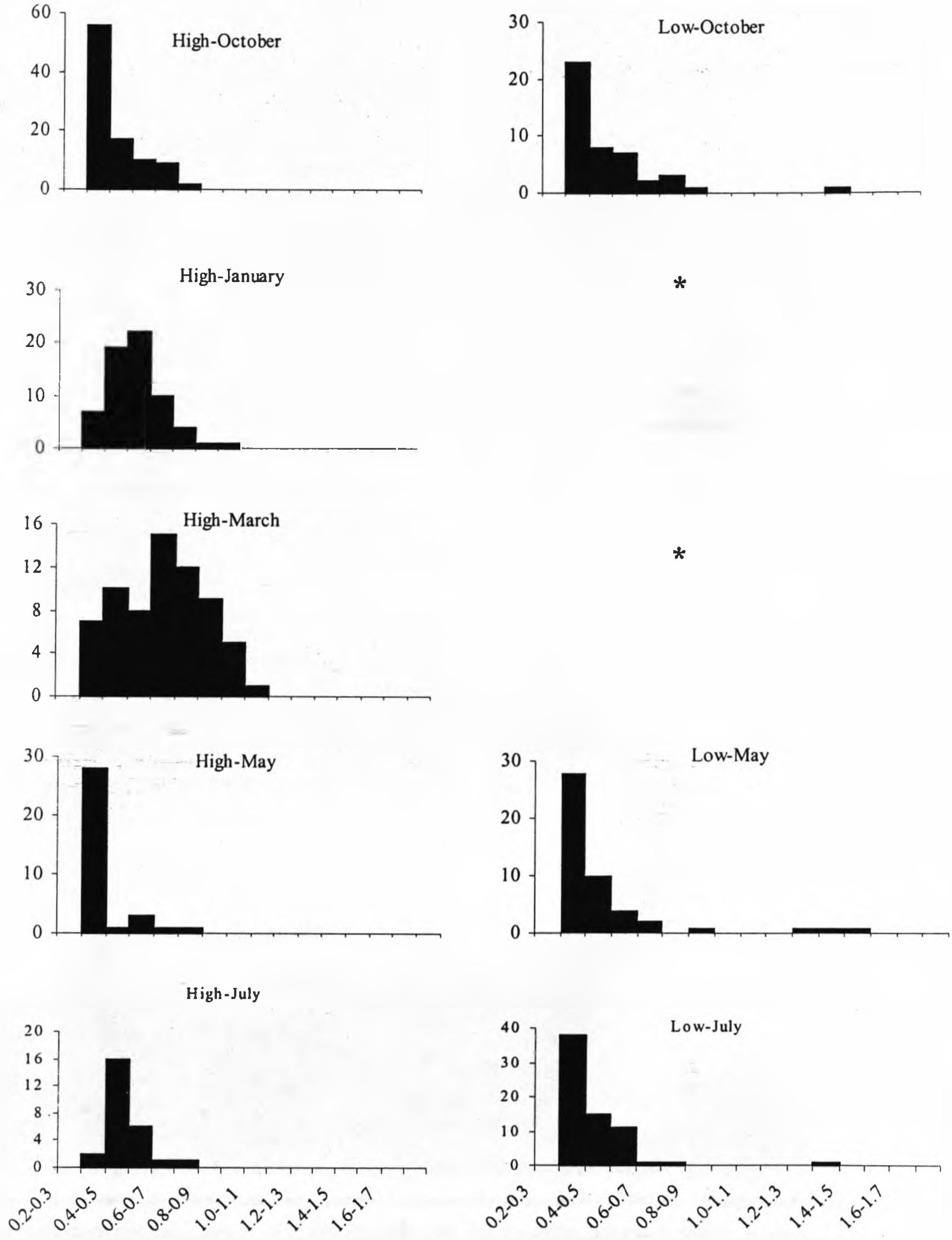


Fig 2.8 Size frequency distribution of *Littorina obtusata* at two shore levels at Scarlett Point between October 1999 and July 2000 *=too few organisms for analysis. Categories for shell width are given on the x-axis and measurements per category on the y-axis.

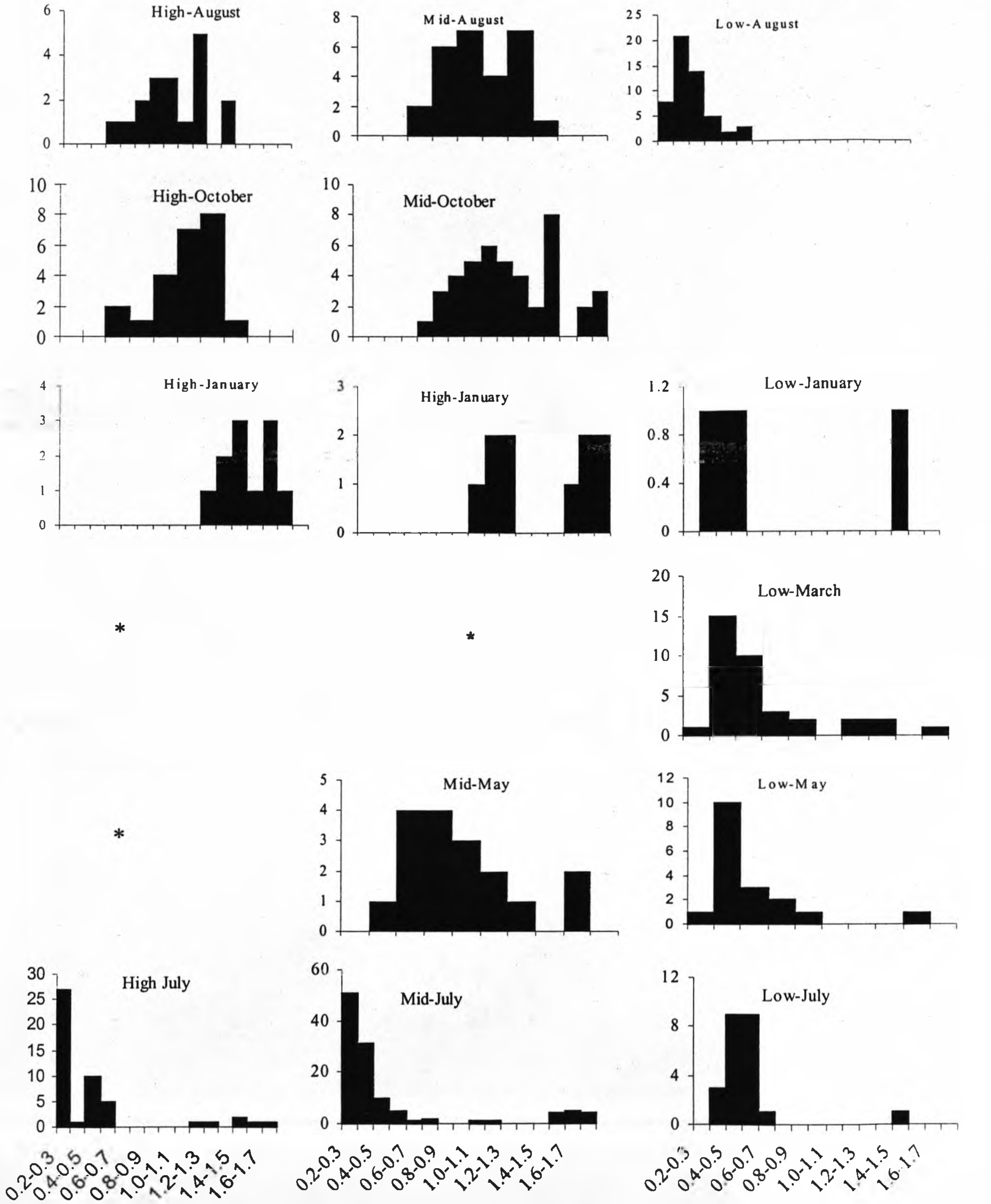


Fig 2.9 Size frequency distributions of *Idotea granulosa* with shore level at Fort Island between August 1999 and July 2000. Months marked * did not contain enough animals for the analysis. Categories for body length are given on the x-axis and measurements per category on the y-axis

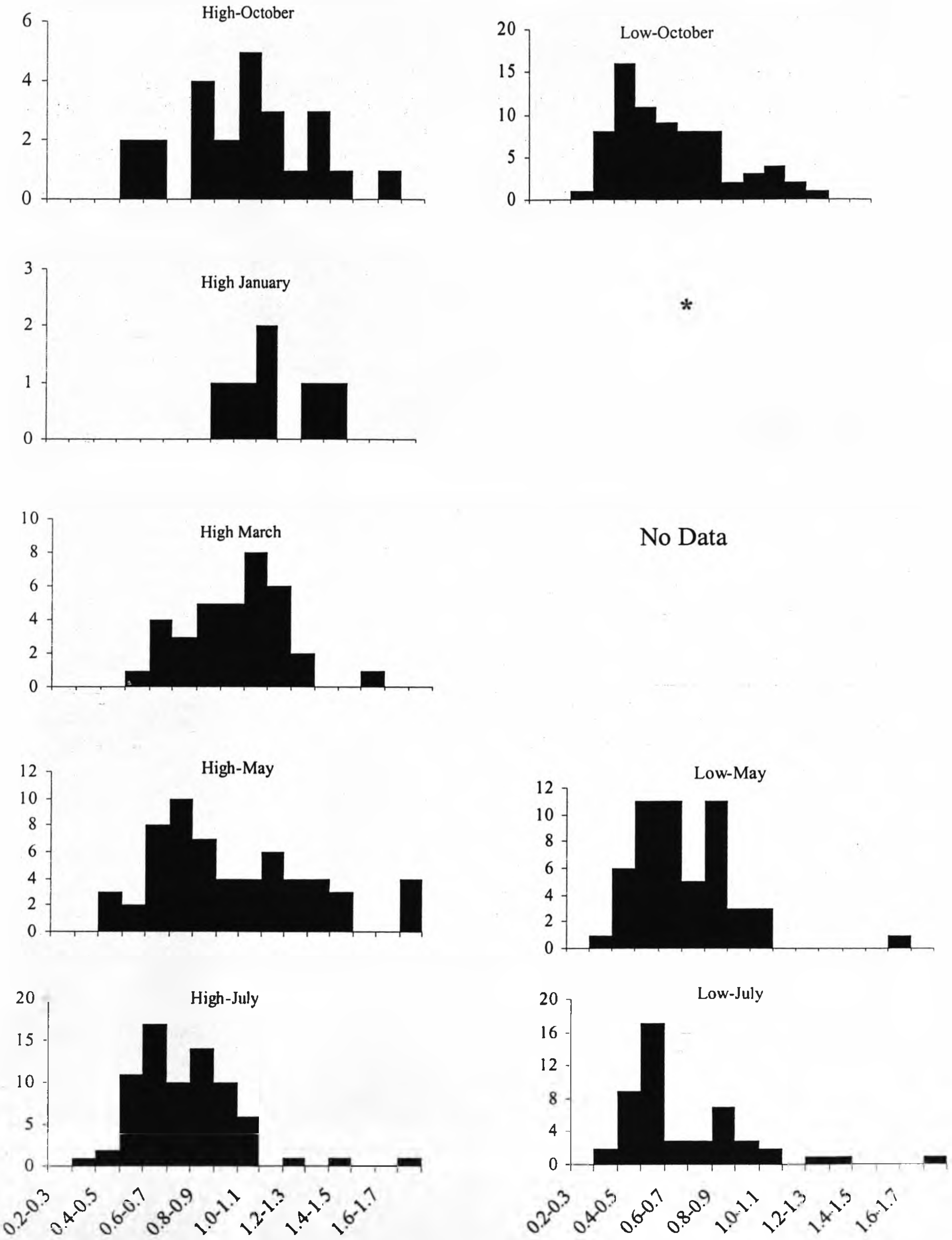


Fig 2.10 Size frequency distribution of *Idotea granulosa* at two shore levels at Scarlett Point between October 1999 and July 2000. Month marked * did not contain enough animals for the analysis. Categories for body length are given on the x-axis and measurements per category on the y-axis.

2.3.2.5 Co-occurrence of grazer species

At Fort Island the dominant grazer species co-occurred at all shore levels. However, none seemed to be affected negatively by the presence of the others. As an example, for the low shore samples at Fort Island a significant positive relationship was found in March between the density of *Idotea granulosa* and *Littorina obtusata* (Figure 2.11). In May the relationship was just significantly positive, while at all other sampling dates no significant relationships were found. No significant relationships were found for the higher shore levels. (Table 2.6a-c, Figure 2.11).

Table 2.6: Results of correlation analyses using Pearson's coefficient for the relationship between densities of *Littorina obtusata* and *Idotea granulosa* at High and Mid zone levels in Fort Island in May and July 2000 and at low zone level between August 1999 and July 2000, at the time of highest juvenile abundance

a. High zone

Month	r	p-value
May	0.1745	0.630
July	0.3732	0.288

b. Mid zone

Month	r	p-value
May	-0.3556	0.348
July	-0.4999	0.141

c. Low zone

Month	r	p-value
August 1999	0.1059	0.771
October	0.1351	0.710
January	-0.0588	0.872
March	0.9191	<0.0001
May	0.6276	0.052
July	0.3135	0.378

Fort Island

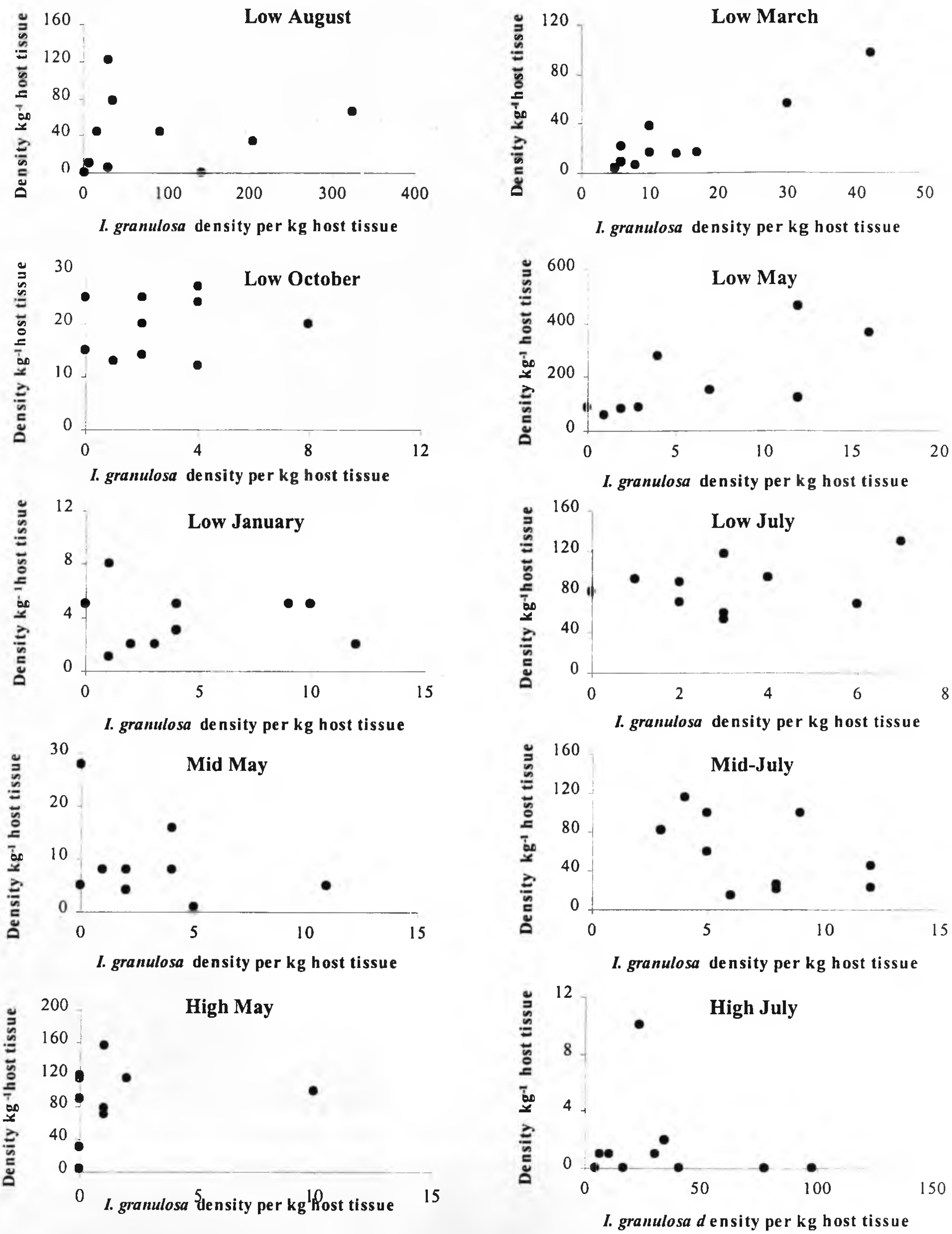


Fig 2.11 The relationship between *Littorina obtusata* (on y-axis) and *Idotea granulosa* densities at Fort Island in the low zone (August-July), mid zone (May-July), and high zone (May-July).

2.3.2.6 Multivariate community analysis

The correspondence analysis carried out for all sites and sampling dates revealed a strong species-environment correlation with the first axis of CCA. The first 4 axes of the ordination explained 48.1% of the total variation in species composition and all of the species-environment interactions (Table 2.7).

Table 2.7: Eigenvalues and species environment correlations for the first 4 axes in the canonical correspondence analysis carried out for the shore survey data

Axis	1	2	3	4
Eigenvalues	0.393	0.199	0.141	0.028
Species –environment correlation	0.907	0.752	0.786	0.52
Cumulative % variance				
Of species	24.9	37.4	46.3	48.1
Of species-environment relation	51.6	77.8	96.3	100

The Monte-Carlo permutation test showed significant results for exposure, epiphytism and sampling date, but not for shore height (Table 2.8).

Table 2.8: Results of the Monte-Carlo Permutation test for the Canonical Correspondence Analysis carried out on the data from Fort Island and Scarlett Point

Factor	Lambda	T	p-value
Epiphytism	0.37	7.76	0.005
Date	0.19	4.39	0.005
Exposure	0.17	4.44	0.005
Shore height	0.03	0.93	0.510

As an arch effect had occurred in the biplot of the first axes (Figure 2.12), the analysis was run again as a detrended canonical correspondence analysis (Table 2.9). An arch effect can occur due to an approximately quadratic dependence of the 2. axis on the first. The detrended canonical correspondence analysis lead to a decrease in eigenvalues for all but the first axis. Species-environment correlations were also found to be very low for axes 2-4. Epiphytism and exposure were most highly correlated with the firs axis. Shore height was most closely correlated with the second axis, while the sampling date related to the third axis, albeit less strongly than the others (Table 2.10).

Table 2.9: Eigenvalues and species environment correlations for the first 4 axes in the detrended canonical correspondence analysis carried out for the shore survey data

Axis	1	2	3	4
Eigenvalues	0.393	0.032	0.003	0.001
Gradient length	1.918	0.029	0.618	0.403
Species –environment correlation	0.903	0.624	0.7	0.437
Cumulative % variance				
Of species	24.9	26.9	27.1	27.2
Of species-environment relation	51.5	60.9		

Table 2.10: Correlation coefficients for the 4 environmental variables with axes 1-4 in the detrended canonical correspondence analysis

Axis	1	2	3	4
Environmental variable				
Exposure	0.7630	0.2327	-0.0619	0.2229
Shore height	0.1025	-0.1645	-0.3126	-0.3071
Epiphytism	0.8748	0.0137	-0.3659	-0.0272
Date	0.2874	-0.1117	-0.1311	0.2449

The species-environment biplot in which the environmental variables are shown as arrows, revealed an association of several species including *Idotea granulosa* and *Dynamene bidentata* with the epiphytised sites and the same species were also more abundant at exposed than at sheltered sites. *Hyale prevostii*, *Littorina fabalis* and *D. bidentata* were also associated with exposed shores. Juvenile littorinids were positively correlated with epiphytised sites whereas the adults were associated with the sites containing clean plants of *A. nodosum*.

2.3.2.7 Feeding Experiments

Feeding rates of the three species were similar with respect to food type in the single diet experiments (Figure 2.13 a). A two-way ANOVA examining the effect of grazer species and food type found no significant result for either food type or grazer species (Table 2.11). In the feeding experiment with *Polysiphonia lanosa, Idotea granulosa* consumed considerably less than *Eulimnogammarus obtusatus* (Figure 2.13 b-c). Separate t-tests for comparison of the feeding rates of the two grazer species and the growth of the control plants (indicated by negative consumption rates in figure 2.13) revealed significant differences in both cases (*I. granulosa*: df = 28, p = 0.017; *E. obtusatus*: df = 14, p = 0.031).

Table 2.11: Two-way ANOVA results for feeding rates of *Idotea granulosa*, *Littorina obtusata* and *Eulimnogammarus obtusatus* in single diet experiments using either vegetative tissue or receptacles of *Ascophyllum nodosum*

	DF	MS	F	P-value
Tissue	1	0.000011	0.074	0.786
Species	2	0.00085	0.579	0.563
Interaction	2	0.000075	0.474	0.624
Error	73	0.000147		

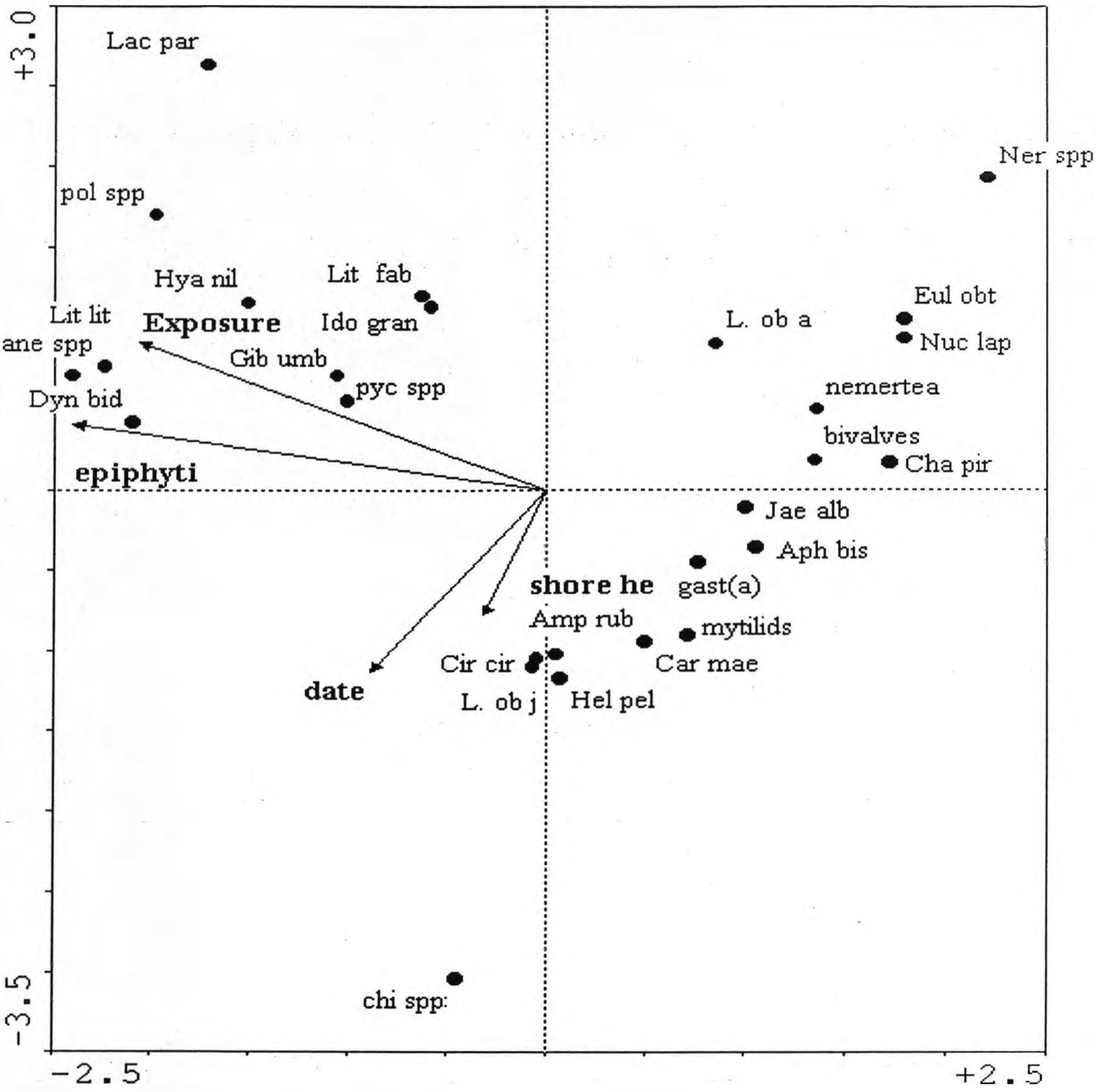


Fig 2.12 Species-environment biplot resulting from a canonical correspondence analysis carried out on the complete data set (6 sampling dates, 3 sheltered locations, 2 exposed locations); the species-environment biplot shows the 2. axis plotted against the 1. axis. Symbols: Amp rub=*Amphithoe rubricata*, ane spp.=anemone spp., Aph bis=*Apherusa bispinosa*, bivalves=other bivalves, Car mae=*Carcinus maenas*, Cha pir=*Chaetogammarus pirloti*, chi spp.=chiton spp., Cir. cir=*Cirratulus cirratus* Dyn bid=*Dynamene bidentata*, , Eul obt=*Eulimnogammarus obtusatus*, gast(a)= unidentified gastropod, Gib umb=*Gibbula umbilicalis*, Hel pel= *Helcyon pellucidum*, Hya nil= *Hyale nilssoni*, Ido gra=*Idotea granulosa*, Jae alb=*Jaera albifrons*, Lac par=*Lacuna parva,* Lit mar=*Littorina fabalis*, Lit lit=*Littorina littorea* L. ob a=*Littorina obtusata*, L ob j= *Littorina* obtusata juveniles, mytilids= mytilid spat, nemertea=nemertea spp., Ner spp=*Nereis* sppNuc lap=*Nucella lapillus*, pol spp=Polychaete spp, pyc spp.=pycnogonid spp.

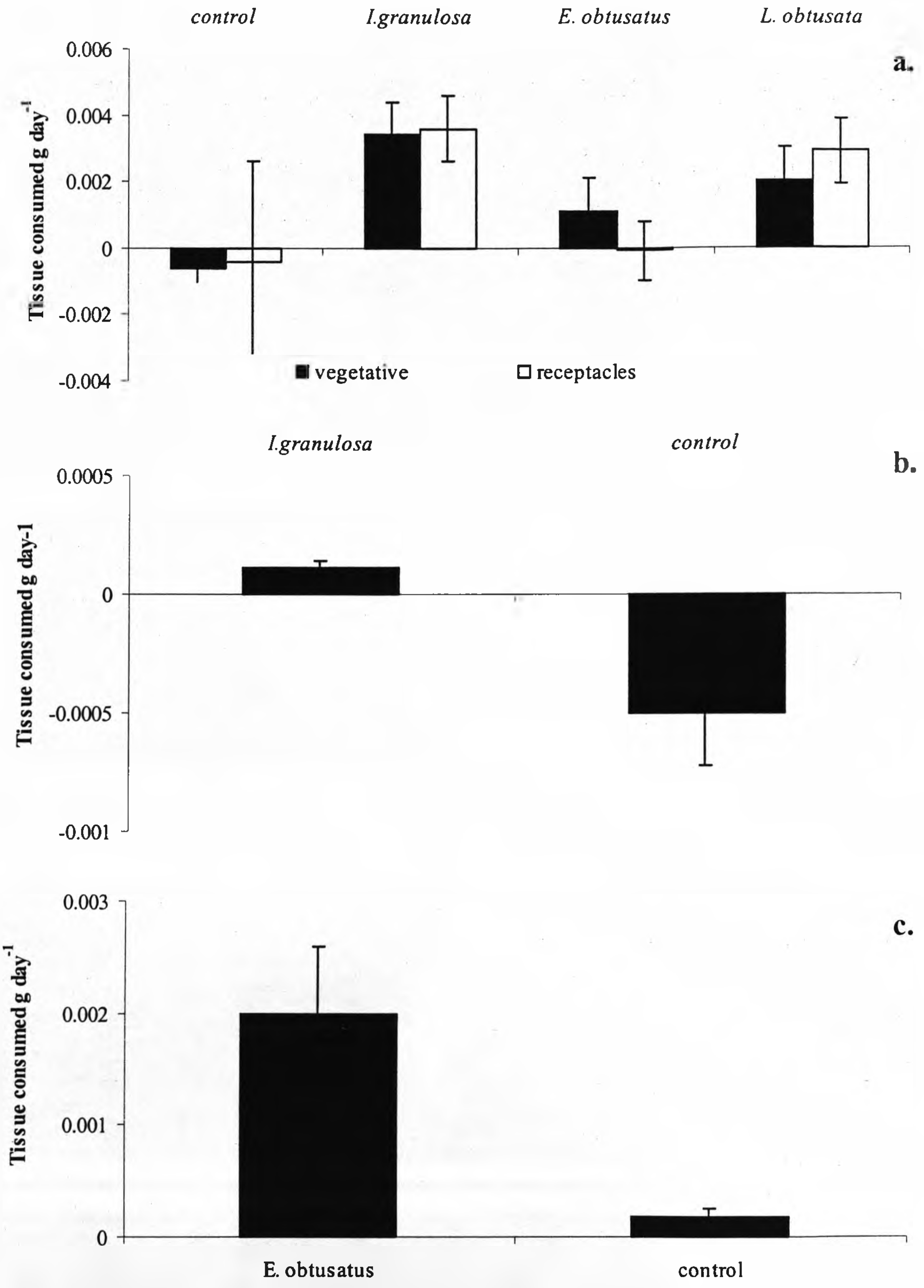


Fig 2.13 **a.** Feeding rates of *Littorina obtusata*, *Idotea granulosa* and *Eulimnogammarus obtusatus* in a single diet experiment. Grazers were presented with either vegetative tissue or with receptacles of *Ascophyllum nodosum* Error bars are ±1 standard error, **b.** and **c.** single diet experiments with *E. obtusatus* and *I. granulosa* on *Polysiphonia lanosa.*

In the multiple choice experiment *Idotea granulosa* showed the highest feeding rates on vegetative tissue, while *Littorina obtusata* and *Eulimnogammarus obtusatus* fed on *Polysiphonia lanosa* (Figure 2.14a). However these patterns were only partly borne out by the two-way ANOVA carried out to examine the relationship between diet and grazer species which revealed significant differences in feeding rates on differenty tissue types, but not between grazer species(Table 2.12).

Table 2.12: Two-way ANOVA results for feeding rates in the multiple choice test of *Idotea granulosa*, *Littorina obtusata*, and *Eulimnogammarus obtusatus* on vegetative tissue and receptacles of *Ascophyllum nodosum* and a piece *Polysiphonia lanosa*. Data are presented for feeding rates after 10 days.

	df	MS	*F*	p-value
Tissue type	2	0.076	7.310	0.002
Species	2	0.010	0.998	0.378
Interaction	4	0.030	2.893	0.035
Error	37	0.010		

The absence of significant effects between grazer species, might have been due to the small number of replicates, which had been the result of random sub sampling of the original non-independent data. The only significant effect was an interaction effect, which is to be expected, when there are significant feeding preferences. A comparison of feeding rates in the single diet experiment and the total average amount of tissue consumed in the multiple choice experiments showed that feeding rates of *Idotea granulosa* were very similar in both studies. Feeding rates for *E. obtusatus* seemed to be slightly higher in the single diet study. However these differences were not tested statistically.

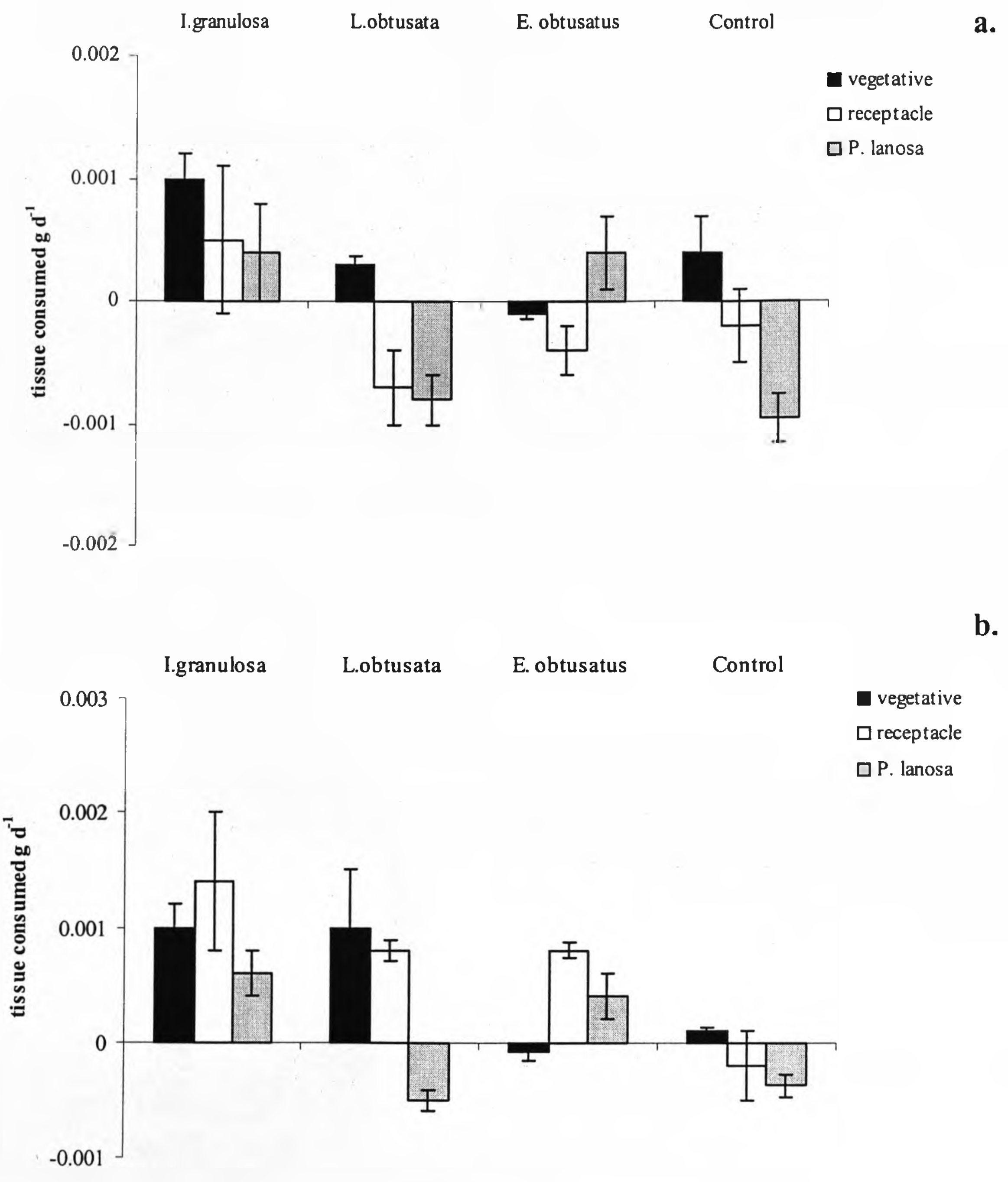


Fig 2.14 Feeding rates of *Idotea granulosa*, *Littorina obtusata* and *Eulimnogammarus obtusatus* in a multiple choice feeding experiment. The grazers were presented with vegetative tissue (black bars) and receptacles (white bars) of *Ascophyllum nodosum* and *Polysiphonia lanosa* (grey bars). Feeding rates are given in mg consumed per day. **a.** after 10 days (data presented are means of 15 replicates), **b.** after 20 days. Error bars are 1 standard error.

2.4 Discussion

The grazer species found in the present study appeared to be a typical assemblage on *Ascophyllum nodosum*. Previous authors for a wide range of geographical locations have reported similar assemblages and seasonal dynamics of grazers such as *Idotea granulosa* and *Littorina obtusata* (Johnson and Scheibling 1987, Pavia *et al.* 1999) As in the study by Johnson and Scheibling (1987) my study also revealed a degree of association of grazers with the epiphyte. This was the case particularly for *Idotea granulosa* and occasionally for *L. obtusata*, but such significant associations were not found for the amphipod species, mainly because in the low zone, where the epiphyte occurred, amphipods were not very abundant, although in the feeding experiments they consumed the epiphyte.

Positive relationships between *Idotea granulsoa* density and *Polysiphonia* load were found during times when juvenile grazers were abundant but not when larger individuals dominated the populations. An association of juvenile organisms with microhabitats such as filamentous algae has been described for a number of aquatic systems. Juvenile fish for instance are often found in vegetation rather than open water (Werner and Hall 1988) and it has also been found for mussels (Petersen 1984) and intertidal snails (Martel and Chia 1991) that survival was higher among filamentous algae than in open areas or on macroalgae.

The selection of microhabitats such as filamentous algae by juvenile grazers can be the result of a variety of factors, a common one being predation (Lewis 1964). Predation by the shore crab *Carcinus maenas* for example is common on many rocky shores (Williams 1992). This crab, which is known to feed on *Littorina obtusata* and small crustaceans (Gee 1987), occurred at all shore levels at Fort Island in May and July, when the abundance of juveniles was high. Reimchen (1982) has argued that the size range of *Littorina obtusata* most susceptible to *Carcinus* was between 16-17 mm shell diameter. However, juvenile crabs are able to feed on smaller sized prey such as juvenile isopods or newly hatched littorinids (van der Meer *et al.* 1998). Therefore crab predation might have caused the correlation between *I. granulosa* and the epiphyte. It has to be noted that the distribution of the epiphyte on *Ascophyllum* is very patchy (Lobban and Baxter 1983) and therefore *Polysihonia lanosa* is a spatially very unpredictable habitat as a refuge from predation. Salemaa (1987) found that *Idotea baltica* fed predominantly on filamentous algae as juveniles, but switched to a macroalgal diet when adult. If it is assumed that this also holds for *I. granulosa*, then feeding by large numbers of juveniles are unlikely to affect *A. nodosum* biomass significantly.

Correlations between the abundance of *Polysiphonia lanosa* and *Littorina obtusata* were rarer indicating that both as juvenile and adult, this species might feed predominantly on its host plant rather than the epiphyte although this was not specifically tested. *Littorina* juveniles often accumulate in air bladders and injured sites on their food plant, which could provide an alternative refuge from predators . *L. obtusata* lays its eggs directly on *Ascophyllum* fronds even if there are no epiphytised fronds nearby. The offspring emerge as crawling young, and large numbers of juveniles were found throughout the *Ascophyllum* zone even on the higher transect where *P. lanosa* was absent.

Significant relationships between juvenile littorinids and *Idotea granulosa* on one hand and *Polysiphonia lanosa* on the other were also found at Scarlett Point in July although no *Carcinus maenas* was found in any of the samples. It is possible that at the exposed site physical factors such as wave action and increased desiccation rather than predation caused the association between the grazers and *P. lanosa*.

Desiccation is likely to be a greater risk on exposed shores without dense canopies of *Ascophyllum nodosum*. As with predation, the risk of mortality from desiccation can lead to aggregations of individuals in refuges such as rock crevices (Lewis 1964, Moore and Seed 1985). Again, such refuges might be more important for juveniles than for adults. Berry and Hunt (1980) and Atkinson and Newbury (1984) for example showed that crevices are likely to increase survival of juvenile but not adult *Littorina rudis*. The importance of filamentous algae as a refuge from desiccation is studied less often, but Gosslin and Chia (1995) have found that mortality from desiccation was significantly lower in *Nucella emarginata* juveniles in tufts of *Cladophora* than on open surfaces or mussel clusters. In addition, the higher wave action on exposed shores will increase the risk of dislodgement (Trussell *et al.* 1993). Salemaa (1986) for example attributed the different size ranges of *Idotea granulosa* on different algal substrates to the physical characteristics of these algae, particularly their structural complexity and the ability of the organism to avoid dislodgement rather than to food preferences or avoiding predation. However, the risk of dislodgement might be less important for adults. In fact *Idotea granulosa* has been observed leaving its algal substrate and swimming freely during nightly foraging excursion (Holyck 1973) and should therefore be able to relocate onto *A. nodosum* fronds if dislodged, at least where the seaweed is very abundant.

While living in filamentous algae is clearly advantageous for juveniles, being associated exclusively with a dense algal canopy brings some obvious benefits to the adult. The environment provided by *Ascophyllum nodosum* is stable and predictable all year round. On a sheltered shore the thick layers of seaweed prevent penetration of light and desiccation within the canopy (Cousens 1985, Johnson and Scheibling 1987). It is known that *Littorina obtusata* and other littorinids have no capability for respiratory temperature acclimation. Instead they enter into a metabolic diapause at temperatures above or below their tolerance range (McMahon *et al.* 1993). While this adaptation might be advantageous to avoid respiratory stress, it also means that the snail cannot feed. Association with a dense seaweed canopy might therefore extend the conditions that allow foraging. However, these benefits will only be apparent during periods of emersion. When immersed, living on the seaweed might become disadvantageous. Davies and Case (1997) have shown that the tenacity of *L. obtusata* would not be sufficient to remain attached in breaking waves.

If dense *Ascophyllum* stands on sheltered shores are indeed providing a stable environment for the associated herbivore species , then this should lead to higher densities and therefore intense competition between the grazers (Huisman and Olff 1998). However, this was not the case. On the contrary, grazer densities were generally low compared with previous studies (Johnson and Scheibling 1987, Pavia *et al.* 1999) and there was a high degree of co-occurrence of grazer species; *Littorina obtusata* and *Idotea granulosa*, were commonly found in the same samples on *Ascophyllum nodosum* although feeding on the same type of food (Trussell 2000). One possible reason for the low overall densities is predation, which has been well documented in other studies (e.g. Hay *et al.* 1989, Trussell 2000). The low densities of grazers indicate a lack of competition between them showing that it is unlikely that the species were limited by food (Polunin and Klumpp 1992).

Some authors have argued that small marine grazers feeding on abundant seaweeds are unlikely to be food limited in the wild e.g. (Hay *et al.* 1987, Brawley 1992). Cruz-Rivera and Hay (2001) also showed that low nutritional quality of the alga can be compensated for by higher feeding rates without reducing fitness in the amphipod *Amphithoe longimana*. However, even if their densities are low, they might still have the capacity to significantly affect a plant by feeding predominantly on meristematic tissue or receptacles (Arrontes 1999, Pavia *et al.* 1999). Poore (1994) also described such behaviour for the amphipod *Hyale rubra* when feeding on *Zonaria angustata*. Pavia *et al.* (1999) found higher feeding rates of *Idotea granulosa* on meristematic than older tissue of *Ascophyllum nodosum*. Such selective feeding could mean that, even at low densities, competition between grazers might occur. In addition, removal of reproductive biomass would be expected to lead to a reduction in fitness of the food plant. However, considering the low feeding rates of the grazers and the high reproductive effort of the host plant, consumption of receptacles is likely to be a trivial source of loss. The feeding experiments in the present study had to be carried out in late autumn and it might be argued that low feeding rates, particularly those of *Littorina obtusata* were more an inability to acclimate to the temperatures at which the experiments were carried out since littorinids will decrease their metabolic rate and reduce feeding rather than acclimate to changes in temperature. However, similar feeding rates for *I. granulosa* and *L. obtusata* have been found by other authors, where feeding experiments were carried out in the summer Salemaa (1987) and Pavia *et al* (1999).

Santelices and Correa (1985) and Santelices (1992) demonstrated that the propagules of a number of seaweeds could survive the passage through the digestive tract of a grazer. Algal propagules excreted alive in fecal pellets might even benefit as the fecal coating protects them from desiccation and the stickiness of the pellets might aid attachment of the propagule to the substratum (Santelices and Paya 1989).

Nonetheless, considering the large standing crops of *Ascophyllum nodosum* on sheltered shores such preferences are still considered unlikely to have a significant direct impact, especially as the host plant is capable of producing new meristems from damaged sites on its fronds (Van Alstyne 1989).

The findings in this chapter have some implications for possible evolutionary relationships between the intertidal grazers and *Ascophyllum nodosum*. The densities of grazers are not high and the grazing rates appear to be too low to create a direct selection pressure either as individuals or collectively, as a set of species, on the host plant to evolve specific anti-grazer defences (Fox 1981, Janzen 1980). The absence of selection pressures resulting from feeding by herbivores would make reciprocal interactions between the host plants and its grazers unlikely.

However, even if grazers do not exert selection pressures on the host plant directly, they could do so indirectly by weakening fronds and increasing the risk of breakage during high flow conditions (Koehl 1986, Lowell *et al.* 1991). The latter authors found for *Ascophyllum nodosum* that loss of biomass from branch breakage could be up to 100 times higher than losses resulting from direct consumption by grazers. But they also demonstrated that damage to the frond surprisingly increased the tensile strength of the frond. This could ameliorate the effect of the damage and several authors have shown wound healing properties in a number of seaweeds (Fagerberg and Dawes 1976, DeWreede *et al.* 1992, Correa *et al.* 1999). The study by DeWreede *et al.* (1992) has shown that cuts in the stipe tissue of three kelp species increased loss rates for a period of one to two months, but after that period of time, the healed tissue could restore the mechanical properties to normal levels. In *Ascophyllum nodosum* old wounds that have healed can be frequently observed on the stipes. Although grazing can jeopardize some vegetative biomass, which will lead to the loss of some reproductive tissue as well, receptacles are produced on such a large scale that their production and fate is likely to be controlled by other factors.

Chapter 3: Reproductive patterns in *Ascophyllum nodosum*: roles of epiphytes and phlorotannins

3.1 Introduction

In most plants the production of vegetative growth is considered a vehicle to lead to reproduction. According to life-history theory, reproduction will incur a cost that will usually be manifested in trade-offs with other life history functions such as a reduction in vegetative growth, longevity and future reproductive effort (Stearns 1992). This implies that there is a limited resource, which prevents the allocation of materials to reproduction in competition with other processes in the organism's life (Pimentel 1976, Coley 1986, Bazzaz *et al.* 1987).

In many terrestrial and aquatic plants including brown algae, chemical defence against herbivores is one such competing process. More specifically, in brown algae phlorotannins are a characteristic feature, often occurring in large quantities, and it has been widely accepted that they play a role as an anti grazer deterrent (Coley 1983, Ragan and Glombitza 1986, Hay and Steinberg 1992, Targett and Arnold 1998).

A small number of studies have investigated the possible costs of phlorotannin production for vegetative or reproductive growth in seaweeds. But usually costs could not be established unequivocally, either in terrestrial or in marine systems (Simms and Rausher 1987, Pfister 1992, Pavia and Åberg 1996). However, costs associated with reproduction and the production of defensive chemicals would be a prerequisite for reciprocal evolutionary interactions, which have been postulated for a number of plant-herbivore interactions in terrestrial systems (Hay and Steinberg 1992).

Both, studies investigating the effects of phlorotannins on grazers and those examining the costs of phlorotannin production have shown inconsistencies with the interpretation of the functions of phlorotannins as anti-grazer defences. The induction of chemical defence following grazer attack for instance, while demonstrated in some species (van Alstyne 1988), has not been found in others (Lowell *et al.* 1991, Steinberg 1994). Hammerstrom *et al.* (1998) even found a decrease rather than an increase in phlorotannin concentrations after injury in the kelp *Hedophyllum sessile.* Furthermore Yates and Peckol (1993) and Peckol *et al.* (1996), who found induction of phlorotannins in the fucoid *Fucus vesiculosus*, noted that the phlorotannin concentrations were partially dependent on nutrient levels, which could influence the magnitude of the response of the plant to grazing.

The failure to demonstrate a cost may be due to several factors: that there really is no cost, or that a cost is incurred, but the wrong parameters have been measured and therefore do not show it or the parameter by which costs are assessed could also serve an alternative function not considered i.e. phlorotannins vary in response to some other factor.

Some additional functions of phlorotannins have been investigated e.g. protection from UV-B (Pavia and Brock 2000). Such alternative functions could account for inconsistencies in the theories regarding phlorotannins as antiherbivore defences, but do not necessarily explain why the costs of phlorotannin production could often not be demonstrated.

A further possible role is related to reproduction. Such a role has been alluded to by Ragan and Jensen (1978) and Peckol *et al.* (1996) who described decreases in phlorotannin concentrations during the period of maturation of receptacles in *Fucus vesiculosus* and *Ascophyllum nodosum*. Moreover, Clayton and Schoenwalder (1999); Clayton and Ashburner (1994) and Schoenwalder and Clayton (1998) found phlorotannnins in eggs or embryos of a number of fucoids and concluded that their possible function was to act as a polyspermy block. Since reproduction in both of the fucoids they investigated is seasonal, such a role could account for both the seasonality of the response of some brown algae to attack by herbivores and the perceived absence of costs of phlorotannin production. It is therefore worth considering whether different phlorotannin concentrations, rather than varying with grazing damage, are indirectly caused by factors influencing reproduction.

Many fucoids such as *Ascophyllum nodosum* have male and female plants and it is conceivable that the production of male and female gametes poses quite different costs on the parent (Mathieson and Guo 1992, Steinberg 1994, Åberg 1996, Campbell 2000). However, few studies have investigated the possibility of differential reproductive growth/ phlorotannin relationships in male and female plants. *A. nodosum* also harbours high concentrations of phlorotannins in its tissues and is therefore a suitable study organism with which to investigate the relationship between phlorotannins and reproduction. Although some evidence of trade-offs between vegetative production and phlorotannin production has been found by Pavia *et al.* (1999), relationships between phlorotannins and receptacle production were not investigated. In *A. nodosum* the situation is further complicated by the presence of its obligate epiphyte *Polysiphonia lanosa*, which might also affect the reproduction of the host and may possibly respond to phlorotannin concentrations. Some authors have in fact argued that phlorotannins might serve as an anti-epiphyte defence (Sieburth and Conover 1965, McLachlan and Craigie 1966).

The aim of this study is therefore to investigate possible differences in the allocation patterns of biomass to vegetative and reproductive structures in 'clean' and epiphytised plants and to compare them with the phlorotannin concentrations in these plants

Two specific hypotheses were tested:

1. Allocation of resources to reproductive biomass, represents a cost which will lead to reduced allocation to vegetative growth.

2. Phlorotannins are a requirement for the reproductive process they will therefore not cause trade-off with the allocation of biomass to reproductive growth.

3.2 Materials and Methods

3.2.1 Field collection

The present study was the result of an initial investigation into possible defence mechanisms with respect to the epiphyte *Polysiphonia lanosa*, for which plants were sampled from the shore in November 2000 and February 2001. However in the course of the study it became apparent that the sex of the plant might play a role in the results obtained. Therefore samples were taken in March 2001 to investigate both epiphytism and possible relationships between epiphyte and the reproductive state of the host plant. Despite the much larger total sample size in March than in previous months, limited comparisons between months can be made with respect to the relationship between phlorotannins and epiphyte loads as all samples were processed in exactly the same way.

Two year old internodes, i.e. tissues of the same age classes were used in the analysis. These are usually the youngest internodes to be heavily epiphytised (pers. obs). In November eight epiphytised and eight clean internodes were excised. Twelve further sets of clean and epiphytised internodes were collected in February. In March, male and female plants were collected separately. Mature receptacles were identified by their colour, which is yellow/ orange in males and green in females.

Internode segments were excised in the field and transported back to the laboratory in polythene bags (separated into male and female plants). They were frozen at -18°C prior to phlorotannin analysis. This was done with all samples to avoid the possible confounding effects of changes in phlorotannin concentrations between fresh and frozen material and different degrees of hydration.

For each internode, the length (to the nearest mm), number of receptacles and *Polysiphonia* load (to the nearest gram) were recorded. Where receptacles were present, their dry weight was also measured. Both wet and dry weights were recorded, the latter after drying in an oven at 60°C until a constant weight was reached, usually after approximately 48 hours.

3.2.2 Phlorotannin analysis

The *Ascophyllum nodosum* samples were analysed with the widely used Folin Denis method (Appendix 1). One gram fresh weight of each sample was macerated in 10 ml of methanol in a tissue homogeniser and then extracted at 4°C in the dark for 72 hours. A second plant fragment of the same size and from the same internode age class was removed from an adjacent branch and its dry weight determined to produce a wet: dry mass conversion factor to convert phlorotannin concentrations from mg per ml of methanol to % phlorotannins of plant dry weight. Only undamaged plant material was used to avoid possible confounding effects of induced elevations in phlorotannin levels (van Alstyne 1988, Hammerstrom *et al.* 1998).

After extraction the samples were filtered (Watman GF/A) to remove solids and then rotary evaporated (Buchi RE rotavapor) at 35°C to a small volume (2-3 ml). This volume was then made up to 35 ml with distilled water and filtered again. To the resulting aqueous solution, 5 ml of sodium-potassium tartrate were added immediately followed by 0.5 ml of the Folin-Denis reagent. After inverting the sample twice to evenly distribute the reagent, it was allowed to stand at room temperature for 30 minutes before the absorbance was measured in a Philips PU8670 VIS/NIR spectrophotometer at a wavelength of 700 nm to determine the total phlorotannin concentration (mg per ml methanol).

3.2.3 Data analysis

Data were log transformed where necessary (calculated as log (x+1)) with the exception of the phlorotannin data, which were arcsin transformed as they were recorded as percentages (calculated as $\arcsin\sqrt{p}$ of phlorotannin concentrations).

A two way ANOVA was used for the comparison of phlorotannin concentrations in epiphytised and clean plants in samples from three months (November, February and March).

Prior to carrying out the analysis of variance the data were tested for homogeneity of variances using Cochran's C test. As data had originally been recorded separately for epiphytised and clean plants, t-tests were carried out initially to see whether data could be pooled into single categories for males and females for the analyses in March to increase sample sizes.

ANCOVAs revealed data from the two groups (epiphytised and clean) could be pooled into single regression analyses on the basis of plant sex to analyse the relationship between phlorotannin concentrations and reproductive characteristics in male and female plants (numbers of receptacles, total receptacle dry weight or receptacle dry weight per g vegetative tissue). On the basis of these ANCOVAS data could be pooled (see Appendix 2a-b). It is appreciated that the absence of a significant interaction between main factor and covariate might have been at least partly the result of weak relationships between the dependent variable and covariate in the epiphytised and clean groups. Plots of complete datasets and plots of both the data pooled for epiphytism and unpooled data are presented for all characteristics examined.

In two cases (total receptacle mass vs total receptacle mass and total receptacle mass per gram vegetative tissue vs vegetative tissue) variances were not homogeneous after (log+1) transformation. However, since these data sets contained no zero values, a simple log transformation was carried out which resulted in homogeneous variances.

3.3 Results

3.3.1 Receptacle production in male and female plants: impacts of the epiphyte Polysiphonia lanosa

Considerable variation occurred in the numbers of receptacles on male and female internodes. Data were analysed both as pooled data sets (i.e. clean and epiphytised plants combined) and with clean and epiphytised internodes separately. Analysis of the pooled data showed that male internodes carried more than twice as many receptacles as female internodes (Table 3.1). Receptacle biomass per gram vegetative tissue was also higher in males but the receptacles were significantly larger in females.

Table 3.1: Differences in morphological characters in male and female plants in March 2001, p-values for t-tests (on the transformed data) between male and female internodes are given for each character

Variable measured	male	female	p-value
No of receptacles g^{-1} vegetative dry wt	65.61±7.43	30.87±7.49	0.0004
Receptacle size (g)	0.034±0.003	0.045±0.002	0.0009
Total receptacle dry weight (g)	0.47±0.07	0.315±0.035	0.093
Receptacle dry wt per g vegetative tissue	0.235±0.03	0.144±0.030	0.036

When only clean male and female plants were compared, only receptacle size differed significantly between male and female plants being larger in females than in males. In epiphytised plants all measured characteristics varied significantly between males and females with the exception of receptacle size. Receptacles were more numerous in males and receptacle biomass was greater (Table 3.2a-b). Epiphytism generally had a markedly different effect on internodes from male and female plants. When the same data were analysed for differences between epiphytised and clean males and females, numbers of receptacles per gram vegetative tissue, total receptacle biomass and receptacle dry weight per gram vegetative tissue were all significantly higher in epiphytised than in clean male plants (Table 3.3). This accounted for the differences in the pooled data. No such differences were found in internodes from female plants.

Table 3.2: T-test for differences in reproductive structures male and female internodes in March 2001 with clean and epiphytised internodes analysed separately

a. clean plants only

	Male	Female	p-value
Number of receptacles per g vegetative tissue	39.893±4.43	31.595±6.23	0.278
receptacle size (g)	0.031±0.004	0.045±0.004	0.025
total receptacle dry weight (g)	0.224±0.037	0.273±0.043	0.418
receptacle dry weight per g veg wt	0.128±0.144	0.126±0.027	0.607

b. epiphytised plants only

	Male	Female	p-value
Number of receptacles per g vegetative tissue	81.436±33.62	30.231±5.21	0.0005
receptacle size (g)	0.036±0.012	0.051±0.003	0.008
total receptacle dry weight (g)	0.626±0.322	0.35±0.06	0.074
receptacle dry weight per g veg wt	0.302±0.130	0.163±0.02	0.008

Table 3.3: Differences in 4 measures of reproductive output in *A. nodosum* in epiphytised and clean males and females in March 2001. Results of T-tests comparing the differences between the variables in epiphytised and clean plants are also given

	male			female		
	+epi	clean	p-value	+epi	clean	p-value
Number of receptacles g^{-1} vegetative tissue	0.97	0.70	<0.0001	0.60	0.62	0.980
receptacle size (g)	0.015	0.013	0.384	0.022	0.019	0.274
total receptacle dry weight (g)	0.20	0.09	0.003	0.13	0.10	0.309
receptacle dry weight per g veg wt	0.02	0.01	0.002	0.057	0.061	0.691

Regression analyses were also carried out on pooled data and separately for female/ male and clean/epiphytised internodes (Figures 3.1-3.3). With respect to the relationship between total receptacle dry weight and vegetative dry weight per internode, the pooled data suggested an increase in receptacle dry weight with increasing vegetative dry weight ($p = 0.001$, figure 3.1a). Separate analyses, however, showed that, although receptacle dry weight seemed to increase faster in male internodes than in female ones, only the slope for internodes from male plants was statistically significant ($p = 0.002$, Figure 3.1b).

Again viewing the data separately for epiphytised and clean plants revealed very different patterns for males and females. In male plants receptacle biomass increased significantly with increasing internode weight for epiphytised but not for clean plants ($p=0.0006$, Figure 3.1c). In females no significant relationships were found between total receptacle dry weight and total vegetative dry weight for either epiphytised or clean internodes (Figure 3.1d).

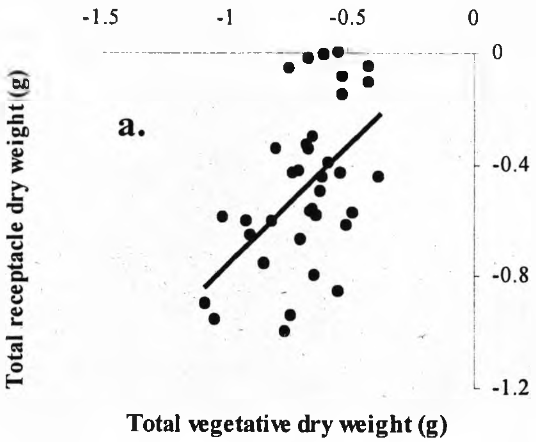
Regression analyses

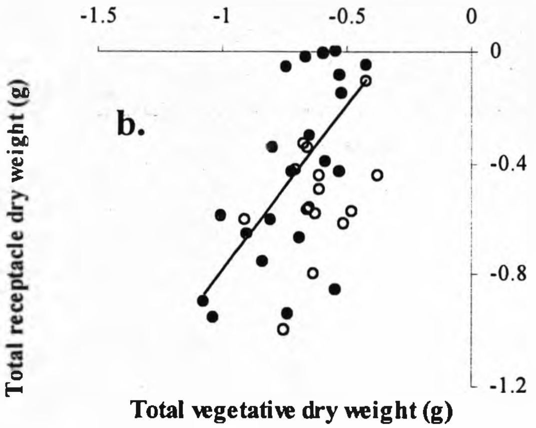
Plot a:
R^2=26.46, $F_{1,34}$=12.24, p=0.001
Y=0.514x+0.103

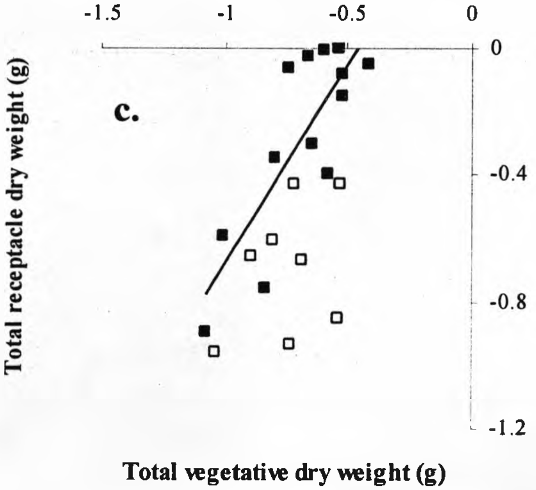
Plot b:
Males: R^2=41.24. $F_{1,19}$=13.335, p=0.002
Y=0.642x+0.375
Females: R^2=10.30, $F_{1,13}$=2.609, p=0.130

Plot c:
Epiphytised: R^2=64.03, F $_{1,11}$ =22.36, p=0.0006
y=0.819x+0.576
Clean: R^2=0.27, $F_{1\ 11}$=1.019, p=0.352

Plot d:
Epiphytised: R^2=0.092, $F_{1,6}$=0.413, p=584
Clean: R^2=0.098, $F_{1,5}$=0.462, p= 0.527







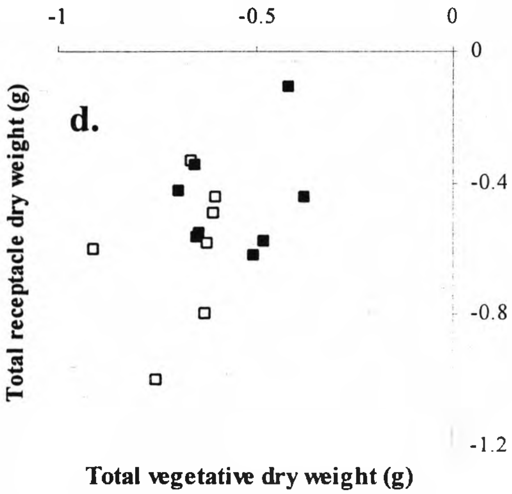
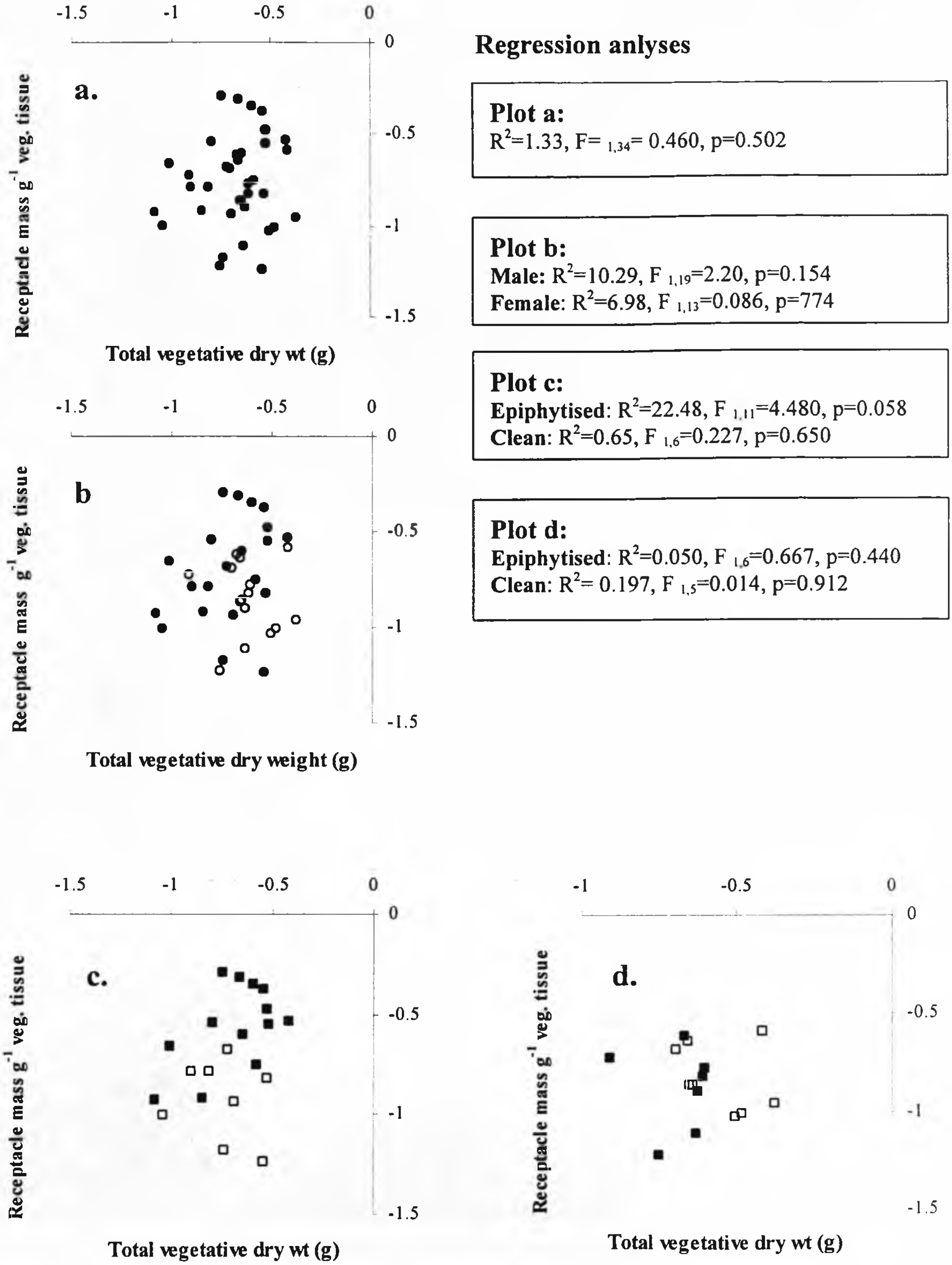


Fig 3.1 Regression for log total receptacle dry weight vs. log total vegetative dry weight: **a**. all data pooled, **b**. females (open circles) and males (closed circles) plotted separately, **c**. epiphytised (closed squares) and clean (open squares) males, **d**. epiphytised and clean females. Data are log transformed (not log+1)

When the receptacle weights were adjusted for the different dry weights of internodes no significant trends were visible for either the pooled data or those analysed separately for epiphytised and clean plants (Figure 3.2a-d) . For male internodes there was no significant increase in the receptacle biomass per g with increasing vegetative dry weight in epiphytised or in clean plants. No patterns emerged in clean or epiphytised females either.

Receptacle size was also highly variable depending on the total biomass of receptacles per internode. The regression on the pooled data revealed a slight but not significant increase in receptacle size with increasing receptacle biomass per gram vegetative tissue (Figure 3.3a). Splitting the data into females and males resulted in a significant increase in the receptacle size for males (p=0.002, Figure 3.3b), but not female plants.

Similarly, for internodes from male plants, both epiphytised and clean samples showed an increase in receptacle size with increasing receptacle dry weight per gram vegetative dry weight, although only significantly so in epiphytised internodes (p=0.007, Figure 3.3c). In females only weak non-significant trends were found.



Regression anlyses

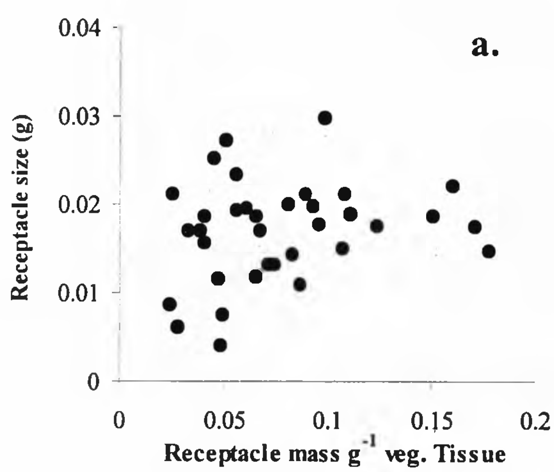
Plot a:
R^2=1.33, F= $_{1,34}$= 0.460, p=0.502

Plot b:
Male: R^2=10.29, F $_{1,19}$=2.20, p=0.154
Female: R^2=6.98, F $_{1,13}$=0.086, p=774

Plot c:
Epiphytised: R^2=22.48, F $_{1,11}$=4.480, p=0.058
Clean: R^2=0.65, F $_{1,6}$=0.227, p=0.650

Plot d:
Epiphytised: R^2=0.050, F $_{1,6}$=0.667, p=0.440
Clean: R^2= 0.197, F $_{1,5}$=0.014, p=0.912

Fig 3.2 Changes in receptacle dry weight per g vegetative dry weight with vegetative dry weight: **a.** all data pooled, **b.** females (open circles) and males (closed circles) plotted separately, **c.** epiphytised (closed squares) and clean (open squares) males, **d.** epiphytised and clean females. Data are log transformed not (log+1)



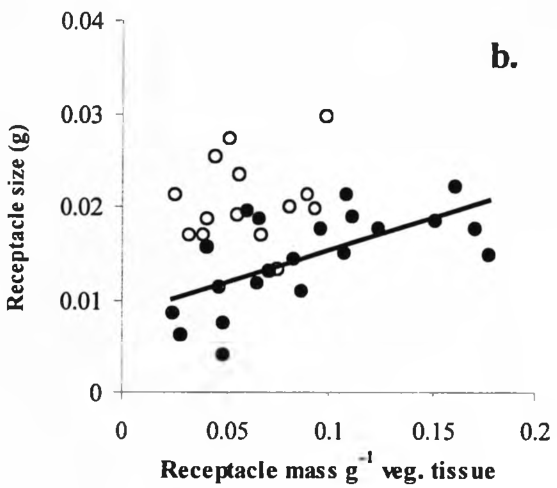
Regression analyses

Plot a:
$R^2=2.65$, $F_{1,34}=0.968$, $p=0.758$

Plot b:
Male: $R^2=36.85$, $F_{1,19}=12.673$, $p=0.002$
$Y=0.633x+0.009$
Female: $R^2=2.53$, $F_{1,13}=1.368$, $p=0.264$

Plot c:
Epiphytised: $R^2=45.86$, $F_{1,11}=11.165$, $p=0.0007$
$Y=0.710x+0.008$
Clean: $R^2=31.79$, $F_{1,6}=4.26$, $p=0.085$

Plot d:
Epiphytised: $R^2=8.85$, $F_{1,6}=1.680$, $p=0.243$
Clean: $R^2=11.63$, $F_{1,5}=0.375$, $p=0.568$



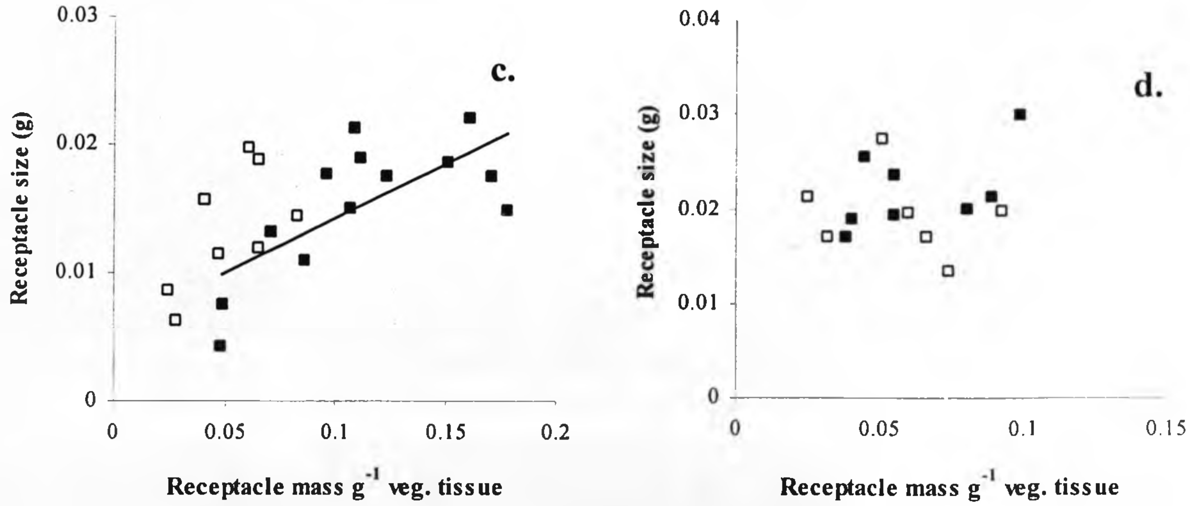


Fig 3.3 Changes in receptacle size with receptacle biomass per gram vegetative tissue: **a.** all data pooled, **b.** females (open circles) and males (closed circles) plotted separately, **c.** epiphytised (closed squares) and clean (open squares) males, **d.** epiphytised and clean females

3.3.2 Correlations between phlorotannin and receptacle production

On the basis of preliminary t-tests which showed no significant differences in the mean Phlorotannin concentration of clean and epiphytised internodes, the data for these two groups were pooled (Table 3.4).

Table 3.4: Results of preliminary t-tests for phlorotannin concentrations in clean and epiphytised plants in November, February 2000 and March 2001

	Phlorotannin concentrations (% dry weight of *Ascophyllum* tissue)				
	Clean	epiphytised	df	T	p
November	5.76	5.02	12	1.33	0.55
February	5.33	5.09	18	-0.883	0.388
March	5.15	5.12	32	0.34	0.7340

Phlorotannin concentrations were significantly higher in female than in male internodes (Table 3.5) when data for epiphytised and clean internodes were pooled. When data were analysed separately for epiphytised and clean internodes of the two sexes in a two-way analysis of variance there was no significant effect of sex. Only the effect of epiphytism was significant (p=0.029, Figure 3.4, Table 3.6).

Table 3.5:T-test for differences in phlorotannin concentrations (arcsin transformed) between males and female vegetative tissue measured in March 2001

	Phlorotannins % dry weight)	df	T	p
Males	2.743	32	2.037	0.019
Females	3.128			

Table 3.6:Two-way ANOVA for the comparison of phlorotannin levels (% of dry weight) of female and male epiphytised and clean internodes in March 2001

Source of variation	df	MS	*F*	p-value
Epiphytism	1	1.058	5.237	0.029
Sex	1	0.002	0.010	0.921
Interaction	1	0.458	2.267	0.143
Error	30	0.202		

Regression analyses were also carried out to investigate the relationship between phlorotannins and reproductive characteristics in more detail. Again, data pooled for both sexes and epiphytism failed to show any significant trends between phlorotannin concentration and total receptacle dry weight (Figure 3.5a). Considering males and females, but ignoring epiphytism revealed just significant positive relationships between phlorotannin concentrations and total receptacle dry weight (Figure 3.5b) and receptacle dry weight per g vegetative tissue (Figure 3.6b) but only in females. When epiphytised and clean internodes were analysed separately, there were no significant relationships between phlorotannins and total receptacle dry weight for either males or females (Figure 3.5c-d). Similarly when the data were adjusted for the size of the internode no significant relationships were found (Figure 3.6d).

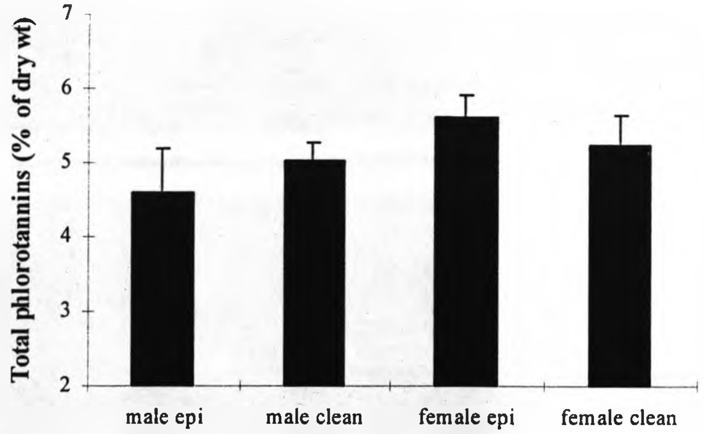
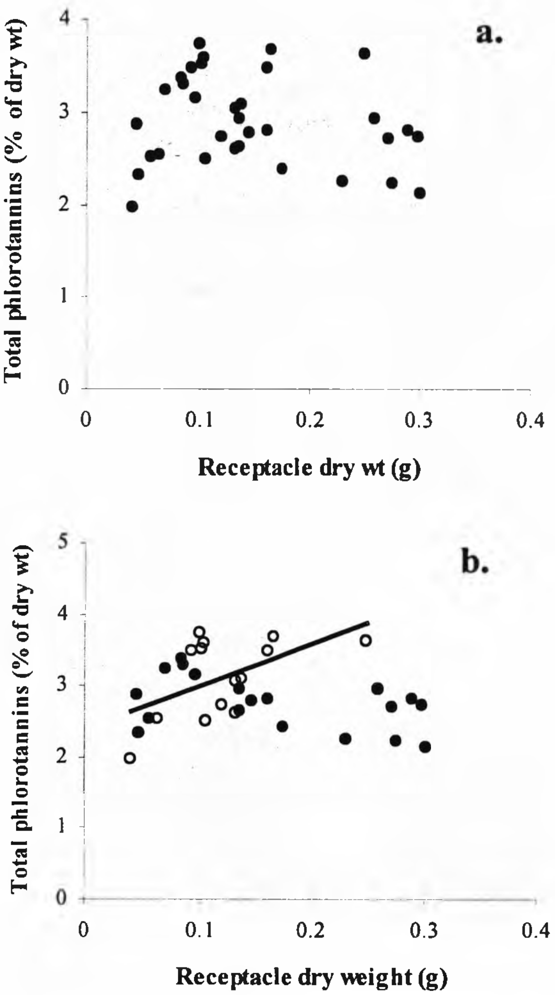


Fig 3.4 The mean phlorotannin concentrations in epiphytised and clean, male and female internodes in March 2001. Error bars= ± 1 standard error



Regression analyses

Plot a:
R^2=8.81, F $_{1,31}$=0.0-1, p=0.348

Plot b:
Males: R^2=14.10, F $_{1,17}$=3.95, p=0.063
Females R^2=21.74, F $_{1,12}$=4.61, p=0.053

Plot c.
Epiphytised: R^2=17.59, R $_{1,9}$=3.135, p=0.110
Clean: R^2=9.01, F $_{1,6}$=0.421, p=0.540

Plot d:
Epiphytised: R^2=8.881, F $_{1,6}$=0.433, p=0.535
Clean: R^2=46.86, F $_{1,4}$=5.41, p=0.080

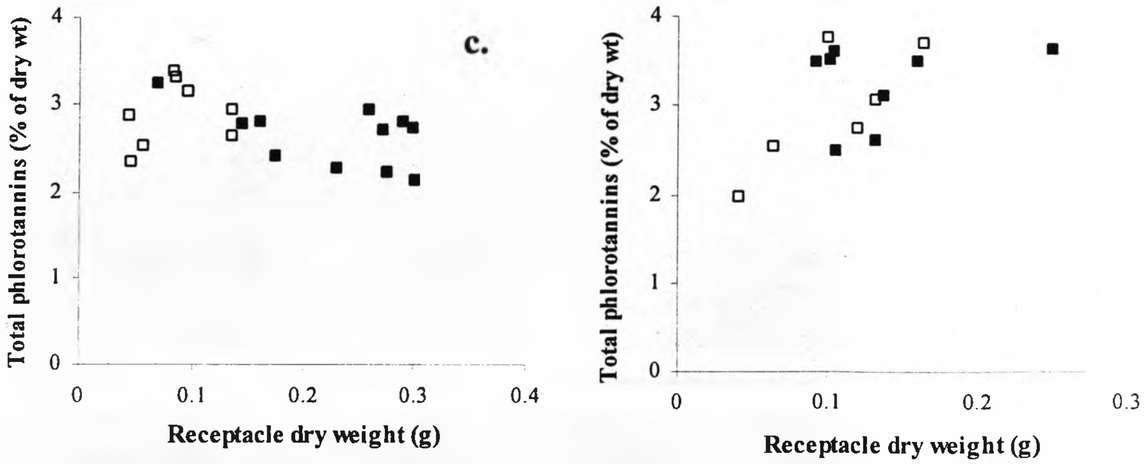


Fig 3.5 Changes in total phlorotannin concentrations with changes in total receptacle dry weight: **a.** all data pooled, **b**. females (open circles) and males (closed circles) plotted separately, **c.** epiphytised (closed squares) and clean (open squares) males, **d.** epiphytised and clean females

Regression Analyses

Plot a:
$R^2=0.098$, $F_{1,31}=0.69$, $p=0.412$

Plot b:
Males: $R^2=2.89$, $F_{1,17}=1.536$, $p=0.232$
Females: $R^2=22.4$, $F_{1,12}=4.76$, $p=0.0496$

Plot c:
Epiphytised: $R^2=4.26$, $F_{1,9}=0.591$, $p=0.461$
Clean: $R^2=5.13$, $F_{1,6}=11.38$, $p=0.285$

Plot d:
Epiphytised: $R^2=-8.6$, $F_{1,6}=0.446$, $p=0.29$
Clean: $R^2=44.68$, $F_{1,4}$ $p=0.088$

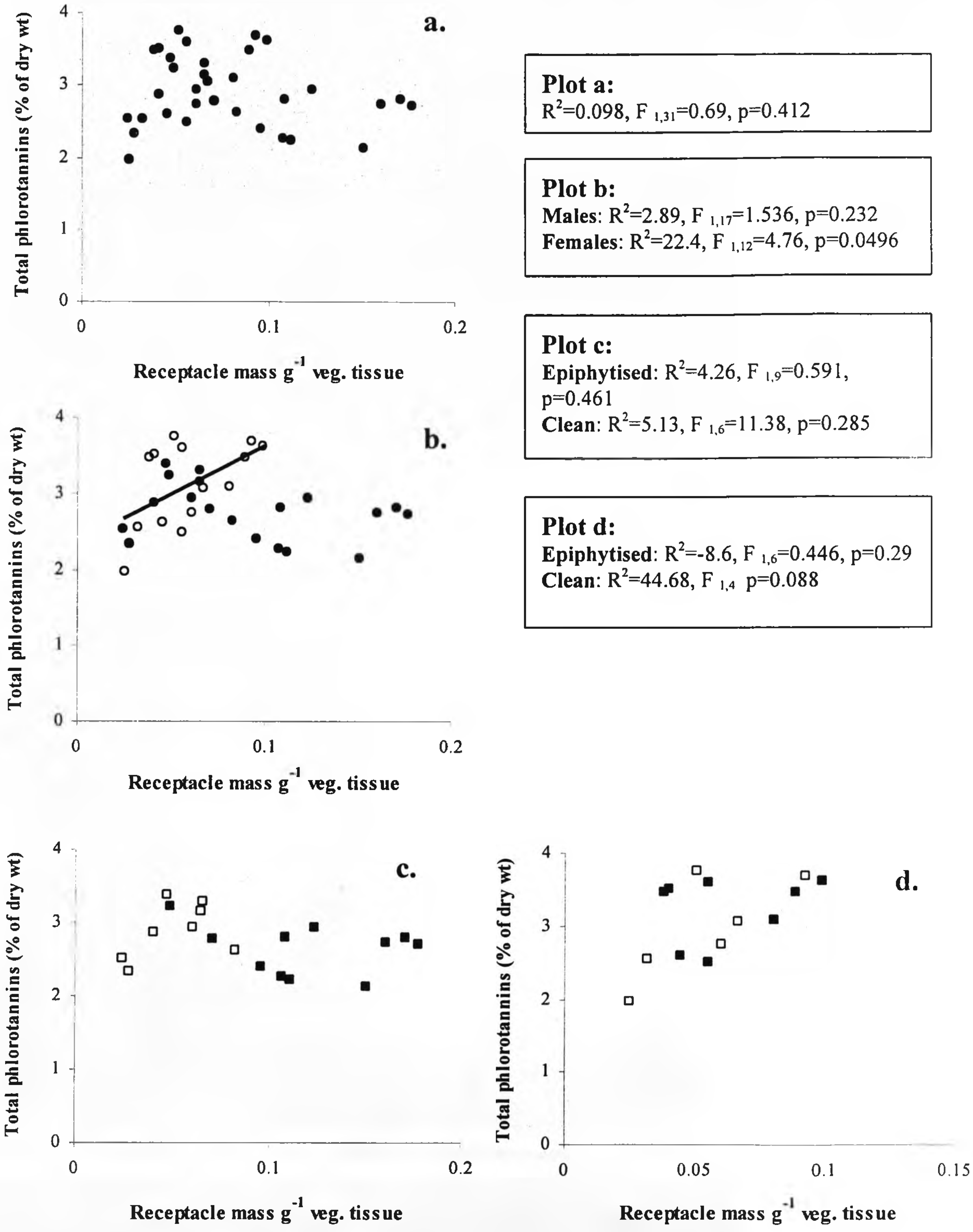


Fig 3.6 Changes in phlorotannin concentrations with changes in receptacle biomass per gram vegetative tissue: **a.** all data pooled, **b**. females (open circles) and males (closed circles) plotted separately, **c.** epiphytised (closed squares) and clean (open squares) males, **d.** epiphytised and clean females

No significant relationship between phlorotannin concentrations and receptacle size was found (Figure 3.7b) in either males or females and no effect of epiphytes was apparent. Lastly, phlorotannin concentrations were compared to the total dry weight of vegetative tissue. Again there were no significant relationships at all (Figure 3.8c).

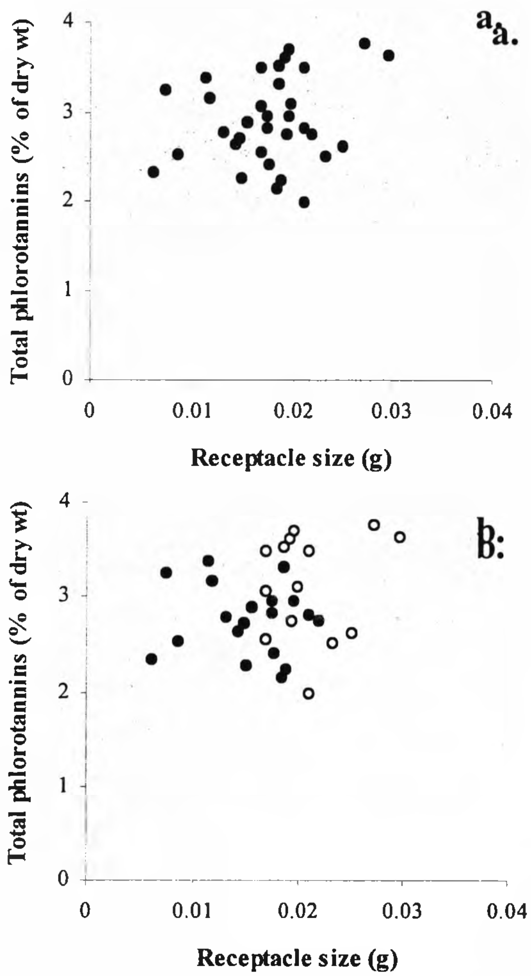
3.3.3 Seasonal Differences between phlorotannin concentrations and epiphyte loads

Figure 3.9 shows a 10% decrease in phlorotannin concentrations in clean plants between November and March. However, a two-way analysis of variance for plants between November and March, did not reveal any significant effects of month or epiphytism on phlorotannin concentrations (Figure 3.9, Table 3.7).

Table 3.7: Two-way ANOVA for Phlorotannin concentrations in *Ascophyllum nodosum* in November 2000 and February and March 2001 in epiphytised and clean plants

Source of Variation	df	MS	*F*	p
Month	2	0.669	0.724	0.489
epiphyte	1	2.874	3.108	0.083
Month – epiphyte	2	0.553	0.598	0.553
Error	65	0.925		

A regression analysis carried out in November to investigate the relationship between phlorotannin concentration and epiphyte load revealed a significant decrease in epiphyte loads with phlorotannin concentrations. However, no such relationship was found in either February or March (Figure 3.10a-c). In March, the data contained one internode with a particularly high epiphyte load but even when the outlier was removed, it did not change the outcome of the analysis.



Regression Analyses

Plot a:
R^2=2.04, F $_{1,31}$=1.67, p=0.206

Plot b:
Males: R^2=4.70, F=$_{1,17}$=4.76, p=0.673
Females: R^2=22.4, F$_{1,12}$=4.76, p=0.0496

Plot c:
Epiphytised: R^2=0.18, F $_{1,9}$=2.00, p=0.191
Clean: R^2=0.2938, F $_{1,6}$=2.50, p=0.170

Plot d:
Epiphytised: R^2=-9.9, F $_{1,6}$=0.365, p=0.568
Clean: R^2=-272, F $_{1,4}$=0.868, p=0.40

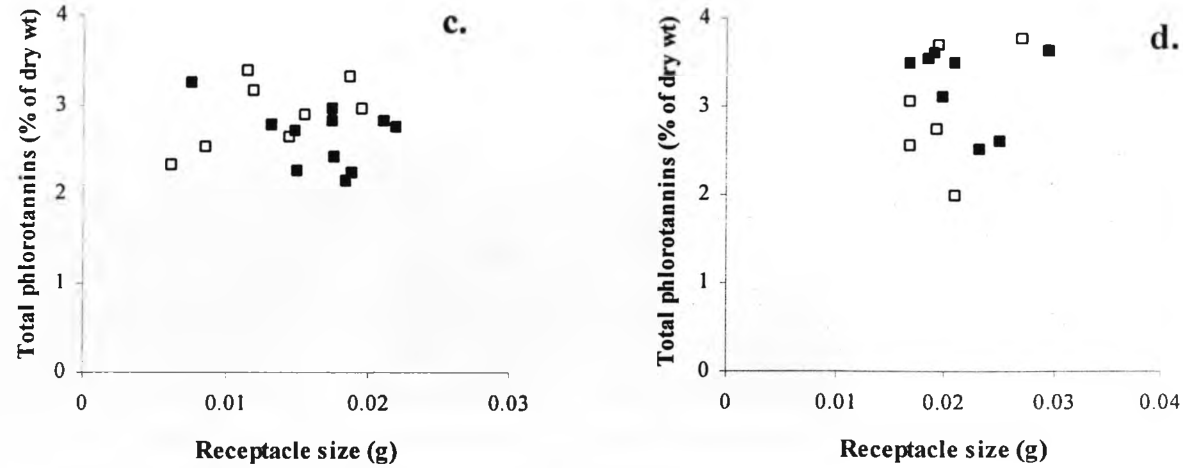
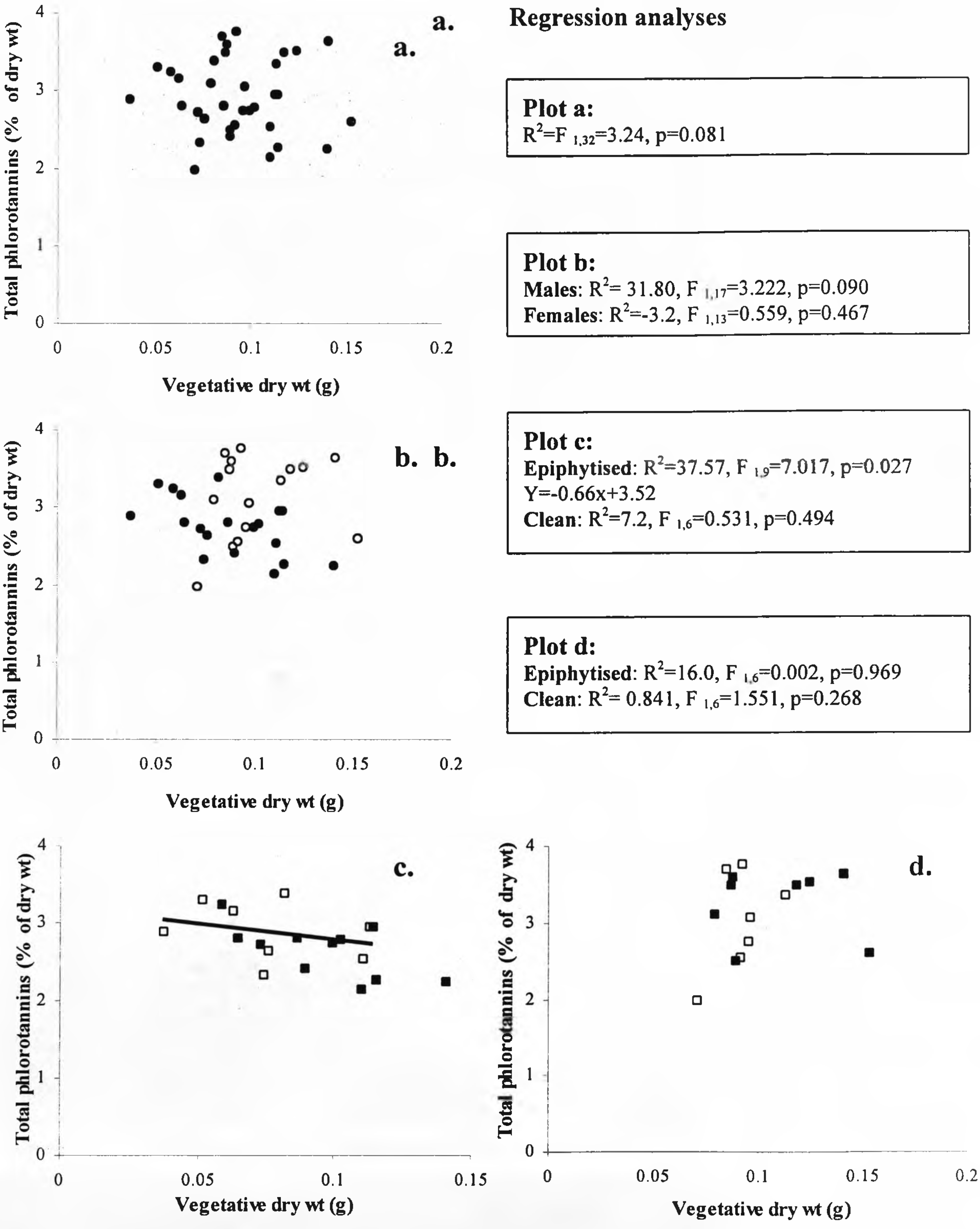


Fig 3.7 Changes in phlorotannin concentrations with changes in receptacle size **a.** all data pooled, **b**. females (open circles) and males (closed circles) plotted separately, **c.** epiphytised (closed squares) and clean (open squares) males, **d.** epiphytised and clean females



Regression analyses

Plot a:
R^2=F $_{1,32}$=3.24, p=0.081

Plot b:
Males: R^2= 31.80, F $_{1,17}$=3.222, p=0.090
Females: R^2=-3.2, F $_{1,13}$=0.559, p=0.467

Plot c:
Epiphytised: R^2=37.57, F $_{1,9}$=7.017, p=0.027
Y=-0.66x+3.52
Clean: R^2=7.2, F $_{1,6}$=0.531, p=0.494

Plot d:
Epiphytised: R^2=16.0, F $_{1,6}$=0.002, p=0.969
Clean: R^2= 0.841, F $_{1,6}$=1.551, p=0.268

Fig 3.8 Changes in phlorotannin concentrations with changes in vegetative dry weight: **a.** all data pooled, **b**. females (open circles) and males (closed circles) plotted separately, **c.** epiphytised (closed squares) and clean (open squares) males, **d.** epiphytised and clean females

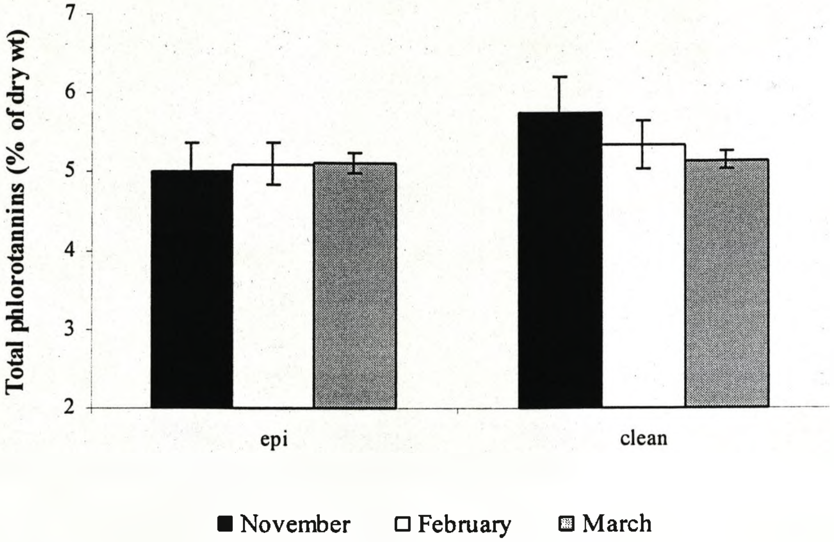


Fig 3.9 Seasonal changes in phlorotannin concentrations in epiphytised and clean plants in November 2000 and February and March 2001. Error bars are ± 1 standard error

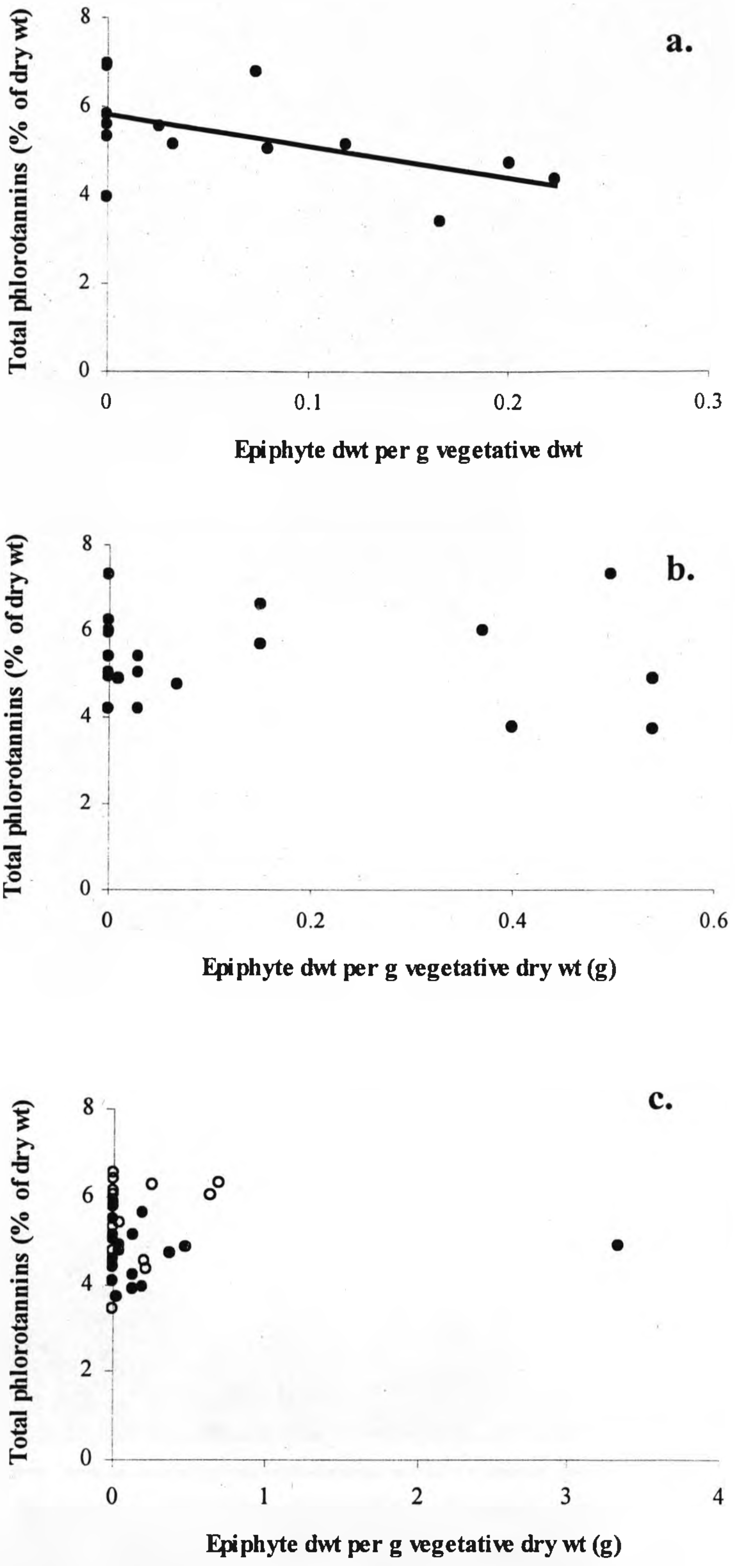


Fig 3.10 The relationship between total phlorotannins and epiphyte dry weight in **a.** November 2000, **b.** February 2001, **c.** March 2001 (open circles=females, closed circle=males)

3.4 Discussion

Ascophyllum nodosum's life history is characterised by a very high fecundity and repeated reproduction over many years but very slow growth compared with some other fucoids (Cousens 1985, Carlsson 1991), which seems to indicate a trade-off. However, the pooled data (containing epiphytised and clean plants) in the present study did not seem to indicate any costs between vegetative growth and reproduction. With respect to the relationship between receptacle dry weight and vegetative biomass it has to be kept in mind that in this study the vegetative biomass of each internode had been produced two years before the receptacle biomass and it might therefore be argued that receptacle production is now merely a function of the thallus surface area available for photosynthesis and should be increasing with increasing vegetative tissue biomass. Therefore no direct trade-offs between growth and reproduction can be measured. However, the costs of reproduction and in particular differential costs of reproduction for male and female plants can be indicated by differences in the slope of receptacle weight vs. vegetative biomass (Schaffer 1974).

If there were no differences in reproductive costs, the same slope would be expected for both males and females. This might be expected as it is well documented that in oogamous species the large eggs are provided with more resources than the small male gametes (Vernet and Harper 1980). Although in the present study the receptacles containing the gametes, rather than individual gametes, were investigated, one might still expect a sex specific trade-off. However, while clean female internodes had larger receptacles, overall reproductive biomass did not differ between the sexes and the regression analyses also did not reveal substantially different slopes as would be expected if there was a trade-off between vegetative growth and reproductive characteristics. In fact when reproductive biomass was calculated per gram vegetative tissue no significant relationships were apparent at all. This is inconsistent with differential costs of reproduction for males and females or reproductive costs in general. Therefore hypothesis one has to be rejected. The only possible trade-off in clean plants was not between total receptacle biomass and total vegetative biomass but the nature of the receptacles, which were fewer, but larger in females.

However, examining the data more closely revealed that one reason for the observed reproductive patterns in male and female plants seemed to be epiphytism, which caused a much greater increase in reproductive biomass relative to vegetative biomass in males than in females. Epiphytised male internodes also had on average the lowest phlorotannin concentrations while epiphytised female internodes had the highest. If it were assumed that the production of phlorotannins was indeed costly in terms of reduced reproduction, then the higher reproductive output in males than in female epiphytised plants might simply have been the result of the lower phlorotannin concentrations, quite independent of whether the internodes were epiphytised or not. However, the above explanation would contradict the findings in the present study, where there was a positive relationship between phlorotannin concentrations and some reproductive characteristics in the host plant in females but not in males.

One of the initial assumptions of this study had been that phlorotannins could be serving as an anti-epiphyte defence. In this case one would have expected settlement and survival of the epiphyte on host plants with low phlorotannin concentrations.

However, while settlement patterns were not investigated specifically, phlorotannin *Ascophyllum nodosum* concentrations were highest at the time of peak epiphyte settlement in November. Ragan and Jensen (1978) also found seasonal changes in phlorotannin concentrations. It might be argued that the reason for this was that recent settlement had caused induction of phlorotannins in the host plant. The most likely trigger would have been penetration of the host plant by a large number of rhizoids from the newly settled epiphytes. It has to be considered though that only relatively few epiphytes get to the stage where they germinate and penetrate the host plant. It therefore seems unlikely that phlorotannins act as a defence against epiphytes as some authors have suggested (Sieburth and Conover 1965). Jennings and Steinberg (1997) also found no evidence of an anti-epiphyte role of phlorotannins in the kelp *Ecklonia radiata* and concluded that epiphyte distribution and abundance was more related to the age of the kelp than the distribution of phlorotannins in it.

The second possible function investigated in this study was a role of phlorotannins in reproduction. A mechanism linking phlorotannin production to reproduction has also been proposed by Clayton (1990), Clayton and Ashburner (1994), Schoenwalder and Clayton (1998). These authors have shown that female gametes of the brown algal species *Hormosira banksii*, *Phyllospora comosa* and also *Ascophyllum* contain large amounts of physodes. These structures are small spherical vesicles containing phlorotannins, and are commonly found in brown algae (Ragan 1976). Physodes are thought to be important in the early development of brown algae, including fucoids. But large numbers of physodes are expelled soon after formation of the zygote.

There are several factors that might select for high phlorotannin levels in eggs. Since the fertilized egg will form the next generation of plants, the phlorotannins in the eggs could serve as a defence to protect these vulnerable stages from grazing. However, even if there are high concentrations of phlorotannins in the eggs themselves, within the receptacle containing the eggs, phlorotannin levels might not be high enough to deter grazing, and indeed grazing damage is frequent on receptacles (pers. obs.). Before release from the receptacles the phlorotannin levels within the egg are therefore unlikely to be an effective defence. Even after release, phlorotannins are unlikely to play a defensive role as they are expelled from the egg soon after fertilization and indeed levels of phlorotannins in reproductive compared with defensive structures have only rarely been described (Steinberg 1984, Ragan and Glombitza 1986).

This fast release of phlorotannins from the egg at fertilization has led some authors to suggest a more direct involvement of these phlorotannins in the reproductive process, namely that they serve as a polyspermy block to prevent more than one sperm from entering the egg (Brawley and Johnson 1992). If phlorotannins do indeed play a considerable role in reproduction then positive relationships between phlorotannin concentrations and receptacle production might be expected rather than trade-offs. Furthermore, since the requirement for phlorotannins only occurs in females, a positive relationship between phlorotannins and receptacle production would only be expected in females. Such a pattern was observed in this study, which means that hypothesis 2 had to be accepted. But the phlorotannin concentrations were only measured in vegetative tissue and not in the receptacles or eggs. Therefore even if it is assumed that phlorotannins are needed for the production of receptacles and the eggs contained in them, the explanation for this positive relationship is not entirely straightforward, since the receptacles are at least partly photosynthetic and will contain 'their own' phlorotannins. However, phenotypes with naturally high phlorotannin concentrations in their vegetative tissue might be expected to also produce high levels in their receptacles.

Trade-offs might still occur as the result of phlorotannin production but possibly in relation to other factors such as defence. Decreases in phlorotannins in vegetative tissue during the period of receptacle production might for example indicate a trade-off between reproduction and defence, i.e. an inability to maintain high phlorotannin concentrations in vegetative tissue while demands for secondary metabolites in the reproductive tissue increase. While seasonal changes in phlorotannins were only slight in the present study, they have been described by previous authors (Ragan and Jensen 1978, Peckol *et al.* 1996, Clayton pers com), with phlorotannins usually decreasing in vegetative tissue during the period of receptacle maturation. However, neither Ragan and Jensen's study nor the present one measured seasonal changes separately for males and females. If phlorotannins are an essential requirement for the production of female but not male gametes, the seasonal patterns might be expected to be quite different.

A role of phlorotannins in reproduction might also explain why some studies have shown seasonal changes in the ' behaviour' of phlorotannins (i.e. induction vs. lack of it) and in the relationship between growth or reproduction and phlorotannin concentrations (Steinberg 1995). These studies might actually have shown changes in phlorotannin concentrations with respect to the reproductive state of the plant rather than in response to being grazed, i.e. the authors tried to identify the wrong trade-off.

The proposed role of *Ascophyllum*'s phlorotannins in the reproduction, e.g. as polyspermy block is a clear alternative to the commonly cited defence function of phlorotannins. All of the above could, if not taken into account, confound studies of the role of phlorotannins as anti grazer defences or any other function. If phlorotannins have a role in essential processes such as reproduction and cell wall construction, they would not just be a 'sink' for unwanted secondary metabolites as has been suggested by some authors (Targett and Arnold 1998), but might play an integral part of the life history of the species. This would have implications for the evolutionary interaction between grazers and their host plants. While the above functions do not mean that phlorotannins cannot be effective as anti-grazer defences, the ability of grazers to exert a selection pressure for changes in phlorotannin concentrations in the host plant might be very limited, as there would be little scope for reciprocal interactions.

Further studies with a number of brown algae, at different levels of organization e.g. that of the individual gamete, zygotes, juveniles and adults are required. More studies on intersex differences in reproductive allocation and phlorotannins are also necessary. Without this type of information the real impact of alternative functions on established theories regarding grazing and fucoid defences cannot be adequately assessed.

Chapter 4: The effect of epiphytism on reproductive and vegetative lateral formation in *Ascophyllum nodosum*

4.1 Introduction

There is one obvious means in which epiphytes can influence the production of receptacles relating to the way in which receptacles are formed. In *Ascophyllum nodosum* the receptacles are initiated on lateral pits on the fronds of the host plant in May to July. Initially all laterals produced from these pits are vegetative, but throughout the autumn and winter most of them become reproductive and gametes are released in the following spring. *P. lanosa* frequently also establishes in the lateral pits from which the receptacles usually arise (Lobban & Baxter 1983). Newly settled epiphytes attach to these lateral pits by means of a rhizoid which penetrates the host tissue. This could cause a physical blockage of the lateral pits (Turner and Evans 1977; Rawlence and Taylor 1970) and prevent the emergence of lateral branches or receptacles.

In addition a dense cover of epiphytes is also likely to cast considerable shade. It has been suggested by several authors e.g. (Russell, 1973 and Mathieson *et al.* 1976) that photoperiod might be involved in the onset of reproduction. This was confirmed by Terry and Moss (1980) who found that short day photoperiods favoured the production of reproductive over vegetative laterals. They also found that with less overall irradiance reaching the plant fewer laterals were produced. Similar observations have been made for another fucoid alga, *Fucus distichus* (Bird and McLachlan 1976).

On the shore such reductions in irradiance can occur in a number of ways. *Ascophyllum nodosum* often occurs in very dense stands on sheltered shores and it is likely that plants on the top of the canopy cast considerable shade onto the plants underneath (Cousens 1985, Robertson 1987, Schroeter *et al.* 1995). In addition high densities of the epiphyte *Polysiphonia lanosa* can reduce the light reaching the underlying *Ascophyllum*. This filamentous, much branched epiphyte often has a very patchy distribution but plants bearing high epiphyte loads can be almost completely covered (Lobban and Baxter 1983, Levin and Mathieson 1991). Considerable reductions in irradiance under a dense cover of epiphytes have been reported by a number of authors (Sand-Jensen and Borum 1984, Sand-Jensen *et al.* 1985, de O. Figueiredo *et al.* 2000).

One way to test whether either of these two possible mechanisms, i.e. shading and physically inactivated pits affect receptacle production and growth, is by carrying out experiments involving the removal of the epiphyte from lateral pits. Very different predictions can be made with respect to the consequences of such removal. Two hypotheses were formulated:

1. Light reduction is the main cause of the absence of receptacles from lateral pits. Removal of epiphytes from these lateral pits during the period of receptacle initiation will lead to the growth of receptacles from previously epiphytised lateral pits.

2. Epiphytism causes the physical blockage of lateral pits caused by *Polysiphonia*'s rhizoid. Removal of external epiphytic tissue will therefore not facilitate the growth of receptacles from these pits.

If shading was having an adverse effect on receptacle production and growth, assuming shading is strong enough to make the host plant light limited, removal of the epiphyte would be expected to lead to an increase in lateral production. In the case of physical blockage on the other hand, epiphyte removal should have no effect. However, either factor, if large enough, could exert significant costs on the host plant in terms of lost reproduction. The magnitude of each factor might also be expected to vary in populations on shores of different exposures (Cousens, 1982, 1985).

The aim of this chapter is therefore to firstly investigate the relationship between the degree of *Polysiphonia infestation* and production of reproductive laterals in the field. Following these observations laboratory experiments will then investigate whether a reduction in irradiance or 'competition' for lateral pits or a combination of both have the potential to significantly decrease host reproduction.

4.2 Materials and Methods

4.2.1 Sample Collection:

All experiments were carried out using plant material, which had been collected on a sheltered shore on Fort Island or an exposed shore at Scarlett, Isle of Man. All plants were collected at low tide, from the lower limit of the distribution of *Ascophyllum nodosum*, where the highest epiphyte loads were found. All plants were different genetic individuals.

4.2.2 *Reproductive effort of A. nodosum in the presence of P. lanosa*

This study was carried out in April 2000 during the peak reproductive period of *Ascophyllum nodosum*. To study possible effects of epiphytism on the production of receptacles, 40 fronds ranging from very lightly to heavily epiphytised fronds were selected. Only sexually mature plants 1m or more in length were used to avoid the confounding effects of plants, which were free of epiphytes but had not yet started reproducing. They were transported back to the laboratory in buckets of sea water to prevent drying and consequent gamete release and were stored at a constant temperature of 10°C until processing. In the laboratory all receptacles and epiphyte clumps were removed from the fronds and the wet and dry weight of all tissues were recorded. Wet weight was measured by repeatedly blotting the tissue between paper towelling until no more residual moisture could be seen on the paper. Dry weights were obtained by drying tissues in an oven at 60°C until constant weight. Total reproductive effort was calculated as the receptacle weight as a proportion of total tissue biomass per frond (Mathieson and Guo 1992; Åberg 1996).

4.2.3 *Measurement of light levels underneath a dense canopy of P. lanosa*

Individual clumps of *Polysiphonia lanosa* were excised from the host plant. They were attached in a straight line to a transparent sheet of Perspex to create an experimental 'frond'. Clumps were attached by boring holes into the plastic through which the base of the clump was passed. A ruler was then attached to the edge of the sheet so that measurements with the probe could be made at precise intervals. The experiment was carried out using three *Polysiphonia* clumps, with the distance between two neighbouring clumps being four cm. The clumps were large enough for adjacent clumps to almost completely cover the space between them.

Measurements were made from 4 cm in front of the first epiphyte clump to 4 cm beyond the third clump giving the experimental area a total length of 16 cm, which, depending on the width of a frond would correspond to a wet weight of roughly 2.4-2.5g in a real internode. The wet weight per clump at the start of the experiment was approximately 1.7 g giving a total of 5.1g epiphyte biomass. In a real frond, weighing 2.5 g, 5.1 g epiphytic tissue would correspond to 2.1 g epiphyte per g vegetative tissue. This means that the experimental set-up was equivalent to internodes on the shore with epiphyte biomass higher than the frond biomass carrying them, which has been found to be the case by Lobban and Baxter (1983) in the most highly epiphytised fronds in their study. The experimental set-up in the present study can therefore be assumed to mimic highly epiphytised fronds of *Ascophyllum nodosum* on the shore.

A white fluorescent tube (Philips 58W/35) was placed 50 cm above the clumps. Care was taken to ensure that the light level (photon flux density) was the same all along the 'experimental branch' and that the equipment used did not cause any shading. The irradiance was measured using a LI-250 light meter (LI-COR Inc.). Measurements were made with the probe facing upwards, through the canopy, from approximately 1 cm below the Perspex sheet. All measurements were made in air.

To obtain light measurements the probe was passed along the three clumps at a rate of approximately 1cm per second. During this time the probe made 15 readings. The measurements were repeated five times. Measurements were made at 5 degrees of desiccation: 1, 2, 4, 6 and 8 hours, corresponding to weight losses in the epiphyte of 19% (after 1h) to 68% (after 8h). The same procedure was followed to obtain control readings in the absence of the epiphyte. Measurements were also made exactly underneath each of the three clumps, with the probe being held still underneath the centre of the clump. These measurements were only carried out at time zero.

4.2.4 Growth of laterals from epiphytised and clean A. nodosum segments

This study was run in the laboratory between June and December 2000, using segments of *Ascophyllum nodosum*, which had been excised from the 2nd. youngest internode of a frond.

To examine whether shading by the epiphyte is the main factor preventing growth of laterals from lateral pits, three treatments were set up:

1. Segments with one epiphytised and one clean lateral pit
2. Segments from which epiphytes had been removed
3. Segments with two completely clean lateral pits.

Each treatment was applied to plants from an exposed shore and a sheltered shore (i.e. six treatments in all). A total of thirty segments were excised for each treatment, resulting in a total of 180 segments. Different fronds were used for each of the replicate segments and each excised segment had two lateral pits. Segments from epiphytised fronds were chosen so that one lateral pit was epiphytised, while the other was completely clean and bore lateral initials. The segments were usually 1 cm in length. However, as it became apparent during the set-up of the experiment that epiphytised fronds often had a wider diameter than clean ones, smaller segments were sometimes cut for the epiphytised treatments to gain segments of comparable size between treatments. But this was only possible to a limited extent as each segment had to contain one clean and one epiphytised lateral pit. Prior to carrying out tests, all segments were examined under a binocular microscope to make sure clean looking lateral pits were actually unepiphytised and that no *Polysiphonia* germlings were present.

For each treatment, five containers, each containing six segments were set up. The initial length and number of lateral initials per lateral pit was recorded for each of the segments. Prior to the start of the experiment *Polysiphonia lanosa* clumps were cleaned manually of contaminating green algae and small grazers using a binocular microscope and forceps.

Studies with such small plant segments have been carried out previously by Terry and Moss (1980), who were able to maintain healthy cultures for six months. Using the same culture conditions, segments were cultured in filtered seawater without any nutrient supplements in a culture cabinet at 10 °C and an 8:16 SD photoperiod using white light. Tanks were rotated periodically in the culture cabinet to compensate for possible small variations in the light conditions within the cabinet. At the end of the study the dry weights of vegetative and receptacle biomass were determined for each segment. Dry weights were determined by drying fragments in an oven at 60°C until constant weight.

4.2.5 Statistical Analysis

Differences in lateral length per lateral pit among and within treatments were analysed using nested Analysis of Variance. Light data were analysed using Repeated Measures ANOVA. Prior to each analysis data were tested for homogeneity of variance using Cochran's C test. Where variances were heterogeneous data were log transformed (Underwood 1981) or in the case of percentage data arcsin transformed. Following the Analysis of Variance an SNK procedure was carried out, where appropriate, to further examine differences between treatments.

4.3 Results

4.3.1 Measurement of light levels underneath a dense canopy of P. lanosa

The light experiments carried out in the laboratory revealed a 65% reduction in the photon flux density under a dense cover of *P. lanosa* compared with control measurements. Desiccation only caused very slight changes in light penetration (Figure 4.1). A one-way repeated measures ANOVA revealed statistically significant differences between treatments. These differences were further examined using a SNK procedure. This post-hoc test revealed that controls were significantly different from all other treatments. But there was no statistically significant difference in light penetration

Table 4.1: One-way repeated measures ANOVA for light levels under a canopy of *Polysiphonia lanosa* after desiccation of 2-8 hours. Results for the post-hoc SNK procedure are also given

a. Average readings taken along the series of tree Polysiphonia lanosa clumps

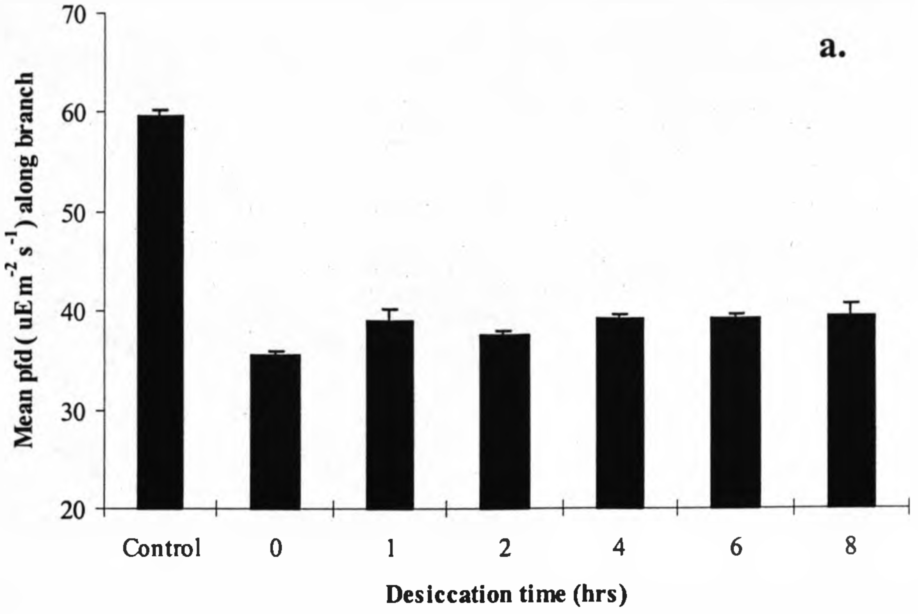
Source of variation	df	MS	*F*	p-value
Desiccation Time	6	0.0447	653.709	<0.0001
Error	24	<0.0001		

SNK Procedure: Ctrl>0-8h and 2h=4h=6h=8h

Table 4.1b. One-way Anova for differences in light penetration underneath the centre of three individual clumps.

Source of variation	df	MS	*F*	p-value
Desiccation Time	2	105.852	48.623	<0.0001
Error	12	2.177		

Clump 1=clump 2 > clump 3



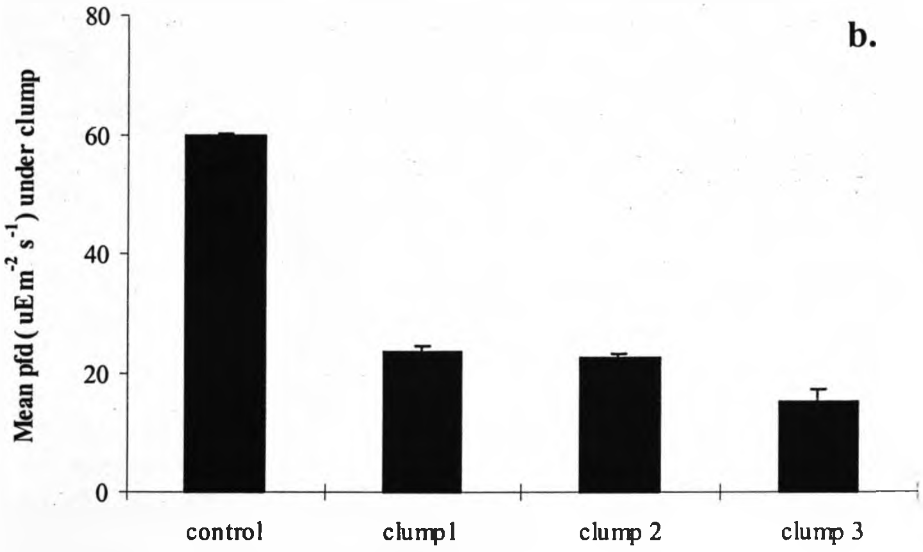


Fig 4.1 Light penetration through a canopy of *Polysiphonia lanosa* **a.** at varying degrees of desiccation of the epiphyte, **b.** under three individual clumps

4.3.2 Reproductive effort of A. nodosum in the presence of P. lanosa

The linear regression analysis carried out on transformed data from epiphytised and clean fronds of *Ascophyllum nodosum* from Fort Island revealed a statistically significant negative linear relationship between the dry weight of *Polysiphonia lanosa* per gram of vegetative tissue of the host plant and the reproductive effort of *A. nodosum* fronds ($F_{1,38}$=5.617, p=0.023, y=-0.36x-3.494). The untransformed data showed that clean fronds or those with very low epiphyte loads could have a range of reproductive efforts from between 10-68% but reproductive effort was generally low at epiphyte loads of above 0.05 g per g of *A. nodosum* tissue (Figure 4.2).

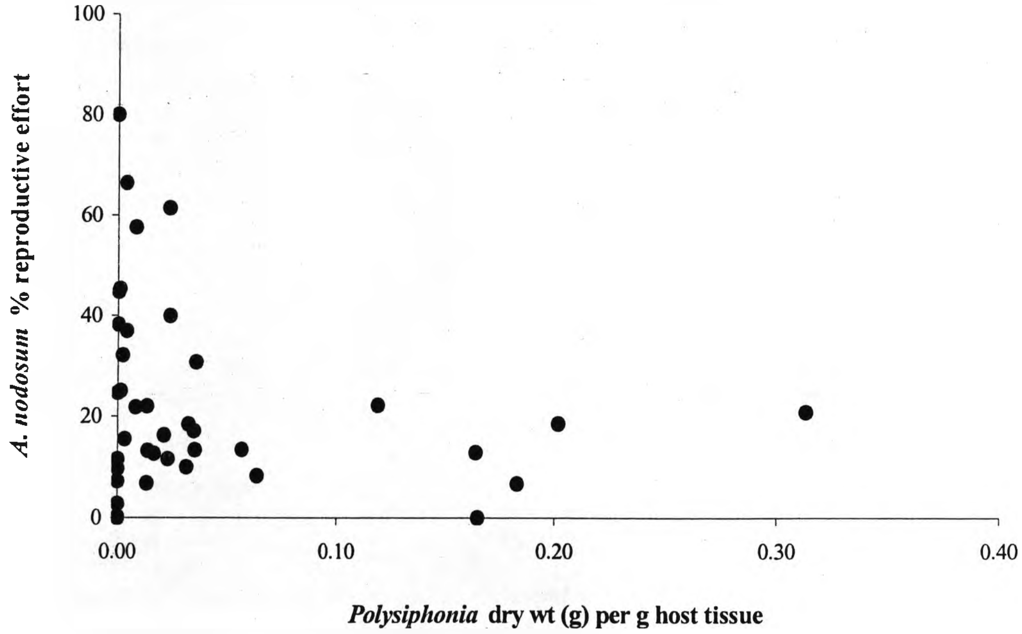


Fig 4.2: The relationship between reproductive effort of fronds of *A. nodosum* (g dry wt receptacle mass per total frond biomass) and their epiphyte load (g dry weight per gram host tissue). Data shown are not transformed.

4.3.3 Morphological differences between clean and epiphytised segments

Within each site, initial numbers of lateral initials were significantly higher in epiphytised than in clean plants (Fort Island: $F_{2,124}$=12.534, p=0.000026; Scarlett Point: $F_{2,194}$= 19.159, p=0.0001). No further statistical analysis was therefore carried out to investigate changes in numbers of laterals over time. However it seems unlikely that differences between epiphytised and clean fronds were significant over the course of the experiment (Figure 4.3). Despite the initial differences in lateral numbers, no significant differences occurred in initial length of segments between treatments. Therefore differences in initial lateral numbers were assumed not to interfere with analysis of length growth (Fort Island: $F_{2,124}$=2.034, p=0.135; Scarlett Point: $F_{2,194}$=2.28, p=0.104, Figure 4.4). The number of replicates in the Fort Island treatment, from which epiphytes had been removed, only had 4 groups , due to mortality of segments in one container within the first month of the experiment. As development of laterals had already started in the other replicates, the segments were not replaced.

4.3.4 Lateral branch development on clean lateral pits

The development of receptacles, judged from the thickening of the tip of laterals in the lateral pits and formation of conceptacles began within a month of the start of the experiment. In all treatments a small proportion of the laterals arising from lateral pits remained vegetative during the course of the study. The proportion of these laterals was higher in the segments from Scarlett than in the corresponding treatments with segments from Fort Island (Table 4.2). There was no clear trend with respect to the effects of epiphytes on lateral development. In the Scarlett samples clean segments produced the highest number of vegetative laterals, in the Fort Island treatments the highest number of vegetative laterals occurred in epiphytised plants. With respect to the numbers of laterals on clean lateral pits there was little change during the course of the experiment. Fragments from Scarlett Point for instance had produced on average 2.5 lateral initials at the time of collection, which only increased to an average of 3 laterals in December. No significant difference occurred in the other treatments. Although some growth on clean lateral pits it was offset by mortality of existing laterals in these cultures.

4.3.5 Lateral development from epiphytised lateral pits

Only a small percentage of plants from Fort Island developed any lateral branches from lateral pits which were epiphytised or from which epiphytes had been removed at the start of the experiment. No growth was found in any epiphytised lateral pits in segments from Scarlett Point (Table 4.2).

Table 4.2: Development of receptacles and vegetative laterals in segments from Fort Island and Scarlett Point

Treatment	No of initial lateral buds		% vegetative shoots of total at end of study	% epiphytised lateral pits with lateral formation
	Initial	Final		
Fort I. clean	69	67	10.45	0
Fort I. +epi	40	40	12.5	5
Fort I. ex epi	28	28	3.45	7.14
Scarlett clean	68	69	18.87	0
Scarlett +epi	68	69	14.49	0
Scarlett ex epi	63	64	10.94	0

4.3.6 Length changes of laterals between June and December

The length of receptacles on segments from both sites had increased considerably. In both sites the length of laterals at the end of the experiment was slightly higher in clean than in epiphytised plants. However, the ANOVA showed that this difference was only significant for segments from Fort Island (Figure 4.5, Table 4.3a-b).

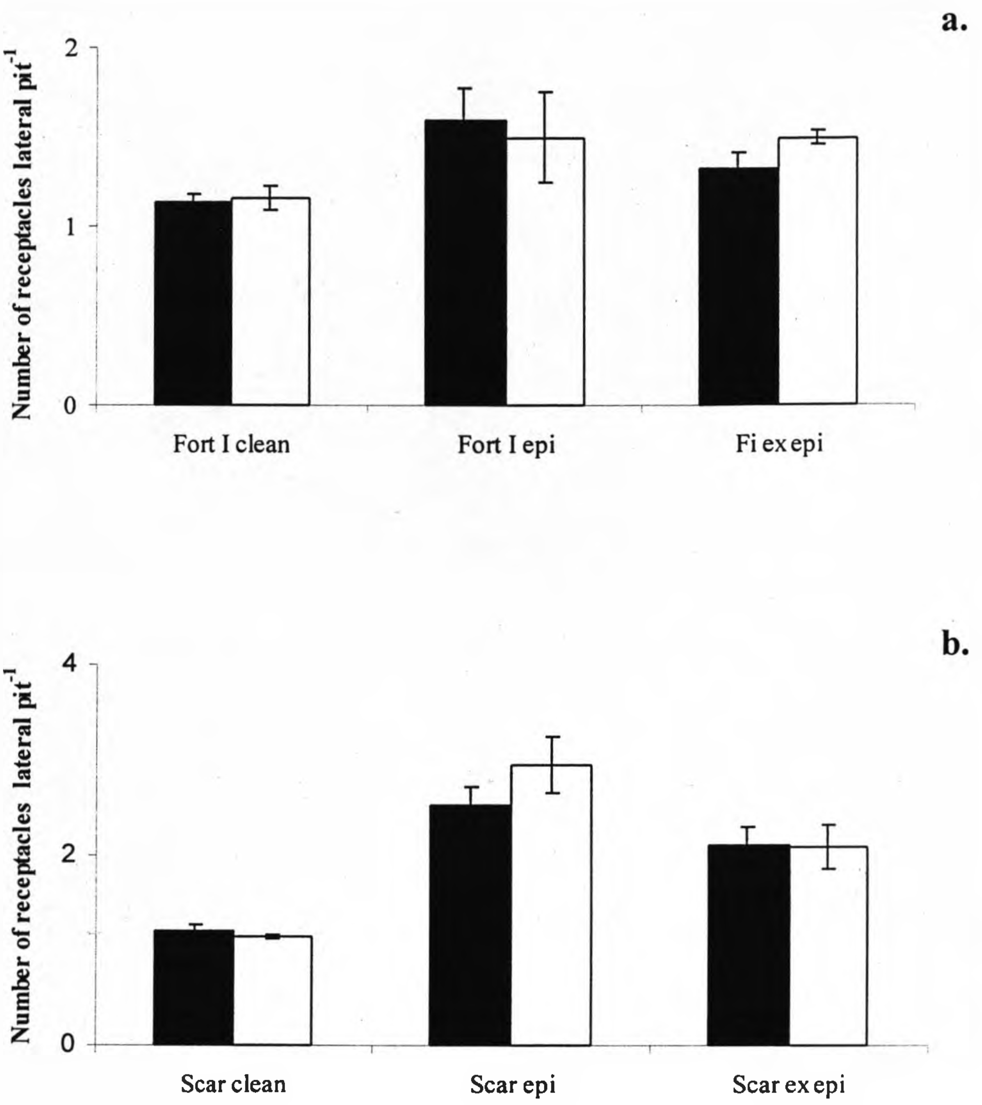


Fig 4.3 The numbers of receptacles per lateral pit in segments from **a.** Fort Island and **b.** Scarlett Point in June (solid bars) and December 2000 (open bars). In both graphs the first set of data (Fort I clean/ Scar clean) shows the results for segments with unepiphytised lateral pits. The second set (Fort I epi/Scar epi) shows results for epiphytised segments and the Fort I ex epi/ Scar ex epi refer to segments from which epiphytes have been removed. Data presented are means of 30 segments (25 for Fi ex epi) Error bars are± 1 standard error.

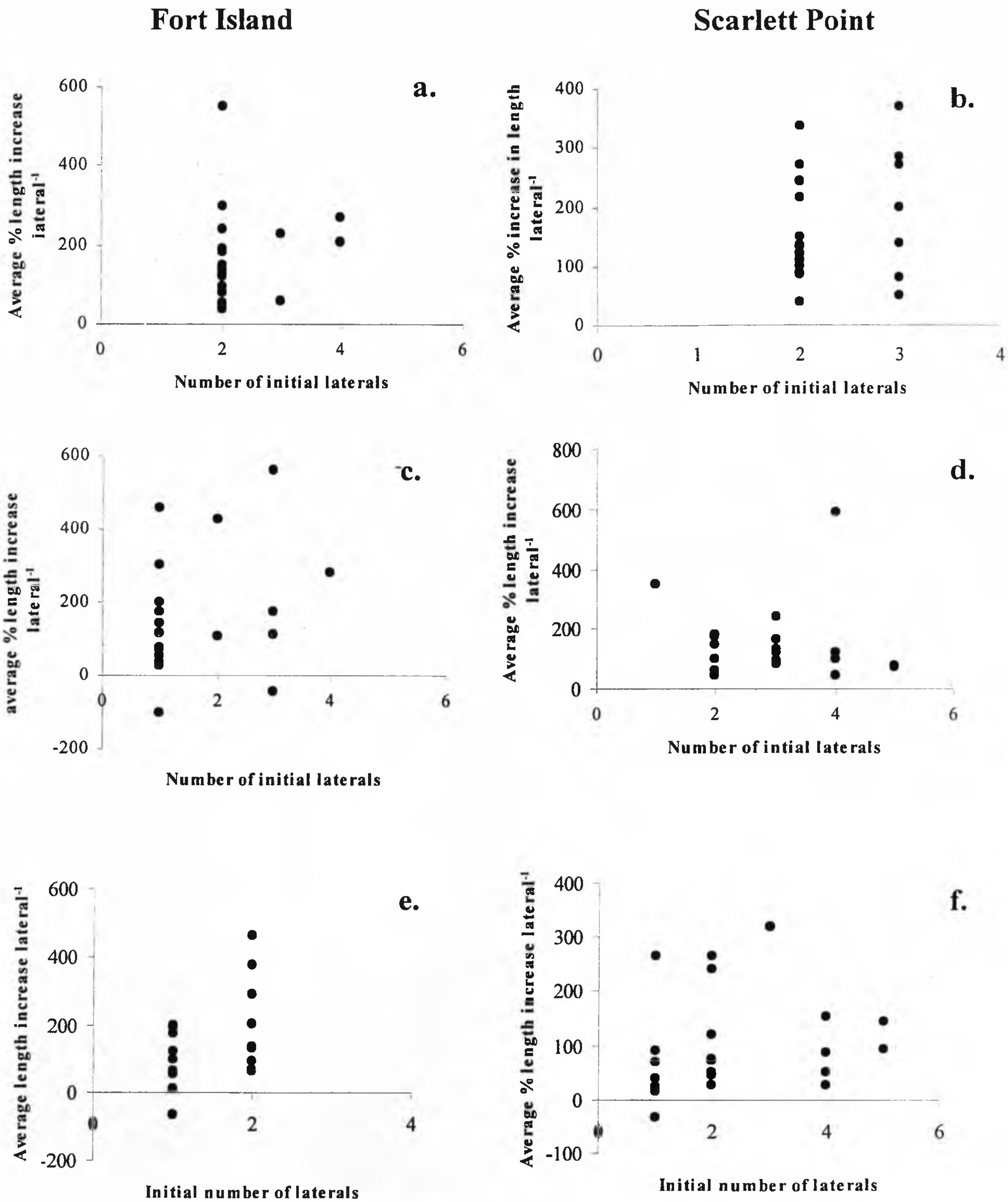


Fig 4.4 The relationship between initial numbers of laterals and average length increase per lateral. **a-b** clean segments, **c-d** epiphytised segments, **e-f** segments from which epiphytes had been removed

Table 4.3 a-b: Nested ANOVA for the final length of laterals in plant segments from Fort Island and Scarlett Point

a. Fort Island

Source of Variation	df	MS	*F*	p-value
Intercept	1	181.435	558.70	<0.0001
Treatment	2	1.192	3.671	0.028
Replicate	10	0.644	1.983	0.040
Error	122	0.3247		

b. Scarlett Point

Source of Variation	df	MS	*F*	p-value
Intercept	1	8.756	32.999	<0.0001
Treatment	2	0.333	1.256	0.288
Replicate	11	0.211	0.796	0.644
Error	162	0.265		

Clean and epiphytised plants within sites exhibited marked morphological variation with respect to the width of the fronds and number of receptacles per lateral pit. Therefore it was unavoidable that plant segments differed in dry weight between treatments. This might be expected to influence the production of receptacles, as more surface area is available for photosynthesis, but with two exceptions (both in segments from Fort Island) regression analyses carried out to compare the relationship between vegetative and reproductive tissue revealed no significant differences at the end of the culture period (Table 4).

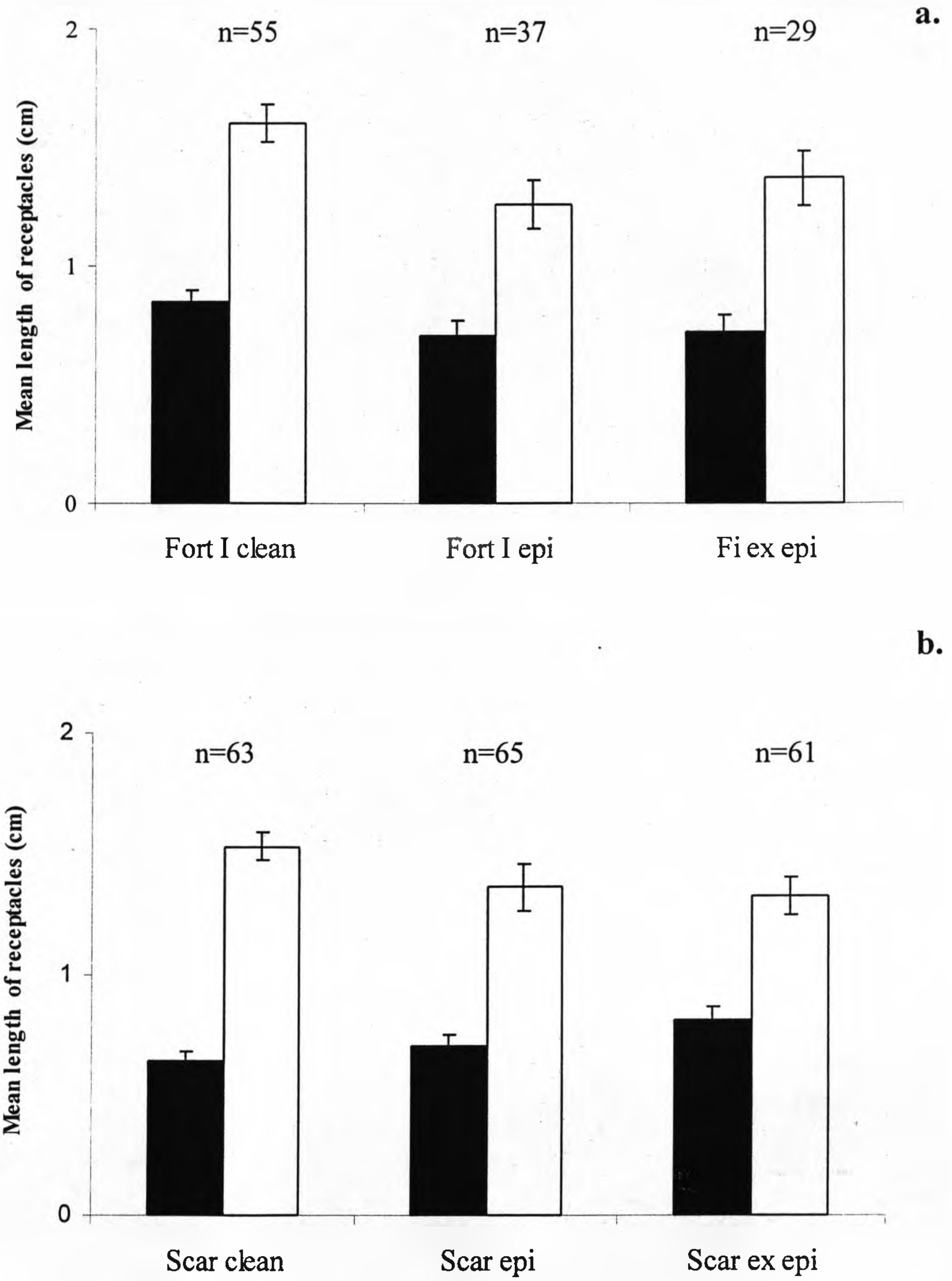


Fig 4.5 The average length of laterals on clean lateral pits In *A. nodosum* segments from **a.** Fort Island and **b.** Scarlett Point in June (solid bars) and December (open bars). In both graphs the first set of data (Fort I clean/ Scar clean) shows the results for segments with unepiphytised lateral pits. The second set (Fort I epi/Scar epi) shows results for epiphytised segments and the Fort I ex epi/ Scar ex epi refer to segments from which epiphytes have been removed. Error bars ± 1 standard error

Table 4.4: Regression analyses for the relationship between vegetative and reproductive dry weight of epiphytised and clean fragments from Fort Island and Scarlett Point

Treatment	R^2	F	p-value	Regression equation
Fort island clean	0.15		0.859	
Fort Island epiphytised	29.17	6.59	0.021	Y=0.002+0.540x
Fort Island - epiphytes	19.14	5.970	0.024	Y=-0.001+0.479x
Scarlett clean	2.57	0.323	0.575	
Scarlett epiphytised	0.06	0.120	0.732	
Scarlett - epiphytes	0.01	0.0004	0.984	

4.3.7 Receptacle dry weights in clean and epiphytised segments

Receptacle biomass expressed as g dry weight per g of host tissue showed that in both sites clean segments carried a lower receptacle mass than epiphytised segments. This effect was slightly more pronounced in plant segments from Fort Island than from Scarlett Point. The ANOVA gave a significant difference for both sites (Table 4.5, Figure 4.6). However, expressed as receptacle dry mass per lateral pit, dry weights in epiphytised plants, where only one lateral pit was available for receptacle production, were significantly higher in epiphytised than in clean fronds. Therefore the fronds could compensate to some extent for the loss of lateral pits by the production of a higher receptacle mass from adjacent lateral pits. Differences between treatments were again significant for both sites. Receptacle dry weight per lateral pit also appeared slightly higher in segments with one epiphytised lateral pit than in segments from which epiphytes had been removed, although this perceived difference was not statistically significant (Table 4.6, Figure 4.7).

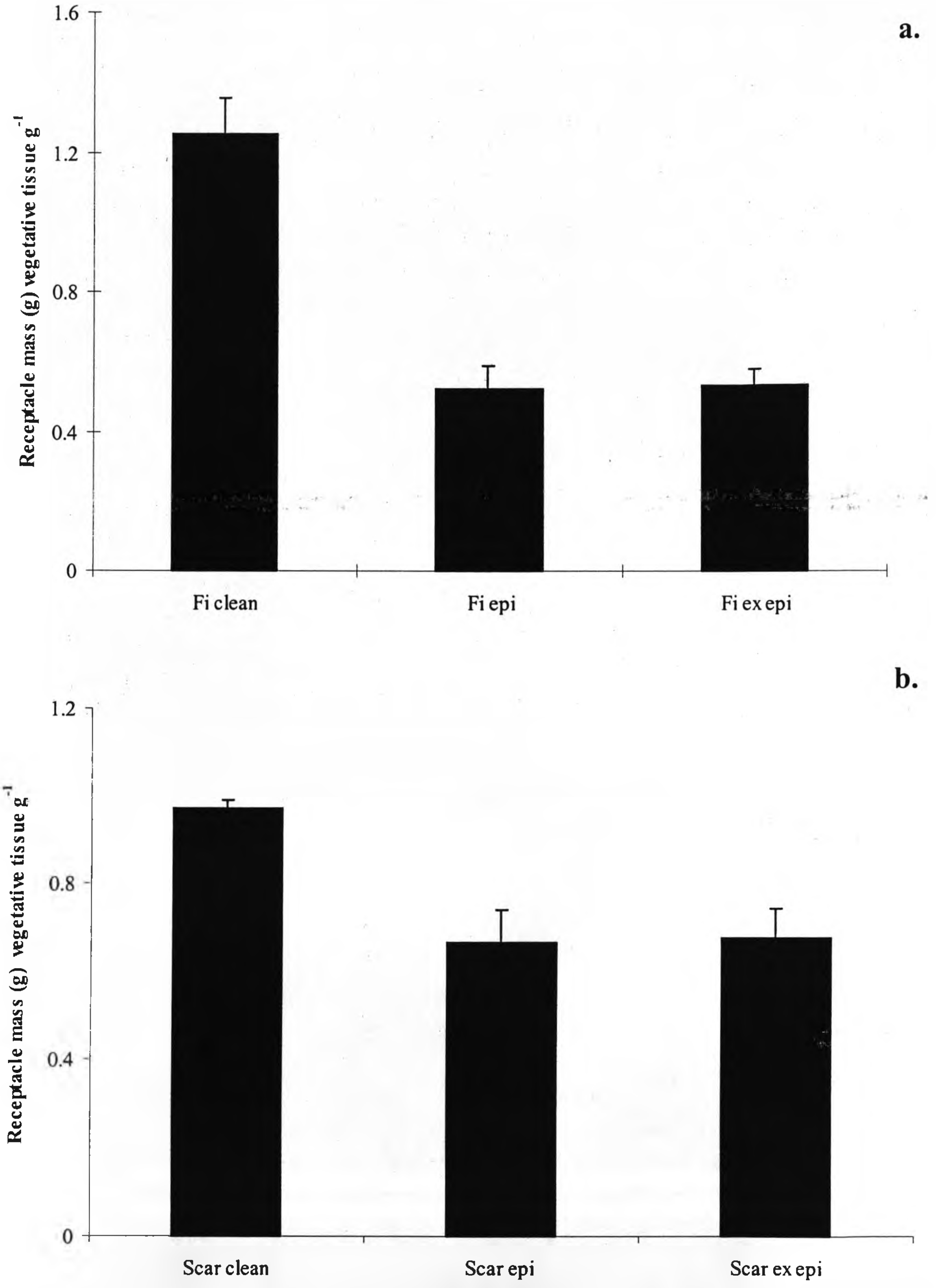


Fig 4.6 Dry weight of receptacle tissue (g) in each treatment, expressed per gram of vegetative tissue **a.** Fort Island, **b.** Scarlett Point; data presented are means of 30 segments (24 for Fi ex epi), error bars are ± 1 standard error

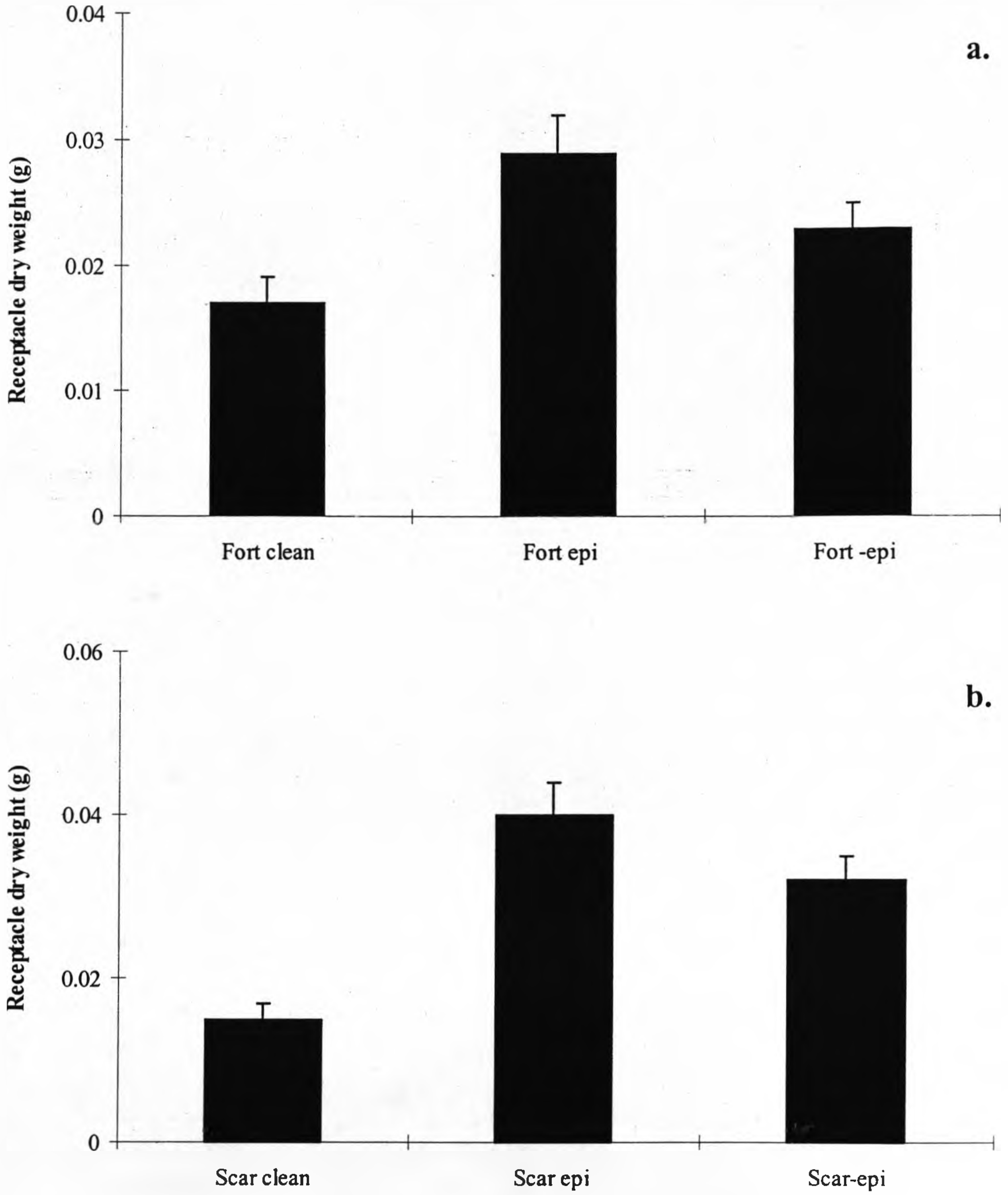


Fig 4.7 Total receptacle dry weight per lateral pit in clean and epiphytised segments in December 2000 **a.** fragments from Fort Island, **b.** fragments from Scarlett Point. Data are the means of 30 segments (24 for Fort I ex epis). Error bars are±1 standard error

Table 4.5a-b: Nested ANOVA for receptacle dry weights per gram vegetative tissue of *Ascophyllum nodosum* in segments from a. Fort Island and b. Scarlett Point

a. Fort Island

Source of Variation	df	MS	*F*	p-value
Intercept	1	36.286	265.12	<0.0001
Treatment	2	3.430	25.061	<0.0001
Replicates (nested in treatment)	10	0.08	0.587	0.817
Error	49	0.137		

b. Scarlett Point

Source of Variation	df	MS	*F*	p-value
Intercept	1	41.160	3.189	<0.0001
Treatment	2	0.697	5.399	0.007
Replicates (nested in treatment)	9	0.214	1.658	0.1219
Error	55	0.129		

Table 4.6a-b: Nested ANOVA for the receptacle dry weight per lateral pit in plant segments from Fort Island and Scarlett Point

a. Fort Island

Source of Variation	df	MS	*F*	p-value
Intercept	1	0.033	220.658	<0.001
Treatment	2	0.0007	4.65	0.014
Replicates (nested in treatment	10	0.00002	0.146	0.999
Error	50			

b. Scarlett Point

Source of Variation	df	MS	*F*	p-value
Intercept	1	1002.38	4122.53	<0.0001
Treatment	2	6.57	27.005	<0.0001
Replicates (nested in treatment	10	0.48	1.977	0.0519
Error	60	0.24		

4.4 Discussion

In the present study an almost complete loss of the ability to produce any type of lateral from lateral pits that have been epiphytised was observed, even when the epiphytes themselves had been removed.

Since the segments were grown at irradiances, favourable for the production of receptacles (Terry and Moss 1980, Cousens 1985), it seems likely that any adverse effect of the epiphyte on host reproduction occurs via physiological changes in the lateral pit the epiphyte has colonised leading to the conclusion that hypothesis 1 rather than 2 is correct. According to Rawlence and Taylor (1972) the host tissue surrounding the penetrating rhizoid of *P. lanosa* degenerates and in some cases completely disappears. Lateral pits, which have been modified in this way, are likely to lose permanently the ability to produce laterals and therefore reproductive bodies if the tissue destroyed by the penetrating rhizoid includes the meristematic region of the lateral pits (Moss 1970). This could explain why on the shore the most severely epiphytised plants often do not consist of more than the epiphytised main axis and one or two laterals (pers. obs.). It might be argued that since the epiphytised pits had, by default, no lateral initials at all, removal of the epiphytes could not have led to production of laterals, because the number of laterals per lateral pits had already been determined prior to the start of the experiment, i.e. the resources had already been allocated. However, limited growth of entirely new laterals did occur from clean lateral pits in the present study and in two cases even from the epiphytised lateral pit itself. Whether such regrowth is possible might depend on the length of time for which the lateral pit had been epiphytised prior to epiphyte removal. Due to the nature of the process of rhizoid penetration, it might actually have been impossible to completely remove the epiphyte from the most heavily epiphytised lateral pits.

Although the lack of growth from currently or previously epiphytised lateral pits is likely to have been the result of the blocking of the lateral pit, a reduction in light intensity could still have affected growth patterns of the clean lateral pits on epiphytised segments. Several authors have reported adverse effects on growth and in some cases morphogenesis in plants in reduced irradiances. Fitzpatrick and Kirkman (1995) for instance found a decrease in the shoot numbers and leaf growth rates in the seagrass *Posidonia australis*, although in their study the shading treatments removed more than 90 % of PAR. But Moore and Wetzel (2000) found similar effects at irradiance reductions of 42 % of incident solar PAR. In an earlier study Critchley (1983) had also found a reduction in length of apical segments and a reduction in the number of air bladders at low light intensities in the brown alga *Sargassum muticum*.

The results indicate that epiphytism does reduce the production of laterals at epiphytised sites. Although significant decreases in light reaching the host plant were demonstrated, light reduction is not necessarily a causal factor in the observed patterns in clean lateral pits. That a reduction in irradiance as a result of epiphytism did not have a major effect on the host plant is borne out by the fact that the ‘clean’ lateral pits on epiphytised segments did not generally show an increase in the number of laterals remaining vegetative, which would have been expected in shaded conditions according to Terry and Moss (1980).

Perhaps most importantly, although epiphytised lateral pits did not produce any lateral branches themselves, be they vegetative or reproductive, clean lateral pits on the same epiphytised internodes produce more receptacles per pit than lateral pits on completely clean internodes. Moreover, while reproduction per gram of vegetative tissue was reduced in epiphytised plants, indicating that epiphytism exerts a cost on the host plant, the receptacle biomass per lateral pit was actually significantly higher in epiphytised plants, as one might expect considering the higher initial number of laterals in these plants. This is inconsistent with adverse effects of shading on the number or length of apical shoots and lateral branches described in earlier studies and indicates that *A. nodosum* seems to be able to compensate to some extent for the loss of lateral pits by increasing production of receptacles from those remaining.

That *Ascophyllum nodosum* is able to increase production of receptacle biomass from clean lateral pits when adjacent pits are blocked, indicates that the cost of epiphytism in terms of lost reproductive output in the host might be considerably lower than estimates from the mere incidence of *Polysiphonia lanosa* would suggest. This ability would, however, be expected to decrease with increasing numbers of lateral pits being epiphytised. The effect of epiphytism on receptacle production might explain the shape of the curve for the data in Figure 4.2 which suggested that beyond a certain threshold epiphyte load reproductive effort would be permanently low. A possible reason for the pattern in Figure 4.2 might then be that, with increasing epiphyte loads, individual lateral pits reach the limit of their ability to accommodate additional receptacles. In addition, light limitation should also become more important in highly epiphytised plants. Therefore, while *Ascophyllum nodosum* might be able to tolerate low to medium epiphyte loads, reproductive inhibition is likely to occur at high incidences of

Polysiphonia lanosa. In considering the possible costs of such reproductive inhibition one has to take into account that receptacle production is not uniformly high along the axes of *A. nodosum*. It is usually only the younger year classes, which are producing receptacles. Therefore the effect of the epiphyte might be expected to have a limited extent. However, since receptacles and vegetative laterals are produced from the same lateral pits, blockage of these pits means inhibition of lateral production in general. Such an effect would therefore not only affect current but also future reproduction as further sites for the production of laterals become reduced and also by preventing the growth of vegetative laterals which would produce new lateral pits.

On exposed shores where epiphyte loads are higher (Levin and Mathieson 1991) and the degree of branch breakage is also considerably increased (Young 1987) the effect of epiphytism might have an even greater impact.

Chapter 5: The Importance of hydrodynamic conditions for the nature of the symbiosis between *Ascophyllum nodosum* and *Polysiphonia lanosa*

5.1 Introduction

The size and morphology of *Ascophyllum* varies with the degree of exposure. While on sheltered shores the plants reach a length of up to two metres, they are commonly much shorter than 1m long on more exposed shores (Levin and Mathieson 1991, Young 1987).

Water flow velocities on rocky shores can vary from only a few centimetres per second to in excess of 10 m s^{-1}, which in effect corresponds to wind velocities of approximately 280 m s^{-1} (Denny *et al.* 1985, Gaylord *et al.* 1994). Seaweeds subjected to water flow will experience drag as the result of pressure differences between their upstream side (near the holdfast of deflected plants) and the downstream side. Such pressure differences will tend to pull the organism downstream (Koehl 1986). The magnitude of drag at a given flow speed depends to a large extent on the surface area of the body and its orientation with respect to the direction of flow. A seaweed with a large surface area perpendicular to the flow will experience considerably more drag than one that is deflected and aligned in the direction of the flow (Vogel 1994). In addition to drag, an organism in flow also experiences forces resulting from the acceleration of water around it. This force can be of particular importance for organisms on wave-swept shores (Koehl 1984 and references therein, Denny *et al.* 1985).

Although marine macroalgae can be subject to extreme drag forces, they are commonly underscaled as well, i.e. the load bearing structures such as stipes do not grow to the same extent as other structures such as fronds and blades. The risk of breakage for a seaweed should therefore increase with increasing size in given flow conditions (Hay 1981, Peterson *et al.* 1982). However, many seaweeds living in high flow environments have evolved adaptations to minimise the effects of drag and underscaling, and enable them to persist in stressful environments. These adaptations include changes in morphology such as shortening of the plant and less branching, greater stiffness of the stipes or greater flexibility and extensibility of the plants. The last two have been described for kelps by a number of authors. Koehl and Wainwright (1977) for instance found that *Nereocystis luetkeana* in strong flows, could extend to more than 15 % of its original length before breaking at a breaking stress of approximately 3 MN m^{-2} (Koehl 1979). Santelices *et al.* (1980) found evidence of both strategies in two kelps co-occurring on shores in Chile and Biedka *et al.* (1987) found that *Pterygophora californica* could avoid breakage by virtue of an extreme ability to bend its stipe. Both flexibility and extensibility allow the plant to become streamlined in flowing water, i.e. to effectively reduce the surface area exposed to the flow (Denny 1993). However, according to some authors this might incur a cost e.g. greater susceptibility to grazing (Biedka *et al.* 1987, Dudgeon and Johnson 1992, Johnson and Koehl 1994).

The effects of large drag forces might be exacerbated by the presence of epiphytes (Witman and Suchanek 1984, Ruesink 1998, Belegratis *et al.* 1999). Filamentous epiphytes, such as *Polysiphonia lanosa* which is commonly associated with *Ascophyllum nodosum*, could considerably increase the drag experienced by a seaweed frond by virtue of their high surface area, and therefore might be expected to reduce the velocities at which the host fronds break (D'Antonio 1985).

However, as results from Chapter 1 have shown, growth of small primary shoots can be considerably enhanced after removal of the canopy fronds. This could be a mechanism for the 'rejuvenation' of the host population by removal of unproductive material. The extent to which this can affect the field population depends on the rate of removal of older axes.

The aim of this chapter is therefore to investigate the effects of host plant morphology and different degrees of epiphytism on the drag experienced by *Ascophyllum nodosum*. It will be attempted to make estimates of the likelihood of breakage in the field and to predict breakage velocities to establish whether the epiphyte plays a role in providing open spaces in the canopy.

The first step was to establish breaking forces of fronds in the field and to obtain an estimate of the scaling properties of *Ascophyllum nodosum*. Then theoretical detachment forces in water were predicted from these breaking forces. Experiments in a flume were used to investigate the actual drag forces at given flow velocities and the effects of epiphytism and plant morphology on the drag to compare with the predictions from field tests.

5.2 Materials and Methods

5.2.1 Breakage study in the field

As in previous studies plants were collected from the sheltered site in Fort Island and the exposed site in Scarlett Point. Plants were taken from the lower limit of the distribution of *A. nodosum* where the highest incidences of *Polysiphonia lanosa* were found. On each of these shores fronds of different length were selected to investigate scaling effects (LaBarbera 1989). On each shore 15 clean and 15 epiphytised fronds were initially selected. A small proportion of the tests resulted in dislodgement of the holdfast from the substratum rather than stipe failure, so tests were carried out until 15 stipes had broken in each category.

All laterals on a frond were aligned towards the tip of the extended main axis (as they would be in normal flow conditions). A 10 kg spring balance was then attached to the distal portion of the frond using electrician's cable padded with several layers of insulation tape. An indelible felt tip pen was attached to the spring balance to mark the point at which the fronds became detached. After the spring balance had been secured around the frond, the spring balance was pulled steadily but quickly (for 2-3s), parallel to the substratum, until the stipe broke.

After they had been removed from the substratum, the fronds were placed in plastic bags and transported back to the laboratory where the epiphytic tissue was carefully removed from all fronds. For each frond the overall plant length and wet weight (after blotting the fronds between tissue paper) were recorded. Prior to wet weight measurements of the epiphyte, the plants were pressed repeatedly between tissue paper until no more residual moisture was visible on the paper. In addition to these data, surface areas for the fronds were calculated using a calibration curve for surface area vs. biomass. The smallest and largest diameter of the stipe at the breakage point was also measured. The equation for the surface area of an ellipse was used to calculate the cross-sectional area of the stipe at the point of breakage. The strength of each frond was then calculated by dividing the breaking force by the calculated stipe cross-sectional area. Log-log plots of the strength of the stipe against the stipe cross-sectional area were used to assess the possibility of underscaling in *Ascophyllum* fronds. It is important to note that these plots are auto-correlated (Dudgeon and Johnson 1992, Johnson and Koehl 1994). They will therefore show negative relationships even if strength does not decrease as the x-variable increases. Their primary use is to compare the slope of the line with a theoretical line, which indicates the independence of breaking force from cross-sectional area of the stipe

To compare the breaking forces from the flume study to the breaking forces measured in situ, it was necessary to adjust the results for the different media. The density of water is approximately 830 times higher than that of air, speeds measured in water would, all other variables remaining equal, be higher by a factor of 15 (Vogel 1994). If it is assumed that drag is proportional to the square of velocity for a given shape (Carrington 1990), the predicted drag in water will be obtained by multiplying any force measurements (resulting from pulling the stipe) on a stipe in air by 830, and then dividing it by 15^2, which results in a conversion factor of 3.7.

5.2.2 Flume experiments

The drag measurements on fronds of clean and epiphytised *Ascophyllum nodosum* were carried out in the high-speed water channel (flume) in the Mechanical Engineering Department in Liverpool. In the flume, water velocities can be accurately controlled for flow velocities from 0.3-6.4 m s^{-1}. Within the working section of the flume, which has a length of 3.96 metres, flow conditions are laminar. A preliminary study was carried out in September 1999. The experimental runs were then carried out between May 9-12 2000 and January 8-9 2001.

For each experiment the seaweed fronds were attached approximately 50 cm below the water surface to a strut, which was in turn attached to a force balance suspended above the flow channel, and consisted of a strip of aluminium milled into a streamlined shape. The strut was connected to the force balance by means of a section of steel pipe. Samples were held in place at the lower edge of the strut using a medal slider. To avoid injury to the plant material the lower end of the strut and the slider were rubber lined.

A transducer on the force balance was linked to a voltmeter and measurements were made as electrical resistance which was then converted to drag measured as Newtons (equation 1). Each day, prior to the start of the experiment, drag on the strut alone was measured. This value was subtracted from subsequent measurements on that day.

Tests were carried out at flow speeds of between 0.5 and 3.0 m s^{-1} in the preliminary study and at 1 and 2 m s^{-1} or, where possible, 3 m s^{-1} for the main experiments. At higher speeds the strut holding the seaweed, produced so much turbulence that drag readings on the samples became unreliable. Each run lasted approximately 15 minutes. Care was taken to ensure that periods of water acceleration were similar for each sample, to avoid differences in breakage as the result of acceleration rather than drag forces.

Tests were carried out on single fronds of two different sizes (50 and 100 cm long) and on axes carrying a fixed number of five laterals located either uniformly or distally on the main axis. For both sets of morphologies, drag was measured for both clean and epiphytised fronds. After the tests in the flume, the wet weight of host plant and epiphytes was measured. This meant that for some of the fronds, which snapped, weights could not be measured at all, as they could not be retrieved from the filters of the flume. Where fronds broke during the run in the flume, the water velocity at the time of breakage was recorded. If fronds broke during acceleration, the last known stable velocity was recorded. The frond diameters at the point of breakage were also measured to calculate cross-sectional surface areas. For the experiments carried out in May, the length of fronds was also measured before and immediately after the run in the flume to obtain an estimate of their extensibility.

For each axis tested in the flume, drag in Newtons and drag coefficients (C_d) were calculated (equations. (1) and (2) respectively). While the drag gives an indication of the forces the frond is exposed to as the result of the water flowing past it, the drag coefficient describes the 'behaviour' of the frond in flow. Specifically it shows how it realigns itself in the direction of the flow in order to minimise the surface area exposed to the flowing water.

$$D = \frac{V_k - V_0}{V_c - V_0} \times 9.81 \tag{1}$$

D= drag, V_k = flume reading in mV, V_c = calibration reading for 1 kg load, V_0 = zero reading

$$C_d = \frac{2D}{pSU^2} \tag{2}$$

C_d=drag coefficient, p = water density, S = plant surface area, U = water velocity

5.2.4 Surface area measurements

For the host plant surface area was measured using images of axes before the experimental run in the flume. The fronds were spread out on a flat white surface and were pressed down with a heavy sheet of glass, making sure none of the branches of the fronds were twisted. Images were taken using a digital camera, transferred to a PC and analysed using Scion Image analysis software.

An indirect method was chosen to estimate the surface area of *Polysiphonia lanosa*. Clumps of the epiphyte were removed from the *A. nodosum* frond, weighed and placed on a piece of paper. The filaments were spread out as thinly as possible without any obvious gaps. The circumference of the clumps was then traced and cut out. The paper was weighed and compared to the weight of cut paper of known area. The resulting surface area measurements were then plotted against wet weight of the fronds to give a calibration curve. After each run in the flume, clumps were removed from the experimental fronds and weighed so that the surface area could be determined using the calibration curve.

5.2.5 Field labelling study

Between November 6 and 10 2000, twenty fronds of different lengths were labelled at Fort Island and Scarlett Point. The labels consisted of orange fluorescent Nylon string to which numbered red insulating tape was stapled. The fronds included the range of morphologies and epiphyte loads used in the flume so that comparisons could be made between the theoretical and actual risk of frond breakage.

For all fronds the total length and an estimate of wet weight (using a spring balance) were obtained in the field. The length range of plants at Fort Island is much larger than for those at Scarlett Point, which is reflected in the results. The longest frond labelled in Fort Island was 190 cm, while the maximum length at Scarlett Point was 140 cm. The length of fronds in the field was obtained by fully extending each frond and measuring the distance between the holdfast and the tip of the longest axis and associated branches (to the nearest cm). The number of laterals and broken lateral tips, as well as the diameter of the narrowest part of the plant was recorded when the study was set up. In addition, an estimate of epiphyte loads was obtained in the field by counting all the clumps of *Polysiphonia* that could be distinguished (including recent settlement).

At the end of the study in March 2001 all remaining fronds were removed from the shore and taken back to the laboratory where the wet weight was measured. Wet weights for *Ascophyllum nodosum* fronds and the epiphyte were measured as in the detachment study. The length was also re- measured, and the wet weight and number of *Polysiphonia lanosa* plants was recorded.

5.2.6 Statistical Analysis

Prior to all parametric tests, the data were checked for normality and, where necessary, homogeneity of variances. Sets of data in the flume and detachment study were compared using regression analysis. To increase sample sizes, data were pooled where possible, e.g. across test dates and exposure. Whether this could be done was tested using Analysis of Covariance in the GLM module of the STATISTICA package

(Version 5.5). For this purpose the homogeneity of slopes option was used. This analysis was used to test for interactions between variables. Where such interactions were found, data could not be pooled (Underwood 1981) and such data were analysed in an ANCOVA with two separate categorical predictor variables and biomass as a covariate.

5.3 Results

5.3.1 Field Study: frond breakage

An initial analysis of the fronds after they had been removed from the shore in the course of the detachment study revealed that the stipe diameters varied considerably between epiphytised and clean fronds and between shores of different exposure, and were noticeably higher in fronds from Scarlett Point than from Fort Island (Figure 5.1a). However, a two-way ANOVA revealed that only the differences resulting from exposure were significant. There was also a significant interaction term, indicating that while there were differences in the effect of epiphytes these differed between shores (Table 5.1a).

The differences in diameters were also reflected in the breaking forces required to break the stipes. At both sites epiphytised fronds did not require higher forces to break the stipe than clean fronds but breaking forces in both epiphytised and clean fronds were higher at Scarlett Point than Fort Island. A two-way ANOVA showed that again only the differences between exposed and sheltered shore were significant (Figure 5.1b and Table 5.1b). The significant relationship between biomass and breaking force allows predictions of the drag force at which a frond of a particular biomass should break, by calculating the corresponding drag value and breaking forces in water.

Table 5.1a-b: Two-way ANOVA for break diameters and detachment forces of epiphytised and clean fronds in Fort Island and Scarlett Point

a. mean stipe diameter at point of breakage

Source of Variation	df	MS	*F*	p-value
Exposure	1	0.124	5.328	0.025
Epiphytism	1	0.072	3.097	0.085
Interaction	1	0.124	5.328	0.025
Error	50	0.233		

b. Mean breaking force

Source of Variation	df	MS	*F*	p-value
Exposure	1	2327.0	7.619	0.008
Epiphytism	1	743.9	2.430	0.125
Interaction	1	195.0	0.638	0.428
Error	52	305.5		

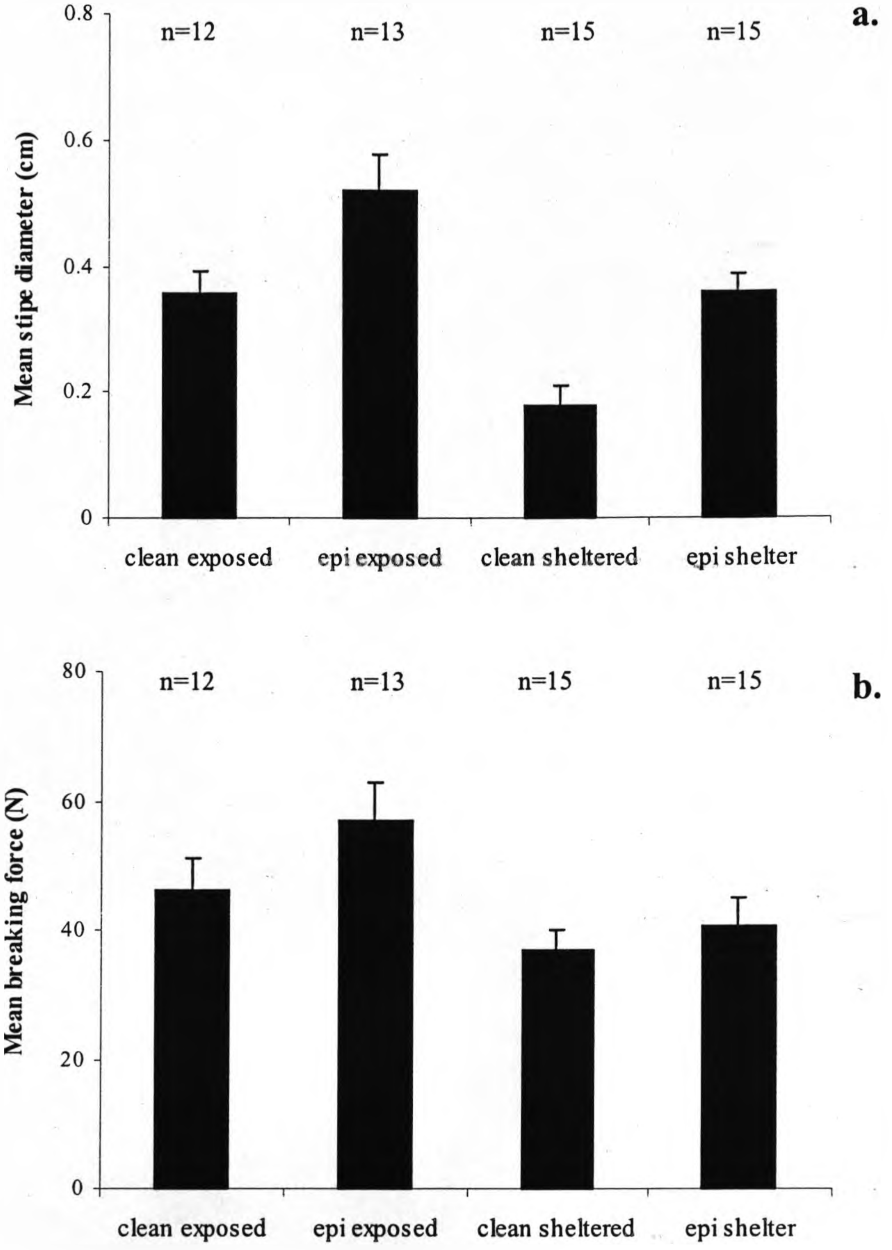


Fig 5.1 Initial measurements in the frond breakage study at Fort Island and Scarlett Point, **a**. average stipe diameter of *Ascophyllum nodosum* fronds at the point of breakage (cm), **b**. average forces (N) required for stipe failure. Error bars are ± 1 standard error

5.3.2 Size effects (scaling)

To investigate the relationship between frond size and mechanical properties, total frond biomass was initially compared with breaking forces. The regression analyses carried out for clean and epiphytised fronds from both sites revealed significant increases in breaking force with increasing biomass for clean fronds from Fort Island and for epiphytised fronds from Scarlett Point (Figure 5.2 a-b, Table 5.2).

Table 5.2: Regression analysis for relationship between detachment force and total biomass of epiphytised and clean *A. nodosum* in Scarlett and Fort Island.

Variable	N	df	R^2	*F*	p-value
Fort Island clean		12	33.78	6.12	0.029
					y=0.581x+24.89
Fort Island epi		12	25.6	4.13	0.065
Scarlett clean		10	20.49	2.58	0.139
Scarlett epi		11	37.54	8.21	0.015
					y=0.654x+32.14

With respect to the cross-sectional surface area of the stipe at the point of breakage, no significant linear relationship between the latter and total frond surface area were found in clean or epiphytised fronds from either site. However, while at Fort Island data for clean and epiphytised fronds formed an indistinct cluster, stipe surface areas were slightly higher in epiphytised than clean fronds at Scarlett for a given frond surface area (ANCOVA: $F_{1,24}$=12.211, p=0.019, Figure 5.3 a-b). Moreover, a larger number of fronds at Scarlett Point appeared to become detached at the holdfast rather than break along the stipe.

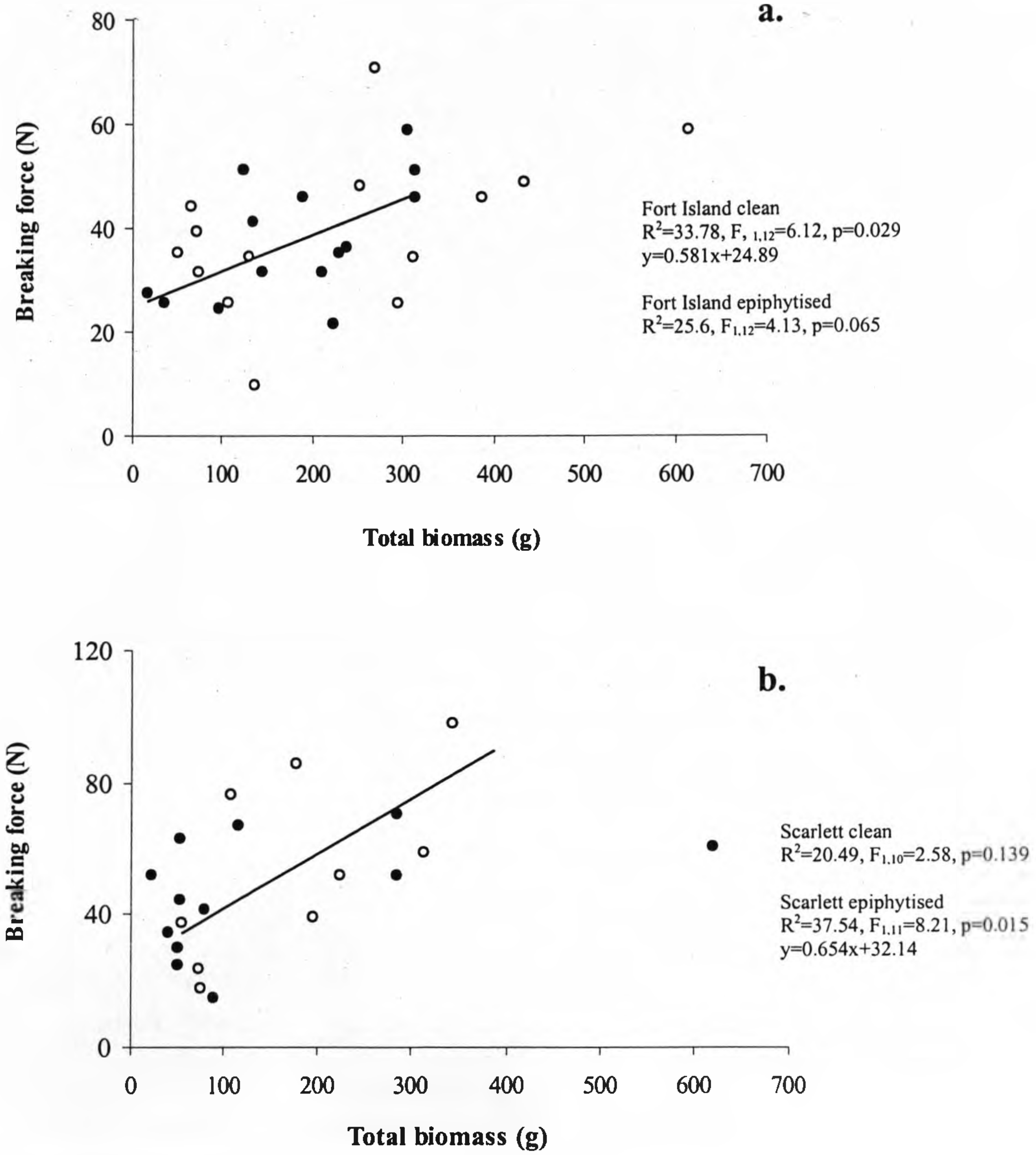


Fig 5.2 The relationship between breaking force (N) and frond biomass (g) for fronds from the frond breakage field study: **a**. fronds from Fort Island and, **b**. fronds from Scarlett Point. Breaking forces are shown separately for clean fronds (closed circles) and epiphytised fronds (open circles).

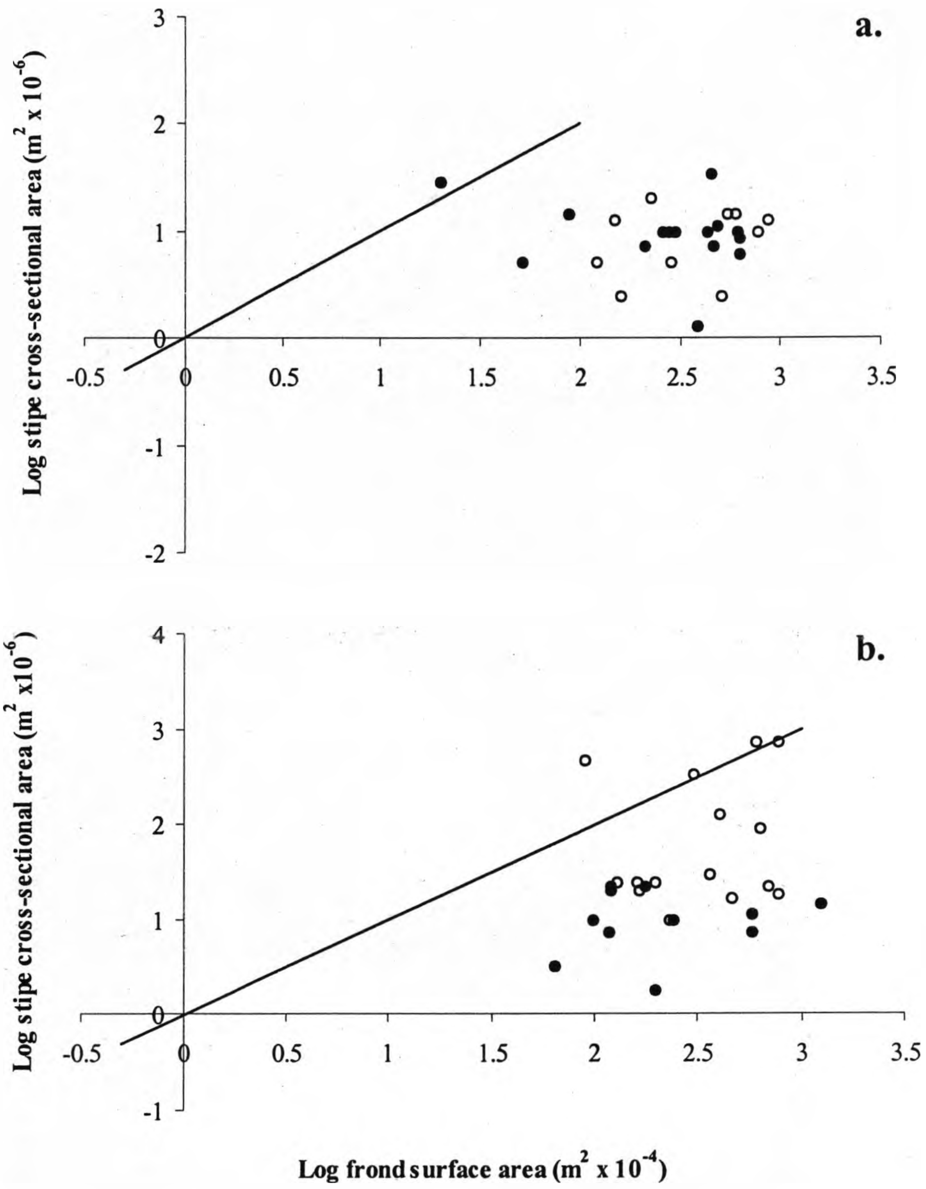


Fig 5.3 The relationship between stipe cross-sectional surface area and frond surface area (frond surface area was calculated from the regression for the relationship between frond biomass and surface area obtained for fronds from the flume study: **a**. fronds from Fort Island, **b**. fronds from Scarlett Point. Data are presented separately for clean (open circles) and epiphytised fronds (closed circles). The solid line shows the slope, at which stipe cross-sectional area would scale perfectly with frond surface area

An analysis of the tissue strength of fronds (i.e. force per unit cross-sectional area required to break the stipe) revealed significant decreases in tissue strength with increasing stipe cross-sectional surface area. No significant differences in the slopes of clean and epiphytised fronds were detected, nor did strength as a function of stipe cross-sectional surface area vary significantly between the sheltered and exposed site (Figure 5.4 a-b). ANCOVAS carried out for both sites did not show any effects of exposure or epiphytism on either shore. Detachment force appeared to be independent of the biomass of the fronds as shown by the insignifican interaction term (Table 5.3 a-b), indicating underscaling of the fronds with respect to tissue strength.

Table 5.3a-b: ANCOVA results for the relationship between strength of the stipe and stipe cross-sectional area for epiphytised and clean fronds from the exposed site in Scarlett Point and the sheltered site in Fort Island

a. Fort Island

Source of Variation	df	MS	*F*	p-value
Intercept	1	9.331	384.638	<0.0001
Epiphytism	2	0.075	3.073	0.0616
Covariate (biomass)	1	4.532	186.839	<0.001
Interaction	2	0.015	0.633	0.538
Error	23	0.024		

b. Scarlett Point

Source of Variation	df	MS	*F*	p-value
Intercept	1	6.586	200.930	<0.0001
Epiphytism	2	0.092	2.816	0.0775
Covariate (biomass)	1	3.198	97.292	<0.0001
Interaction	2	0.072	2.211	0.129
Error	21	0.033		

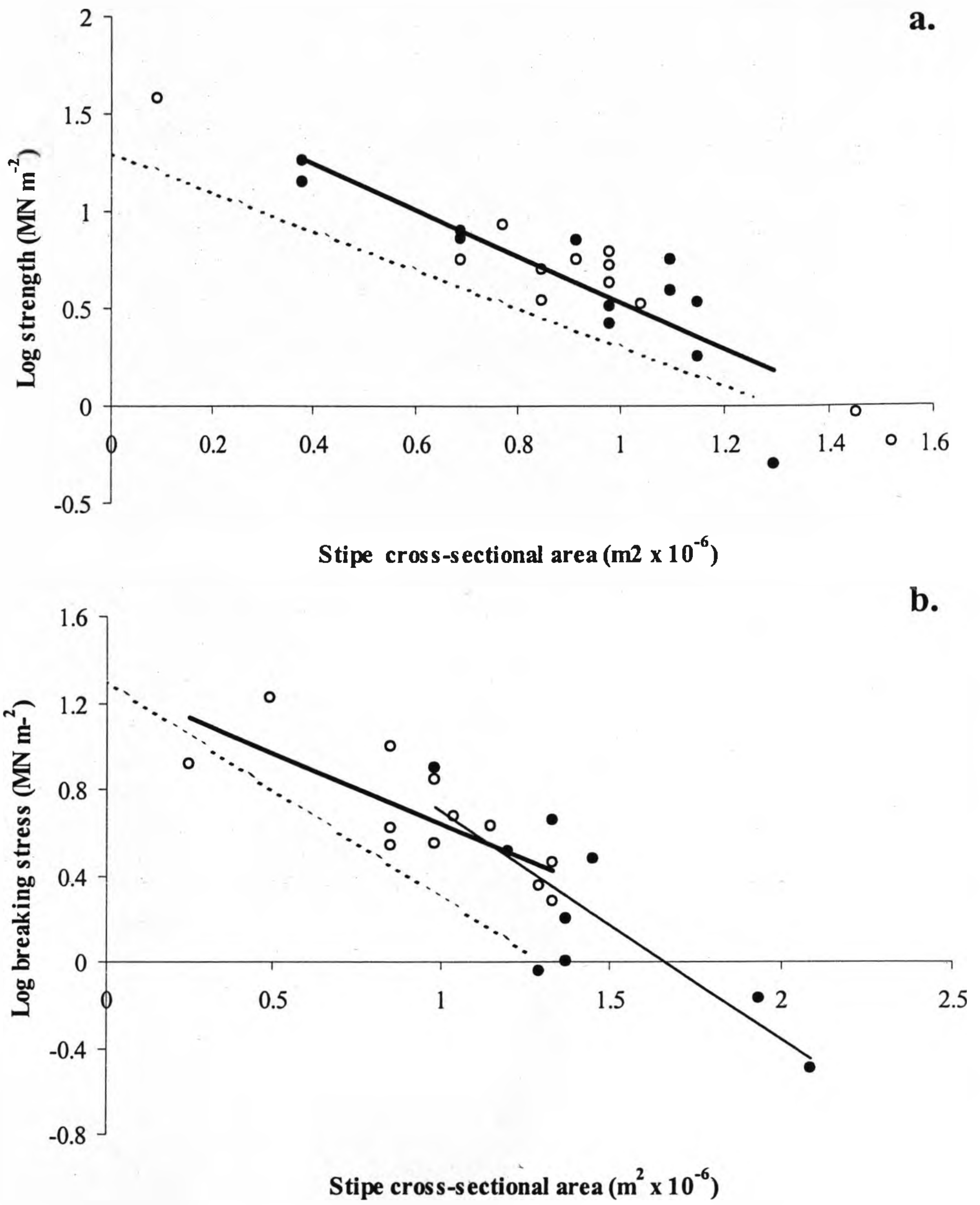


Fig 5.4 The relationship between mechanical strength of the stipe and cross-sectional area of fronds from the frond breakage study. Data are presented separately for clean (closed circles) and epiphytised fronds (open circles). **a**. fronds from Fort Island. Lines represent regression equations for clean fronds (y= 1.72-1.20x, R^2= 0.9144) and epiphytised fronds (y=1.74-1.20x, R^2= 0.9144) and **b**. fronds from Scarlett Point. Lines represent regression equations for clean fronds (y=1.30-0.66x, R^2=0.62) and epiphytised fronds (y=1.75-1.05x, R^2=0.68). The dashed line represents the slope of a line where breakage force is independent of stipe cross-sectional area.

5.3.3 Flume experiments: The effects of frond morphology and epiphytisation

Drag was examined on single, unbranched fronds, 50 and 100 cm in length as well on 50 cm long fronds with five lateral branches. The drag experienced by single unepiphytised fronds increased with increasing water flow velocity with the best fit to the data being given by a power curve (except for 100 cm long axes from Fort Island, which showed a straight-line relationship with increasing velocity). At a velocity of 3 m s^{-1}, 100 cm long plants from Scarlett Point experienced the highest drag, whereas the shorter fronds from the sheltered site experienced the highest drag (Figure 5.5a-c). Single epiphytised fronds exhibited straight-line relationships, again with the exception of 100 cm long plants from Fort Island, for which the relationship between drag and velocity followed a power relationship. Replicates from this treatment carried a lower epiphyte load than fronds in the other treatments (Table 5.4a-b). Again, 100 cm long fronds collected from Scarlett Point, experienced the highest drag. However, despite this, they usually had lower drag coefficients than fronds from Fort Island (Figure5.6 a-d).

Table 5.4a-b: Average total wet weights (g) and average proportion of epiphytes of total biomass

a. Epiphytised plants

Treatment	Mean total wet weight per frond	Mean proportion of epiphyte per host frond	Average drag
Scar 50	23.24	47.59	4.32
Scar 100	15.22	55.01 (single value)	6.66
Fi 50	26.44	45.03	4.22
Fi 100	33.39	18.81	4.79

b. Clean plants

Treatment	Mean total wet weight per frond	Average drag
Scar 50	6.88	0.45
Scar 100	23.33	1.54
Fi 50	7.55	0.82
Fi 100	23.333	1.03

Few measurements of branched fronds were made at flows of 1 m s^{-1}; therefore curves could not be fitted. However, epiphytised fronds clearly experienced the highest drag compared to clean fronds of comparable length. Interestingly, there was no difference in the drag experienced by branched and single fronds of similar overall biomass. For subsequent analyses the morphology of fronds was therefore ignored and only total biomass was used. The drag coefficients calculated for clean and epiphytised fronds did not give clear results, although drag coefficients were often found to increase with velocity in clean fronds and decrease in epiphytised fronds (Figure 5.6a-d).

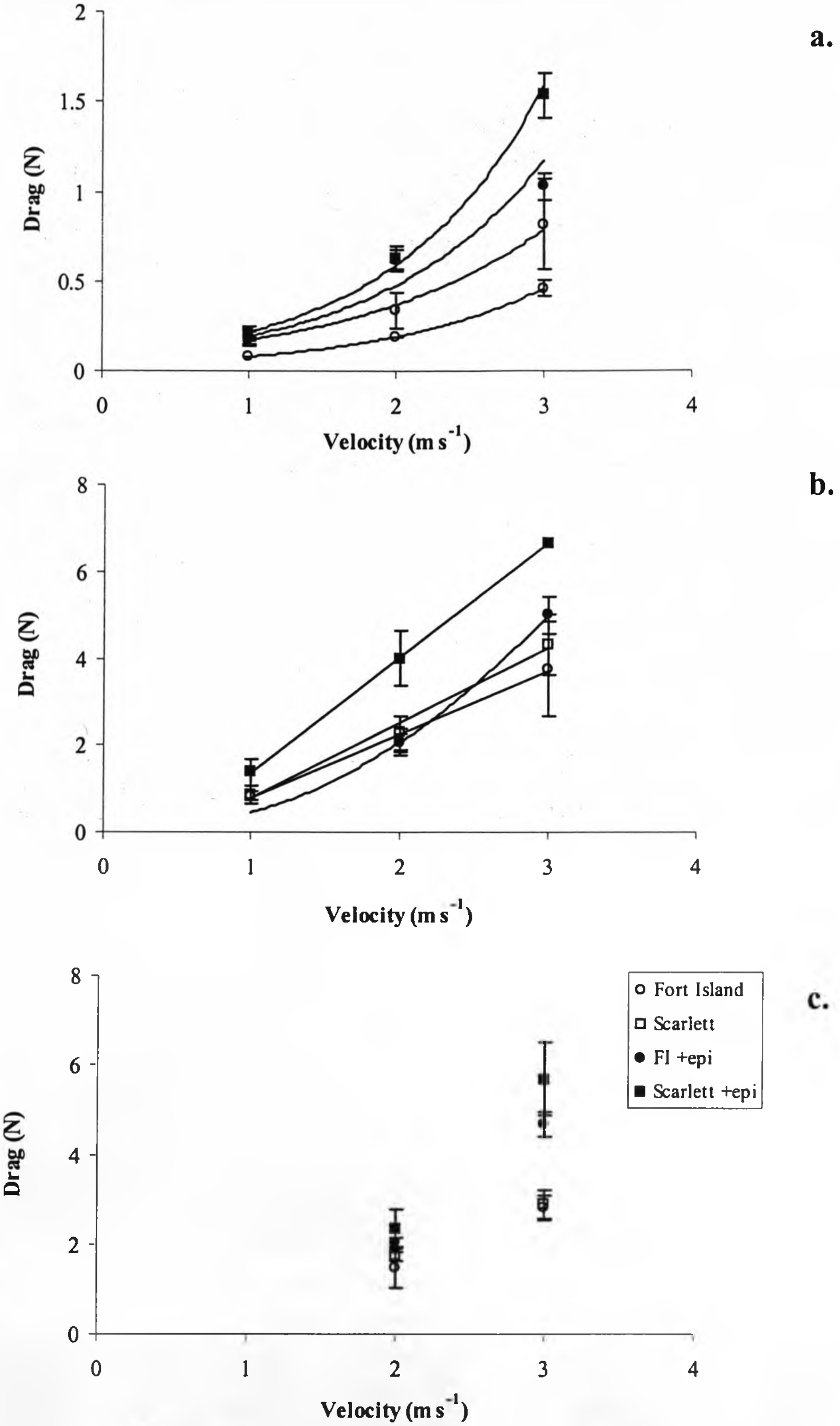


Fig 5.5 The effect of frond morphology and epiphytism on the drag experienced by fronds from Fort Island and Scarlett Point: **a**. single, unbranched fronds without epiphytes, **b**. single unbranched fronds with epiphytes, filled squares=fronds from Scarlett, 100 cm in length, filled circles Fort Island fronds, 100cm, open squares: Scarlett fronds 50 cm, open circles: Fort Island fronds, 50 cm; **c**. 50 cm long fronds with 5 lateral branches with (closed symbols) and without epiphytes (open symbols).

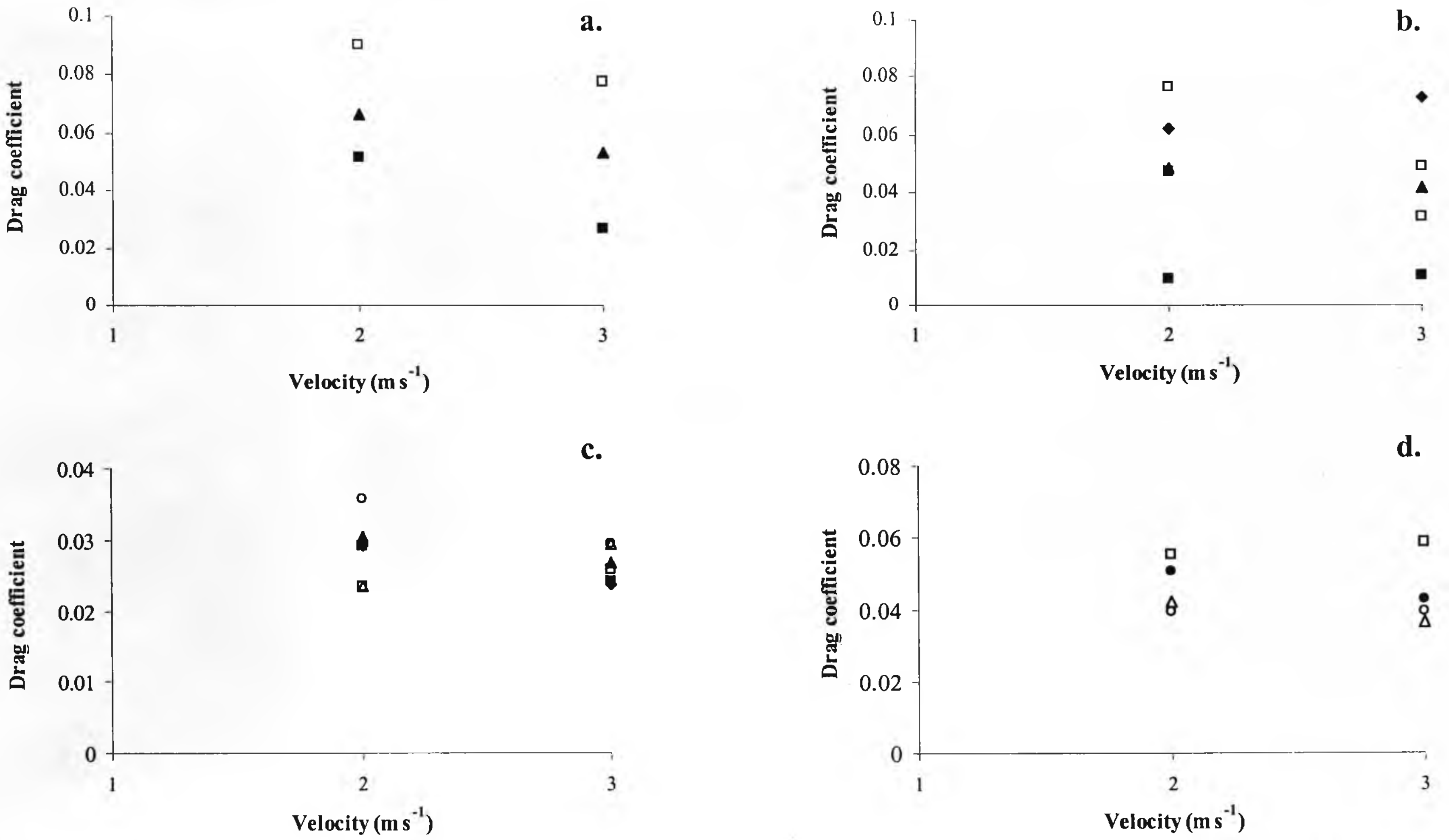


Fig 5.6 Drag coefficients for **a**. 50 cm long unbranched fronds from Fort Island, **b**. 100 cm long fronds from Fort Island, **c**. 50 cm long unbranched fronds from Scarlett Point, **d**. 100 cm long unbranched fronds from Scarlett Point. Open symbols represent clean fronds, closed circles represent epiphytised fronds.

5.3.4 Drag suffered by fronds on sheltered and exposed shores

For the fronds tested in May 2000 differences between drag experienced by fronds from exposed and sheltered shores was also investigated. As before, tests were initially carried out separately on epiphytised and clean fronds (Table 5.5a-b,Figure 5.7a-d). At neither of the high flow velocities (2 and 3 m s^{-1}) did the ANCOVA reveal differences in the rate of increase of drag with increasing total frond biomass, i.e. for a given biomass fronds from the sheltered and exposed site experienced the same drag.

Table 5.5a-b: Analysis of Covariance for plants from a sheltered shore and an exposed shore tested in the flume in May 2000 to test for homogeneity of slopes

a. Clean fronds only

Source of Variation	df	MS	*F*	p-value
Exposure	1	0.0083	0.0430	0.838
Covariate (biomass)	1	17.195	88.814	<0.0001
Interaction	1	0.166	0.85693	0.3684
Error	16	0.194		

b. Epiphytised fronds only

Source of Variation	df	MS	*F*	p-value
Exposure	1	0.0047	12.054	0.943
Covariate (biomass)	1	11.077	0.005	0.004
Interaction	1	0.117	12.345	0.911
Error	13	0.897	0.013	

As the initial tests had revealed homogeneity of slopes, the data could be pooled for exposure with epiphytism as an independent factor, biomass as covariate and drag as dependent variable. ANCOVA revealed significant effects of epiphytism as well as the covariate (Table 5.6 a-b, Figure 5.8a-d).

Table 5.6: Analysis of Covariance for the flume data for epiphytised and clean plants pooled for environmental exposure, measured in May 2000. Biomass was used as the covariate.

Source of Variation	df	MS	*F*	p-value
Intercept	1	11.019	22.528	<0.0001
Epiphytism	1	32.028	65.483	<0.0001
Biomass	1	5.933	106.180	<0.0001
Error	35	0.489		

Drag coefficients varied greatly between sheltered and exposed shores. They were generally more variable at the sheltered site for fronds of similar morphology but reached higher values than at the exposed site.

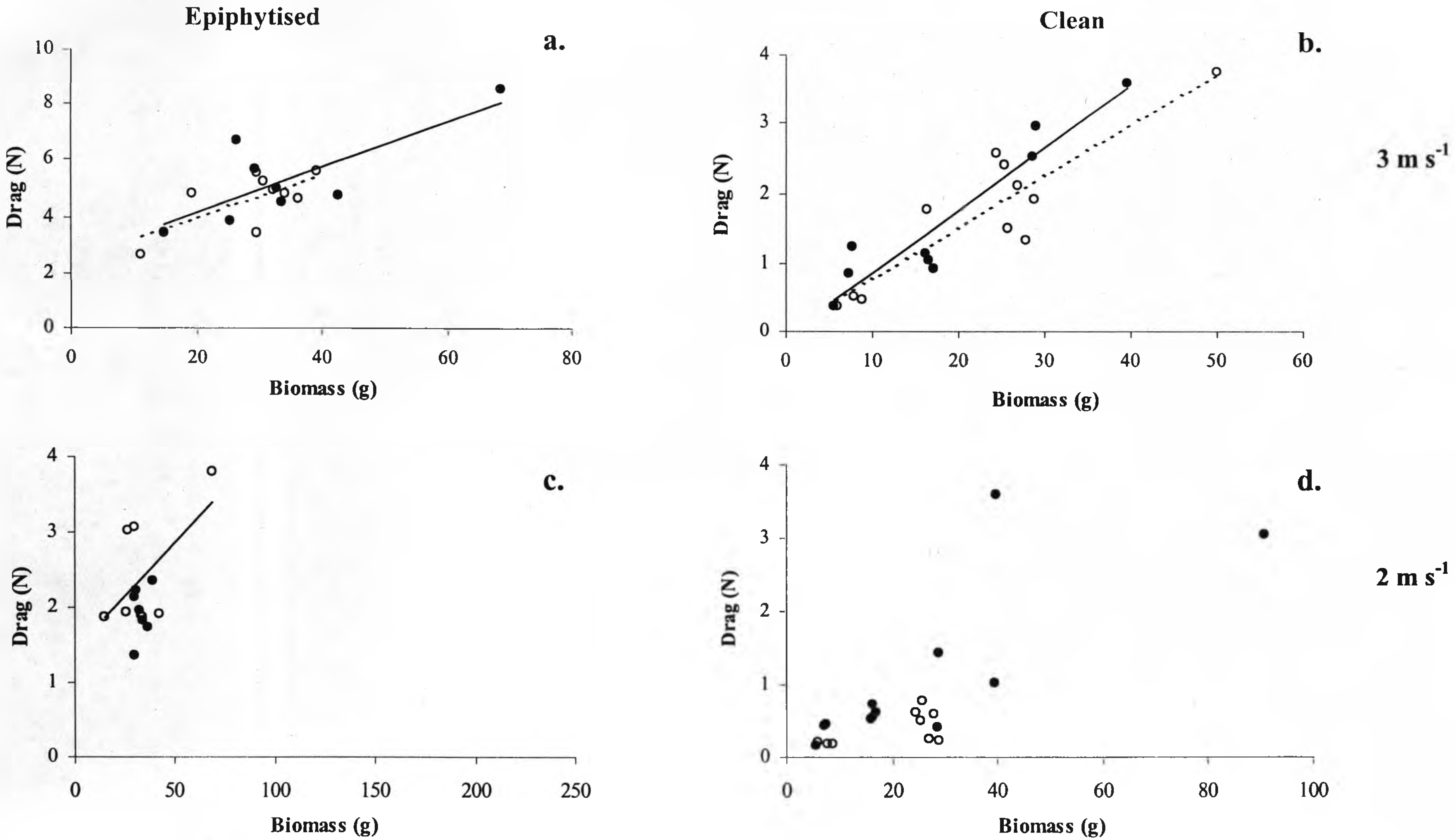


Fig 5.7 Drag experienced by fronds in May 2000: Results are presented separately for exposed shore (closed circles) and sheltered shore (open circles). Plots **a** and **b** show the results for epiphytised and clean fronds at a flow velocity of 3 ms^{-1} and plots **c** and **d** those at 2 ms^{-1}. The lines represent regression equations for **a.** exposed shore, epiphytised fronds (y=2.5413+0.08x, R^2=0.61) and sheltered, epiphytised fronds (y=2.44+0.08x, R^2=0.43) and for **b.** exposed, clean fronds (y=-0.05+0.09x, R^2=0.88) and sheltered, clean fronds (y=0.03+0.07x, R^2=0.82) **c.** epiphytised, sheltered fronds at 2 ms-1 (y=1.44+0.03x, R^2=0.36).

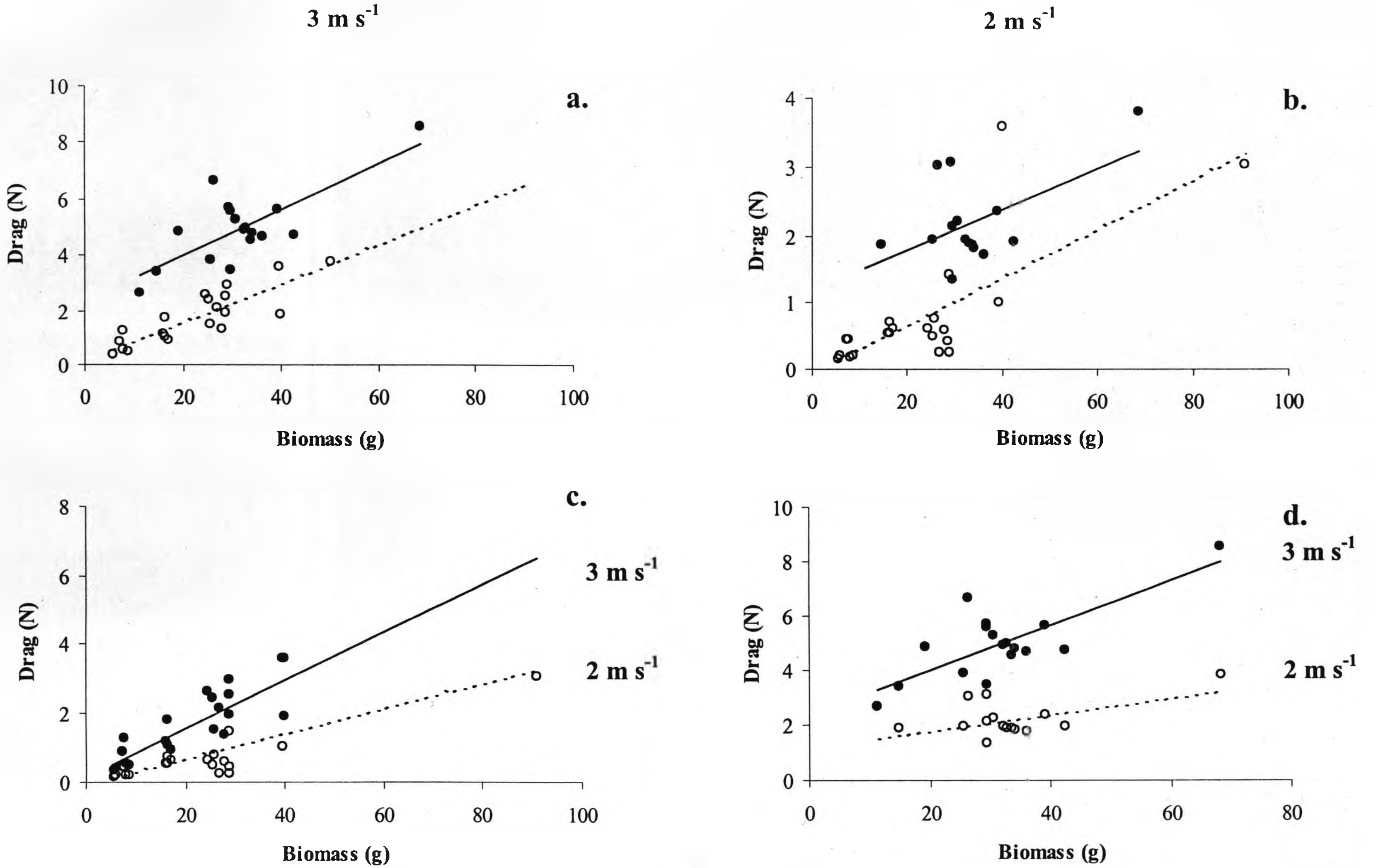


Fig 5.8 Drag experienced by epiphytised (closed circles) and clean fronds (open circles) pooled for exposure in the flume experiments in May 2000. The lines represent regression equations for **a.** epiphytised fronds (y=2.38+0.08x, R^2=0.58) and clean fronds (y=0.14+0.07x, R^2=0.76) at 3 m s^{-1} and **b.** epiphytised fronds (1.19+0.03x, R^2=0.29) and clean fronds (y=-0.007+0.02x, R^2=0.57) at 2 m s^{-1}; **c.** clean fronds at 3 m s^{-1} (y=1.16+0.07x, R^2=0.69) and 2 ms-1 (y=-0.07+0.04x, R^2=0.57), **d.** epiphytised fronds at 3 ms-1 (y=2.38+0.08x, R^2=0.58) and 2 ms^{-1} (y=1.19+0.03x, R^2=0.29).

5.3.5 *Differences between May 2000 and January 2001*

The additional experiments in January 2001 had been carried out chiefly to increase the sample size of the data sets collected in May 2000. Before attempting to pool the data (Figure 5.9a-d, Table 5.7a-b) an Analysis of Covariance was used to check homogeneity of slopes for data from May 2000 and January 2001. This revealed homogeneous slopes for clean fronds at both 3 m s^{-1} and 2 m s^{-1}. For epiphytised fronds, the slopes were homogeneous at 2 m s^{-1}, but not at 3 m s^{-1}. The latter data could therefore not be pooled (Figure 5.10a-b).

Table 5.7a-b:Analysisis of Covariance for fronds tested in the flume in May 2000 and January 2001 with biomass as the covariate to test for homogeneity of slopes

a. Epiphytised fronds

Source of Variation	df	MS	*F*	p-value
Intercept	1	24.349	12.490	0.0009
Date	1	17.165	8.805	0.005
Biomass	1	133.172	68.588	<0.0001
Interaction	1	33.172	17.016	0.0001
Error	48	1.950		

b. Clean fronds

Source of Variation	df	MS	*F*	p-value
Intercept	1	12.668	11.549	0.002
Date	1	0.589	0.534	0.471
Biomass	1	10.085	9.194	0.065
Interaction	1	1.459	1.331	0.259
Error	26	1.097		

5.3.6 *Differences between velocities (pooled data only)*

When the pooled data were plotted at 2 and 3 m s^{-1} (with the exception of epiphytised fronds at 3 m s^{-1}), this revealed significant positive slopes in both the clean and epiphytised fronds, indicating that biomass is a good predictor of the drag experienced by fronds of different morphologies. Epiphytised fronds always suffered a significantly higher drag than clean fronds of comparable total biomass. For epiphytised fronds at both 2 and 3 m s^{-1}, the best fit for the data was a logarithmic curve, i.e. with increasing biomass the rate of increase in drag became smaller (Figure 5.10 a-b). For clean fronds on the other hand, the best fit was a logarithmic curve at 2 m s^{-1} but a straight line at 3 m s^{-1}.

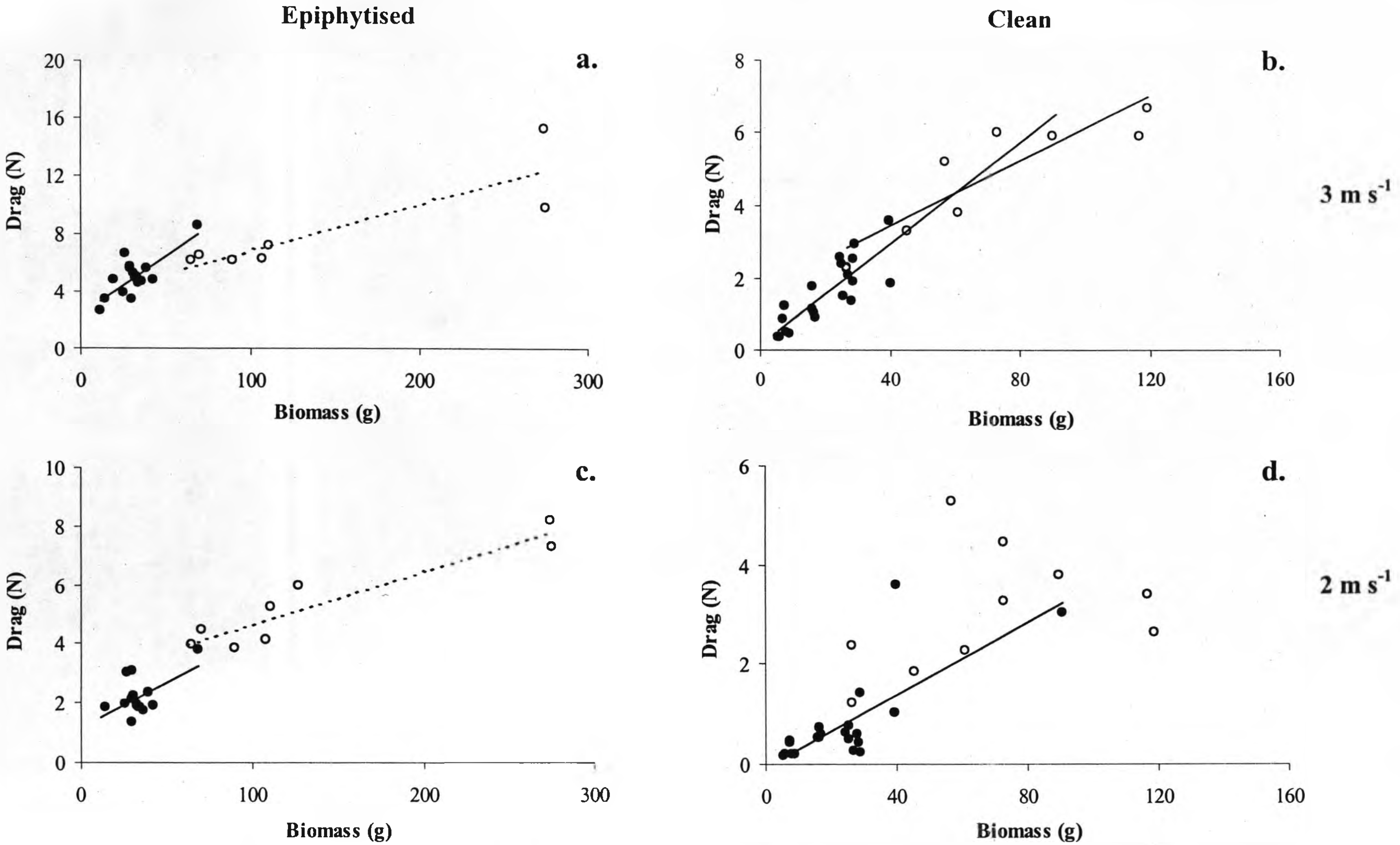
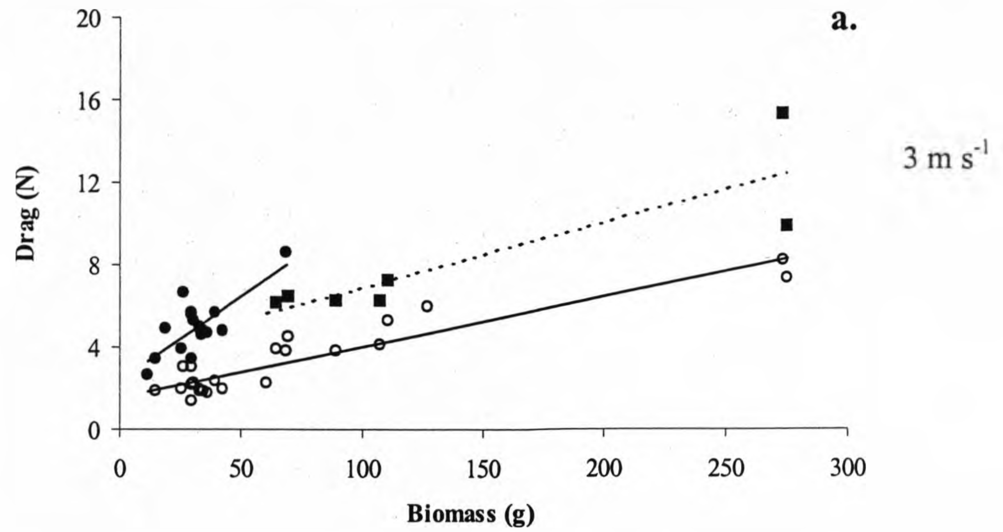


Fig 5.9 Drag experienced by fronds in the flume experiments in May 2000 (closed circles) and January 2001 (open circles). Lines represent regression equations for **a**. Epiphytised fronds at 3 m s^{-1} in May (y=2.38+0.2x, R^2=0.58) and January (y=3.67+0.03x, R^2=0.76), **b**. clean fronds at 3 m s^{-1} in May (y=0.14+0.07x, R2=0.76) and January (y=1.35+0.05, R^2=0.84), **c**. epiphytised fronds at 2 m s^{-1} in May (y=1.19+003x, R^2=0.29) and January (y=2.87+0.02x, R^2=0.88), **d**. clean fronds at 3 m s^{-1} in May (-0.07+0.04x, R^2=0.57).



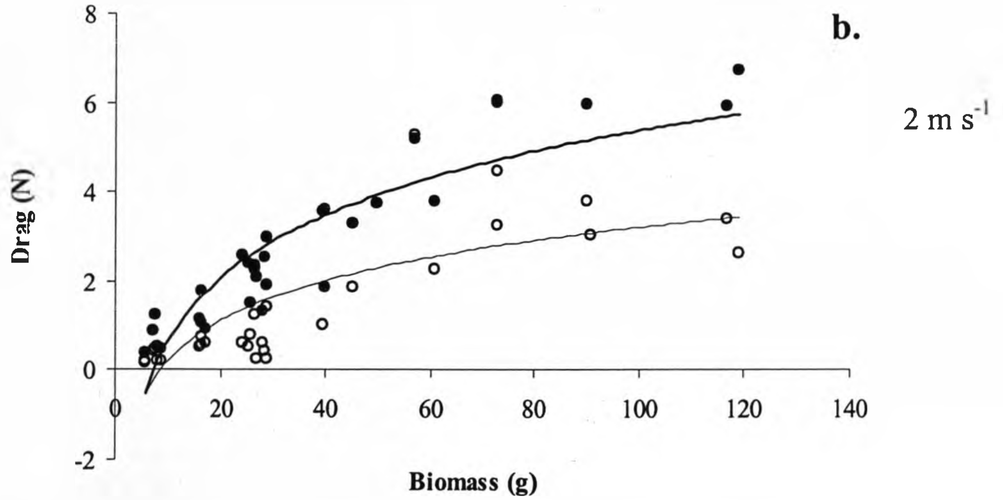


Fig 5.10 Pooled data from the experiments carried out in May 2000 and January 2001 for epiphytised fronds (closed circles), clean fronds (open circles) and epiphytised fronds in January 2001 (closed squares). Lines represent the regression equations for a. epiphytised, May, 3 ms^{-1} ($y= 2.38+0.08x$, $R^2=0.58$) and epiphytised, January, 3 ms-1($y=3.67+0.03x$, $R^2=0.76$) and pooled clean fronds ($y=1.53+0.02x$, $R^2=0.86$ **b**. epiphytised fronds ($y=-4.03+2.04\ln(x)$, $R^2=0.83$) and clean fronds ($y=-2.78+1.3\ln(x)$) at 2ms^{-1}.

5.3.7 Plant breakage

Thirteen fronds broke during the trials (including two in the preliminary study), which constituted 18.6% of the total of 70 fronds tested between May 2000 and January 2001. Mechanical failure occurred at water velocities of between 1.65 to 3 m s^{-1}, which corresponded to drag values of 2.15 to 8.54 N. The majority of the fronds that failed were epiphytised.

While this range of drag forces represents a four-fold difference between the smallest and largest value, there was a ten-fold difference in the size of the smallest and largest broken plants. No significant relationship was found between velocity at the time of breakage and plant biomass (R^2=4.71, $F_{1,6}$=1.297, p=0.306) or between drag forces and flow velocity at that point (R^2=20.4, $F_{1,6}$=3.223, p=0.128). Mechanical failure occurred at very similar flow speeds in all fronds (Figure 5.11 a-b, Table 5.8). Figure 11a shows that the breaking force values for the fronds from the flume were smaller than in the detachment study.

Table 5.8: Characteristics of Fronds failing in the flume tests in May 2000 and January 2001.
Note: the higher number of fronds from Fort Island shown in this table is no indication of a greater risk of breakage for fronds from this site. For the last series of experiments in the flume in January no fronds from Scarlett Point were used as earlier experiments had not shown any differences in the drag suffered by fronds of a given biomass from the two sites.

Frond type	Length	Epiphytised?	Site	No of laterals	Stipe diameter (Min./Max.) at breakage point	Velocity at breakage (m s^{-1})	App. Drag (N)	Test date
Single	100	Yes	Scarlett	0		2.25		May 2000
Single	100	Yes	Scarlett	0		2.6		May 2000
Single	50	Yes	Scarlett	0		2.4	2.93	May 2000
Branched	50	No	Scarlett	5		3.0	8.54	May 2000
Single	50	Yes	Fort Island	0		2.5		May 2000
Branched	50	No	Fort Island	5		3.0	3.0	May 2000
Branched	112	No	Fort Island	11	0.7,0.25	2.7	5.97	January 2001
Branched	165	Yes	Fort Island	12	0.4,0.2	1.65	2.75	January 2001
Branched	223	Yes	Fort Island	8	0.5,0.35	3.0		January 2001
Branched	219	Yes	Fort Island		0.5,0.15	3.0		January 2001
Branched	165	No	Fort Island	25	0.7,0.2	1.8		January 2001

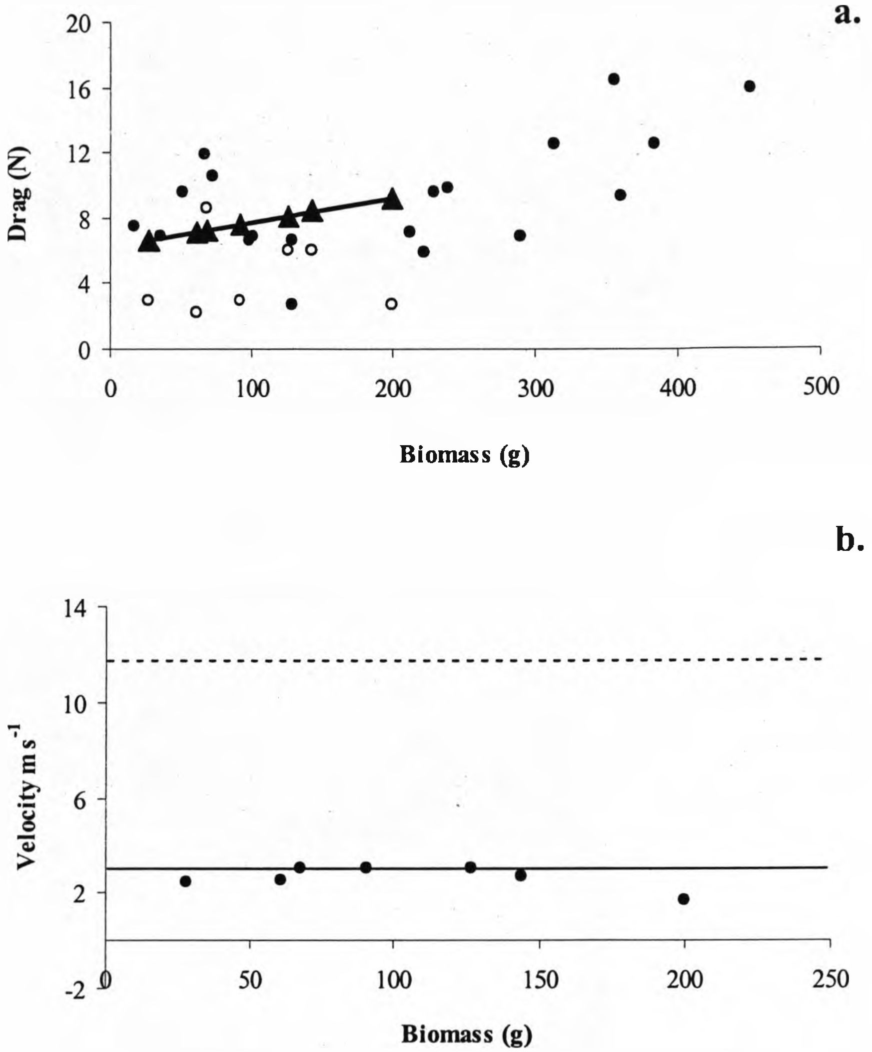


Fig 5.11 a. The predicted forces resulting in stipe failure in water, calculated from the breakage data in the detachment study (closed circles), the drag forces at which failure occurred in the flume (open circles) and the forces predicted for the fronds which failed in the flume study using the regression equation for the relationship between detachment force and frond biomass in the detachment (filled triangles); **b**. the velocities at which fronds in the flume study failed plotted against their biomass. Mean natural flow velocities calculated by Young (1987) are shown for sheltered (solid line) and exposed shores (stippled line) at Fort Island

5.3.8 Stretching in fronds tested in the flume

The degree of extensibility, which had been measured for fronds in the flume study, varied greatly within frond size classes, but showed a similar degree of variation between size classes (Figure 5.12a). In all cases the fronds stretched less than 2.2% of their initial length. There no statistically significant decrease in the degree of stretching in the larger fronds (Regression Analysis: R^2=6.83, $F_{1,16}$=2.246, p=0.153; Figure 5.12b). The only significant negative relationship was found between total length increase and the diameter of the stipe at the point of breakage when all samples were analysed together (Regression analysis: R^2= 31.88, $F_{1,13}$=7.553, p=0.017; Figure 5.12c). There also was no significant difference in the degree of stretching between fronds from Fort Island and Scarlett Point (unpaired t-test assuming equal variances: df=16, p=0.437)

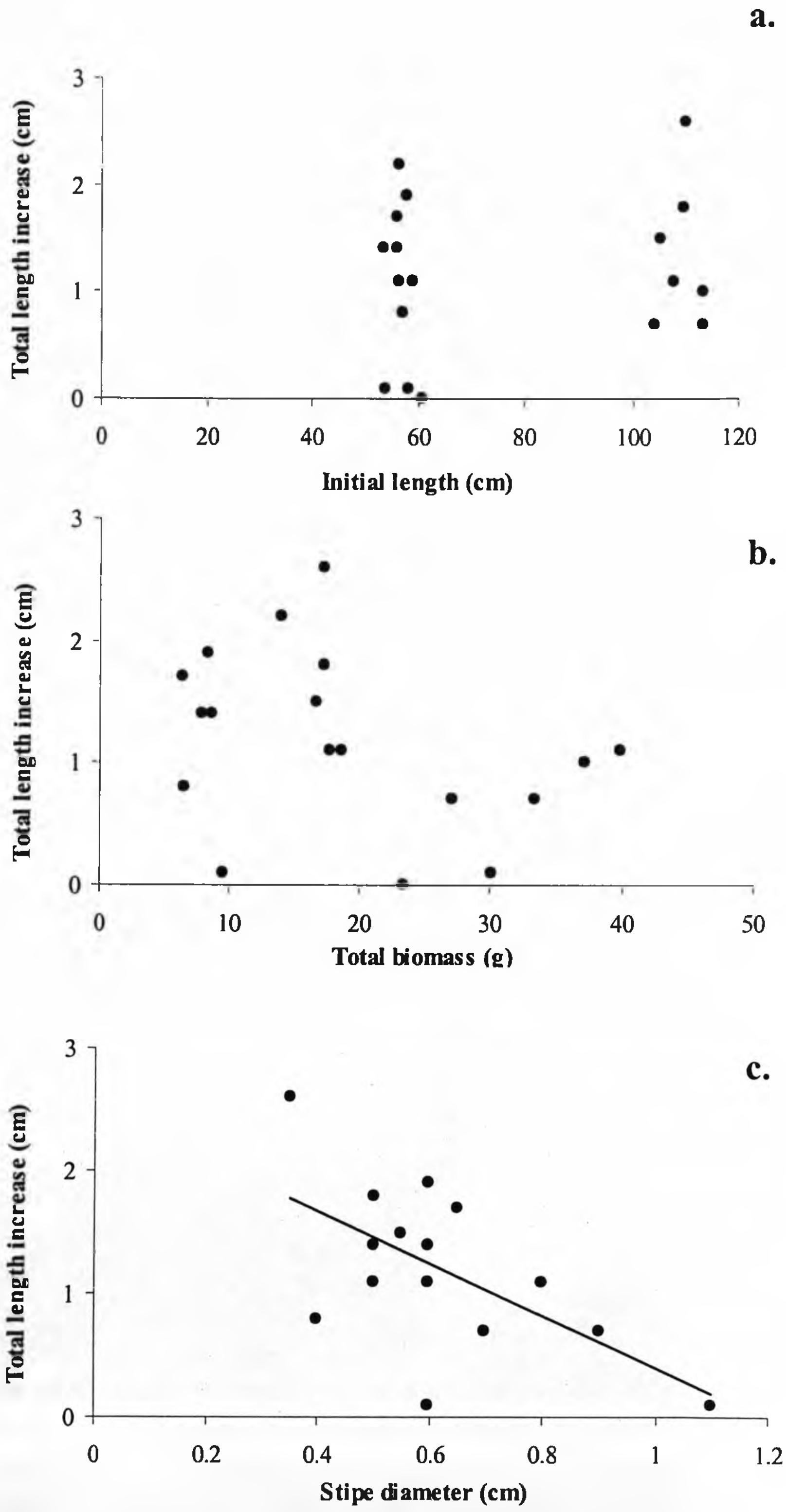


Fig 5.12 The degree of extension of single unbranched fronds in the flume: **a**. the relationship between total length increase and stipe diameter, **b.** total length increases in two size classes of unbranched fronds **c.** the relationship between total length increase and frond biomass

5.3.9 Labelling study

On both the sheltered and exposed shore, fronds showed very similar degrees of damage at the beginning of the study, but their morphology clearly differed, with fronds on the exposed shore being shorter and more highly branched (Figure 5.13a-b, Table 5.9). Stipe diameters were found to be similar in fronds from both shores and did not vary with the biomass of the frond (Figure 5.14a-b). On the sheltered site at Fort Island, five fronds had gone completely at the end of the study and four additional fronds had incurred branch breakages, reducing the overall length of the fronds. On the exposed shore at Scarlett, three branches were lost, but no plants had suffered any reductions in length from breakage. On the contrary, length increases were detected in a few fronds. (Figure 5.15a-b).

Table 5.9: Some characteristics of fronds from Fort Island and Scarlett Point used in the labelling study between November 2000 and March 2001. The p-values given are for two-tailed t-tests assuming equal variances testing differences in characteristics between the two sites with the exception of the results for epiphyte loads which were examined using a Mann-Whitney-U test.

	Fort Island	Scarlett Point	df	p-value
Mean frond length (cm)	121.9	82.7	38	0.0004
No of laterals per frond	0.09	0.27	38	<0.0001
% fronds damaged	46.10	45.31	37	0.935
Mean *Polysiphonia* load (g) per cm frond	0.16	0.29		0.175

There was no clear pattern with respect to the length of the plants that failed mechanically in the course of the study. At Fort Island, the length range of fronds which had been lost during the study was between 36 and 190 cm. At Scarlett it was 51 to 89 cm. However, while all but one dislodged frond in Fort Island were completely free of epiphytes, all dislodged fronds in Scarlett had been carrying at least some epiphytes (Table 5.10 a-b), with the epiphyte loads being in general considerably higher at Scarlett Point than in Fort Island.

Table 5.10a-b: Characteristics of fronds, which became dislodged in the course of the labelling, study in Fort Island (sheltered) and Scarlett Point (exposed) between November 2000and March 2001

a. Fort Island

Length (cm)	Epiphyte load (g)	No of laterals
130	0	2
95.4	0	1
36.2	0	2
119	0	8
73	0	14
141	82	36

b. Scarlett

Length (cm)	Epiphyte load (g)	No of laterals
51	1	33
89	78	26
67	9	17

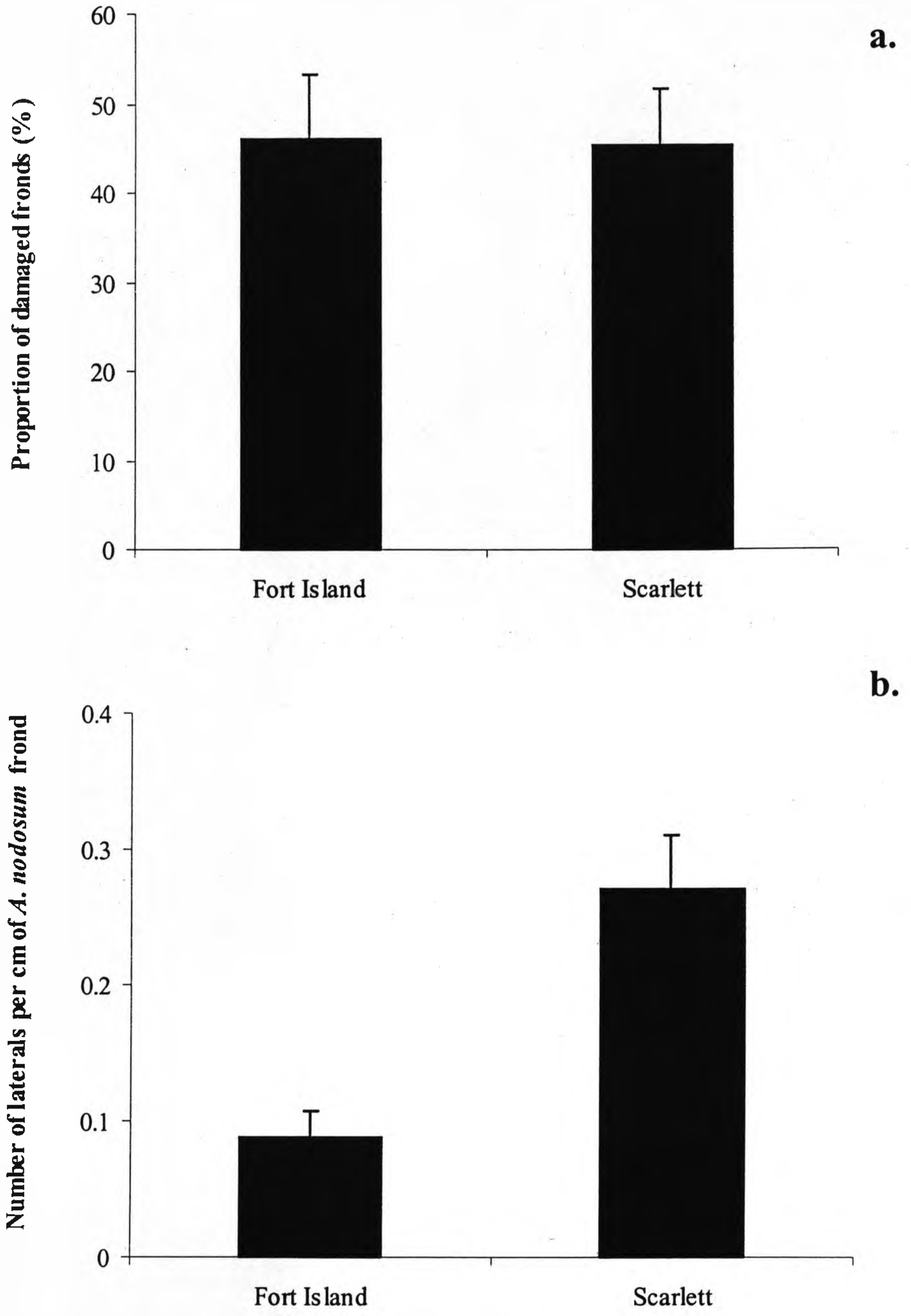
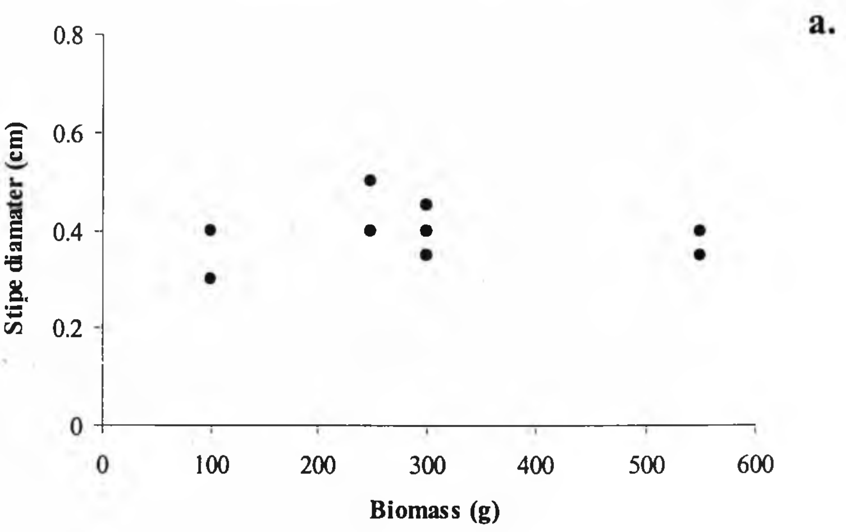


Fig 5.13. The proportion of damaged laterals per frond (**a**) and the number of laterals per cm frond (**b**) in the labelling study carried out between November 2000 and March 2001 at Fort Island and Scarlett; n= 20, Error bars are ± one standard error.



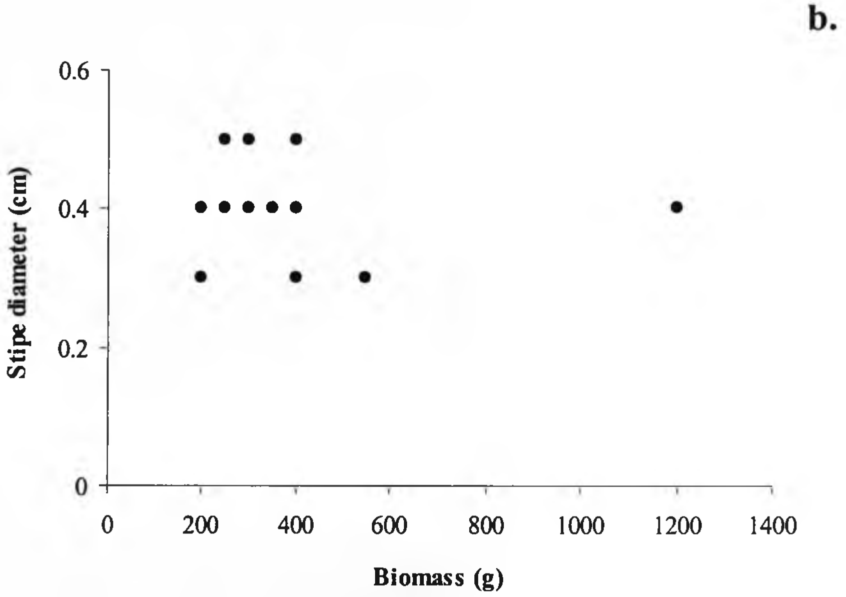
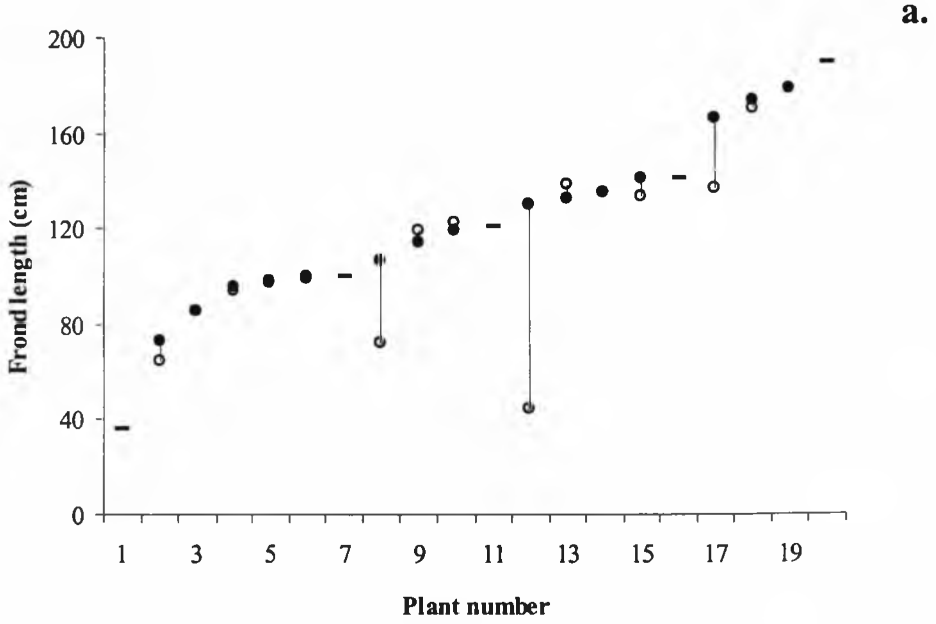


Fig 5.14 The relationship between stipe diameter and frond biomass in the labelling study at **a**. Fort Island, **b**. Scarlett Point



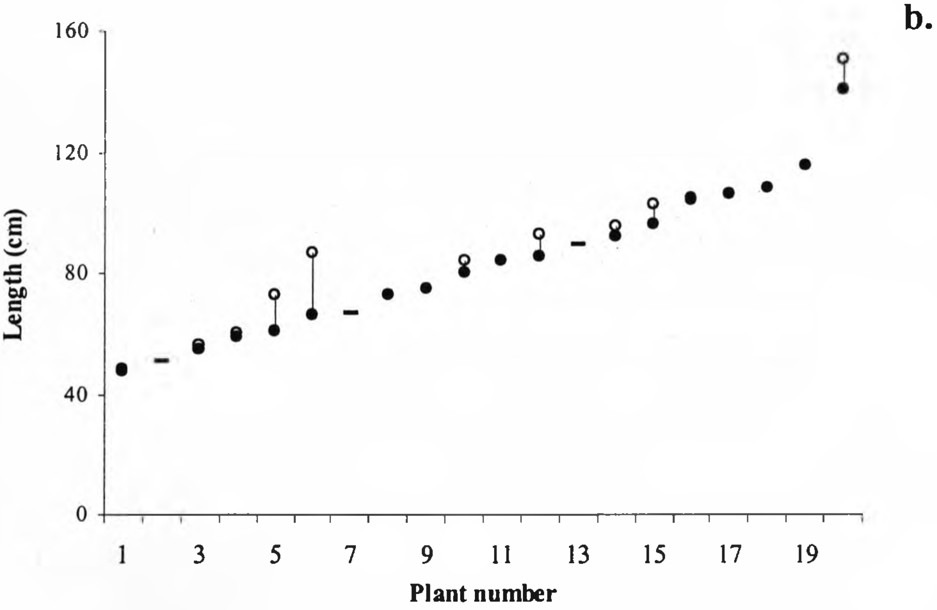


Fig 5.15 The length of individual fronds in the labelling study at **a**. Fort Island and **b**. Scarlett Point. Fronds are sorted by length. Fronds, which had become detached completely, are marked by a horizontal line; closed bars=initial length, open circles=length at end of experiment.

5.4 Discussion

Two components of the forces acting on the stipe of *Ascophyllum nodosum* were investigated: those required to break the stipe, which are dependent on the strength of the tissue as well as its cross-sectional area, and those experienced at non-destructive flow velocities, which are determined by the surface area of the frond and the velocity. The principal forces at play are drag and acceleration reaction forces (Denny *et al.* 1985). As the frond increases in biomass and therefore in surface area, these forces will also increase. Therefore, to avoid breakage at lower and lower velocities, the stipe will have to increase its strength, thickness or flexibility (Koehl 1982, Holbrook *et al.* 1991, Dudgeon and Johnson 1992, Shaugnessy *et al.* 1996).

In the case of *Ascophyllum nodosum*, although the lack of significant relationships between stipe cross-sectional area and frond surface area meant that no statistical comparisons of slopes could be made, the absence of significant increases in cross-sectional area over a wide range of frond sizes is in itself an indication of some degree of underscaling. This means that the girth of the axis (the load bearing structure) does not increase sufficiently as the frond increases in biomass and surface area. This was shown clearly by the relationship between the strength of the stipe and its cross-sectional area. The strength of *A. nodosum* decreased in fronds from both shores with increasing axis girth and more importantly breaking stresses were essentially independent of stipe cross-sectional area. Such underscaling of the stipe of seaweeds has been shown for a number of species, mostly kelps (Carrington 1990, Dudgeon and Johnson 1992, Johnson and Koehl 1994). Therefore in *Ascophyllum* strengthening of the stipe does not contribute to an ability to withstand higher drag forces with increasing

biomass particularly on wave exposed shores. Whether underscaling increases the risk of breakage of larger fronds depends on the performance of fronds in flow.
A further strategy adopted by some seaweeds is a great extensibility of the fronds. Extensibility in high flows has the effect of delaying the point at which the frond becomes fully extended and therefore experiences sufficient stresses on its stipe to result in breakage. However, like the strength of the stipe, extensibility was not different between the two shores and it was low in fronds from both sites compared with a study by Koehl and Wainwright (1977) of the bull kelp *Nereocystis luetkeana* which has a very thin flexible stipe. These authors found an extension of the stipes of up to 25% at stresses of 3 MN m^{-2}. Even if it is assumed that at the time of the length measurements immediately after the flume runs the fronds would already have slightly contracted again, the extreme extension values found by Koehl and Wainwright (1977) were never observed during the actual runs in the flume. Therefore, there seems to be little evidence for extension as a drag or stress reducing adaptation in *Ascophyllum nodosum*.

In general, fronds from the exposed shore required higher forces to break the stipe than those from the more sheltered site, which should compensate for the higher flows encountered. They also had generally lower drag coefficients. Johnson and Koehl (1994) found similar results in fronds of *Nereocystis luetkeana* from more exposed shores, and attributed this to differences in blade morphology rather than mechanical differences between fronds; fronds on exposed shores had smooth narrow blades which collapsed onto themselves in high flow conditions.

Some decreases in drag coefficients with increasing flow velocity were also found in the present study, indicating realignment of the fronds in the direction of the flow, but this was the case with fronds from both exposures. Moreover, these decreases were only slight in all fronds, which may be because even at the lowest velocity measured, they had already realigned in the direction of the flow to some extent. Therefore, there appears to be some evidence that a difference in flexibility rather than stiffness or extensibility might aid the fronds to withstand the high flow velocities at exposed sites.

Whatever the strategy, the majority of 'clean' (i.e. epiphyte-free) fronds were clearly able to remain attached at considerable velocities. If the mean flow velocities of 2.6 m s^{-1} recorded by Young (1987) for the sheltered site in Fort Island are taken as an indication of the drag the fronds must be able to withstand, it seems unlikely that undamaged, clean fronds will dislodge or break on the shore as the result of hydrodynamic forces alone. Even at 3 m s^{-1} they were experiencing drag forces lower than those required to break the stipe, although it has to be kept in mind, that the values given by Young (1987) were mean values, and during storms the flow velocities would have been much higher (Collado-Vides *et al.* 1998). The flume results for fronds of different morphologies (Figure 5.5 and Table 5.8) confirm the findings of Young (1987) who claimed that clean fronds of moderate size should rarely encounter flow velocities high enough to cause frond breakage or dislodgement on sheltered shores. However the picture could be quite different for epiphytised fronds.

The effect of Polysiphonia lanosa infestation

Epiphytised fronds showed straight-line increases in drag with flow velocity, while 'clean' fronds exhibited power relationships. This indicates that epiphytised fronds were more compliant for a given biomass, although of course total frond biomass and therefore drag is considerably increased by the presence of the epiphyte (Vogel 1994). The results have shown that *Polysiphonia lanosa* can considerably increase drag forces, particularly at higher flow velocities. Such epiphytised fronds experienced drag values at 2 m s^{-1}, which were not reached by the clean, branched frond at 3 m s^{-1} (Figure 5.5). The fact that almost all the fronds that snapped in the flume were epiphytised indicates that the epiphyte has the potential to considerably reduce the velocity at which fronds on the shore break or cause whole plants to be uprooted and it can even do so with smaller, morphologically simple fronds.

Increased frond mortality as the result of epiphytism has been found previously for different algae (D'Antonio 1985, Belegratis *et al.* 1999). Having established the influence of *Polysiphonia lanosa* on the host plant in flow, it is worth considering possible long-term consequences resulting from this. If the host plant incurred a reduction in fitness as the result of epiphytism, one would expect adaptations decreasing the incidence of epiphytism or adaptations increasing the chances of withstanding it. However, this study revealed no significant differences in strength or extensibility between fronds from exposed and sheltered shores, be they epiphytised or clean indicating that the costs incurred as a result of epiphytism might not have been high enough to warrant mechanical adaptations (Figure 5.4).

In fact, it could be the hydrodynamic conditions on exposed shores, which contribute to higher epiphyte loads. The fronds of *Ascophyllum nodosum* are usually much shorter on exposed shores than in sheltered sites probably due to abrasion damage. For a given biomass, cross-sectional area and epiphyte load, maximum stress will be less in shorter fronds (Koehl 1979) so that on exposed shores the shorter fronds can tolerate high epiphyte loads despite the greater hydrodynamic forces acting on the frond. In nature therefore, epiphyte infestation might not be as disadvantageous as it obviously was in the flume nor exert sufficient selection pressure to influence the morphologies of the host plant. Since frond morphology seems to influence drag only to a limited degree, it is unlikely to cause trade-offs between hydrodynamically advantageous morphologies and those that for example reduce grazing or enhance photosynthesis (Carrington 1990). Indeed the epiphyte loads on exposed shores are often higher than on sheltered shores (Levin and Mathieson 1991), indicating that hydrodynamic conditions do not select for adaptations to reduce epiphytism, but might be part of the environmental conditions favouring their growth.

Ascophyllum is a very poor coloniser and the main means of replacement of lost material is by means of vegetative growth of shoots from the basal holdfast rather than from sexual reproduction to form new plants. As a host plant therefore, it lacks the ability to adequately and quickly respond to the pressures placed on it by the epiphyte, although some possible defence mechanisms have been investigated e.g. the shedding of the outer epidermal layers to dislodge settled spores (Filion-Myklebust and Norton 1981). *Ascophyllum plants* might persist for more than fifty years. High densities can inhibit recruitment in *Ascophyllum* which can live for fifty years or more (Keser *et al.* 1981, Keser and Larson 1984, Klinger and DeWreede 1988). Therefore a small risk of breakage might be advantageous and not jeopardise the stability of the system as a whole (Bernstein and Jung 1979, Littler and Littler 1980). The patchy distribution of the epiphyte on the host plant will also ensure that losses are confined to relatively small areas, but in these areas to allow the kind of growth enhancement seen in Chapter 1. Indeed, at a smaller scale, the growth form of *Ascophyllum nodosum* might favour such losses, since the areas of attachment of the lateral to the main axis frequently have the smallest diameter of the lateral and might thus present an inbuilt point of weakness at which high loss rates might occur. Such losses could facilitate better light conditions for emerging primary shoots without jeopardising the survival of the whole frond. Such possible differences in strength of different areas of the frond are an interesting area for further studies.

Chapter 6: The effect of frond damage on the distribution of *Polysiphonia lanosa* on the host plant

Chapter 6: The effect of frond damage on the distribution of *Polysiphonia lanosa* on the host plant

6.1 Introduction

After examining the strategies of 'host', epiphyte and grazer independently, this chapter considers how the type of damage produced by grazers might influence the distribution of *Polysiphonia lanosa* on *Ascophyllum nodosum*. The distribution of the epiphyte is patchy. It usually reaches its greatest abundance near the lower limit of distribution of *A. nodosum*, but even within its zone of highest abundance suitable host plants may be clean or heavily epiphytised (Garbary *et al.* 1991, Lobban and Baxter 1983).

6.1.1 Host specificity of Polysiphonia lanosa

Several mechanisms have been suggested to explain the host specificity and observed distribution patterns of *Polysiphonia lanosa*. Skin-shedding in the host plant has been put forward as a possible factor for *Ascophyllum nodosum* (Filion-Myklebust and Norton 1981) and for *Himanthalia elongata* (Russell and Veltkamp 1984). Skin-shedding sloughs the outer epidermal layers of the host plant, which should remove previously settled spores, confining them to sites such as the lateral pits where skin-shedding does not occur. Skin shedding may also explain the common occurrence of *Polysiphonia lanosa* at damaged sites, since skin-shedding does not occur there either (Pearson and Evans 1990). However, shedding can only be a successful antifouling mechanism against *Polysiphonia* if the time needed by its rhizoid to anchor firmly within the substratum, exceeds the normal skin shedding frequency of the host.
More recently,differences in tissue toughness have been put forward as possible explanations for the apparent specificity of a number of epiphytes (Martinez and Correa 1993, Correa and Martinez 1996). Differences in tissue toughness could also provide an alternative explanation for the predominant settlement of

Polysiphonia lanosa on injured sites as damage provides access to the softer internal tissue. All mechanisms proposed so far would lead to the prediction of predominant settlement and survival of *Polysiphonia* spores at sites of injury.

One source of damage to *Ascophyllum nodosum* is grazing by molluscs, isopods or amphipods. Grazers abrade recessed sites for the colonization, which are more sheltered from water flow allowing more time for the epiphyte to penetrate the host, without being dislodged. Pearson and Evans (1990) suggested that the reason for the absence of *Polysiphonia lanosa* from other fucoids may be the lack of appropriate settling sites rather than any chemical dependence on the 'preferred' host.

The present study will investigate the effectiveness of cuts and abrasions to the thallus as facilitators of *P. lanosa* settlement and survival. Shallow abrasions are often the result of grazing by isopods, while a gastropod like *Littorina obtusata* excavates the thallus, producing deeper wounds (Watson and Norton 1987). The settlement and survival of epiphyte germlings on damaged and undamaged tissue will be followed in the field. Experiments will investigate the timing of the settlement processes to discover at what point *P. lanosa* germlings might become invulnerable to mechanisms normally thought to depress settlement and survival of an epiphyte on its host.

6.2 Materials and Methods

*6.2.1 Tetraspore production of **Polysiphonia lanosa***

Collections were made from Fort Island and Scarlett Point, at approximately fortnightly intervals in the summer and autumn of 2000 (June to early October) to study tetraspore production. Ten randomly collected frond segments of *A. nodosum* were taken back to the laboratory where one *Polysiphonia lanosa* plant from each segment was excised and viewed under a binocular microscope. *P. lanosa* produces its reproductive structures towards the tips of its branches. Each clump was spread out in a petri dish and all the reproductive structures visible in one field of view were counted (approximately 30-50 distal branches per count).

6.2.2 Field Studies: Settlement patterns of P. lanosa

A preliminary study was set up at Fort Island near to the lower limit of the *Ascophyllum* zone in July 1999, around the time of peak tetraspore release by *Polysiphonia lanosa*. Six plots at a sheltered site at Fort Island were chosen. In each plot a total of nine axes of *Ascophyllum* were selected, each of which had at least three internodes, an intact meristem and no visible grazing damage. These axes were divided into three groups with three replicates each. One group was used as control, i.e. the internode was labelled, but not in any way injured, in the second group, part of the internode was abraded, and in the third group, a cut was applied to the axis. All treatments were applied to the second youngest internodes, and the axes were labelled with orange fluorescent tape, tied around the base. The study was terminated in October due to high losses of experimental axes from grazing; large numbers of juvenile snails were regularly found on the host fronds, particularly on cut surfaces.

Following the preliminary study, the main field experiment was set up in August 2000, just after the peak of spore release of *Polysiphonia lanosa* and terminated at the end of November 2000. It was hoped that this later set-up date could avoid grazing damage from juvenile littorinids.

In the main study only cuts were applied to the host tissue as they were judged to be more likely to provide shelter for newly settled spores. The number of replicates was increased to 30. Treatments and controls were set up in groups of five axes in each of six plots. In addition cuts were now applied to two different groups of axes: fronds which were already epiphytised above or below the treated internode and fronds which were clean but located in the vicinity of heavily epiphytised fronds. Two sets of undamaged laterals (again on clean and epiphytised branches) were labelled as controls. Two small, shallow incisions marked the location on each axis on which settlement was to be observed.

6.2.3 Application of the damage to the axes in the field experiments

Abrasions in the preliminary study were produced using fine sand paper (grit F2), and deeper cuts with a scalpel. Care was taken to avoid weakening branches, as this could increase the risk of branch loss. Incisions were made along the main axis of plants, which showed as little previous damage as possible. To produce abrasions of uniform size, the sandpaper was cut to a width of 0.3 cm. The sanding was carried out along a length of one centimetre of the internode by moving the sandpaper slightly up and down the frond. The branch was abraded until an obvious change in the colour of the thallus occurred, but without creating a visible 'step' between treated and untreated tissue. For cuts an area of 1cm x 0.3 cm was outlined using the scalpel. Tissue from within this outline was then removed to a depth of approximately 1 mm producing a trough shaped depression in the frond. Cuts and abrasions were applied to the centre of the internodes. All treatments and controls were applied to the second youngest internode of the main axis or to the same age class on first order laterals. The same order internodes were used as controls.

6.2.4 Laboratory study

6.2.4.1 Preliminary study

Polysiphonia lanosa was collected from Fort Island in August 1999. Segments of *Ascophyllum* fronds where 'injured' in the same way as in the field study, i.e. cut and abraded. The plants were washed briefly in filtered seawater to remove debris and small grazers. *P. lanosa* was desiccated for 48 hours at room temperature in the laboratory to encourage fast spore release and was then re-immersed in a Perspex aquarium tank containing 5 litres of filtered seawater. The segments of *A. nodosum* were placed at the bottom of the tank containing the sporulating *Polysiphonia*. The tank was left undisturbed in a CT room at 10°C and a Short Day 8:16h photoperiod to allow settlement and the epiphyte was removed from the tank after 40 hours. Counts of the number of germlings on each of the segments were made after 40 and 88 hours under a binocular microscope. After the second count, a physical disturbance was introduced by vigorously stirring the water in the tank for five minutes. Afterwards the segments were removed from the tank and the numbers of spores in each treatment and the controls recorded again. For this preliminary experiment all segments were kept in the same container. The data were therefore not independent and were not analysed statistically.

6.2.4.2 *Main laboratory study:*

6.2.4.2.1 Experiment 1: The effect of physical disturbances on germling survival

To investigate the effect of a physical disturbance such as water motion in more detail, 30 Segments of *Ascophyllum nodosum* were prepared. Like in the field study, only second order internodes of clean plants were used. Five intact and five damaged segments of *Ascophyllum* were placed at the bottom of each of six 1000 ml plastic containers. Equal amounts of fertile desiccated *Polysiphonia lanosa* were then fastened to the top of each of the plastic containers (filling the whole area) so that they were suspended just below the water surface (Figure 6.1). Spores were allowed to settle and were cultured in a CT room at 10°C and an 8:16 SD photoperiod. *P. lanosa* was removed from the tanks after 48 hours. The *Ascophyllum* segments were then carefully transferred to new containers with filtered seawater. Counts of spores and their germination success were made after 24, 48, 72 and 96 hours. After 96 hours the water in each container was vigorously agitated for 5 minutes and spore counts were carried out immediately afterwards.

6.2.4.2.2 Experiment 2: The effects of different degrees of disturbance

The second experiment investigated both different lengths of undisturbed periods and different degrees of disturbance. The experimental set-up and introduction of spores to the segments were the same as in experiment 1. The *Ascophyllum* segments were divided into four groups with two treatments each. One of the groups was exposed to a physical disturbance 48 hours after inoculation and the second group was disturbed after 72 hours. Two different durations of disturbance were used. Four replicates were used for each treatment combination (Figure 6.2). Immediately after the disturbance period segments were transferred to containers with fresh filtered seawater to prevent dislodged spores from resettling. The number of spores and germlings present on the segments were counted before and after the disturbance. Segments which where exposed to *Polysiphonia lanosa* for 48 or 72 hours but were not physically disturbed were used as controls.

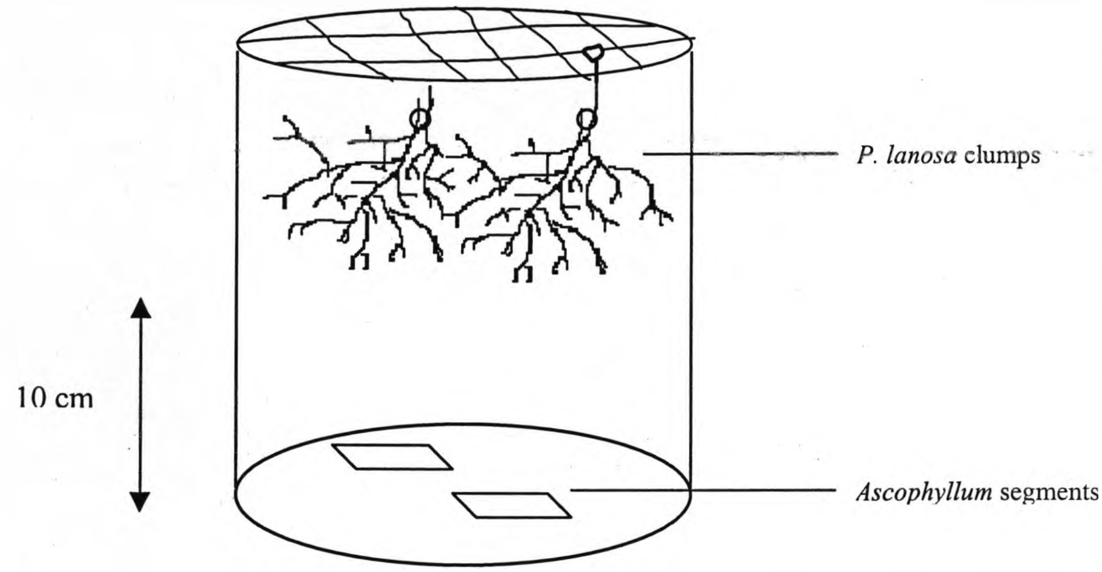


Fig 6.1: Set-up of *Ascophyllum nodosum* segments and *Polysiphonia lanosa* in experimental containers

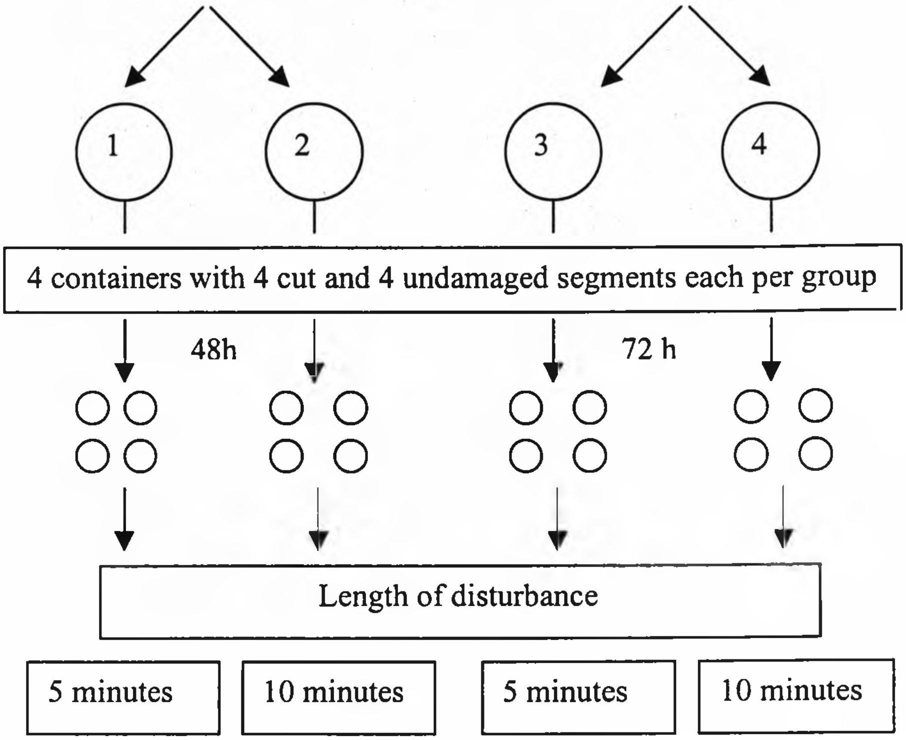


Fig 6.2: Experimental set-up of experiment 2 in the laboratory

6.2.5 Natural settlement densities of Polysiphonia lanosa

To establish the natural settling densities, four untreated axes were removed from the vicinity of six experimental field sites in November 2000 and January 2001. Two of these axes were epiphytised and two were not. The second internode from each of the axes, was excised and used for the analysis. The location of spores on these internodes was scored separately for undamaged thallus tissue, receptacles or cuts and abrasions on the thallus. The outline of each segment and the position of injury sites were traced onto plastic sheets. Overlaying the tissue maps with a transparent sheet of dots arranged at 2 mm intervals and counting the number of dots in each tissue type provided an estimate of the frequency of each tissue type.

Statistical Analysis

The results of experiments 1 and 2 in the laboratory study were examined using Analysis of variance. For experiment 1 a Paired t-test was also carried out to compare loss rates resulting from water motion between injured and undamaged segments. For experiment 2, in which the set-up was fully factorial, a Three-way Analysis of Variance was carried out in order to be able to examine the effects of all factor combinations as well as compare differences between treatments and controls. Prior to the analysis, data were tested for homogeneity of variances using Cochran's C test. Where necessary data were log transformed (calculated as log (x+1)). The frequency distributions of dots and spores in the natural settlement study were compared using a goodness-of fit test. As the frequencies of some expected values were below unity, a log-likelihood analysis rather than a X^2 test was carried out comparing the actual distribution of spores in the different tissue types with a random distribution (Zar 1984). As some expected frequencies were zero a small value (0.00001) had to be added to all data before the analysis could be carried out. To increase the number of counts for the analysis, counts from individual branches were pooled. Normally a test would have to be carried out to check whether different counts can be pooled. This type of analysis is usually performed using a heterogeneity Chi-square test. However, as mentioned above chi square analysis would have been unreliable due to the small expected frequencies. Other tests such as a Poisson heterogeneity test were also deemed unsuitable. Therefore as the trends in individual branches were clearly similar, e.g. vegetative tissue always had lower than expected germling densities, pooling was carried out without any preliminary statistical analysis.

6.3 Results:

6.3.1 The reproductive period of Polysiphonia lanosa

At both, Fort Island and Scarlett Point, spermatangial branches and cystocarps first developed in July 2000. Cystocarp production occurred earlier and was slightly higher at Fort Island than at Scarlett Point. Fertilization success measured as the carpospores formed as a proportion of the total number of available carpogonial branches per female plant, was considerably lower at Scarlett Point than at Fort Island (Table 6.1).

At both sites tetraspore production peaked at the end of July and then declined (Figure 6.3a-b). Small numbers of tips were still producing tetraspores in mid September. Throughout the study in excess of 60 % of all branch tips remained infertile. At most sampling dates some plants were not reproductive at all.

Table 6.1: Numbers of plants out of a sample of ten bearing cystocarps and the fertilization success, calculated as the proportion of branches bearing cystocarps out of the total number of available branches. Percentages of total tips are shown ±1 Standard Error

a. Fort Island

Date	Plants with cystocarps	% of total tips	Completely infertile plants
01/07/00	3	41.92±7.29	2
16/07/00	3	24.79±6.10	0
31/07/00	3	66.41±6.00	4
16/08/00	4	22.66±3.96	2
01/99/00	6	20.13±3.34	2
13/09/00	3	30.11±3.17	3

b. Scarlett Point

	Plants with cystocarps	% of total tips	Completely infertile plants
01/07/00	2	3.60±033	0
16/07/00	0	0	3
31/07/00	4	27.60±1.55	1
16/08/00	2	13.39±4.33	5
01/99/00	2	15.02±6.05	6
13/09/00	3	9.05±2.52	3

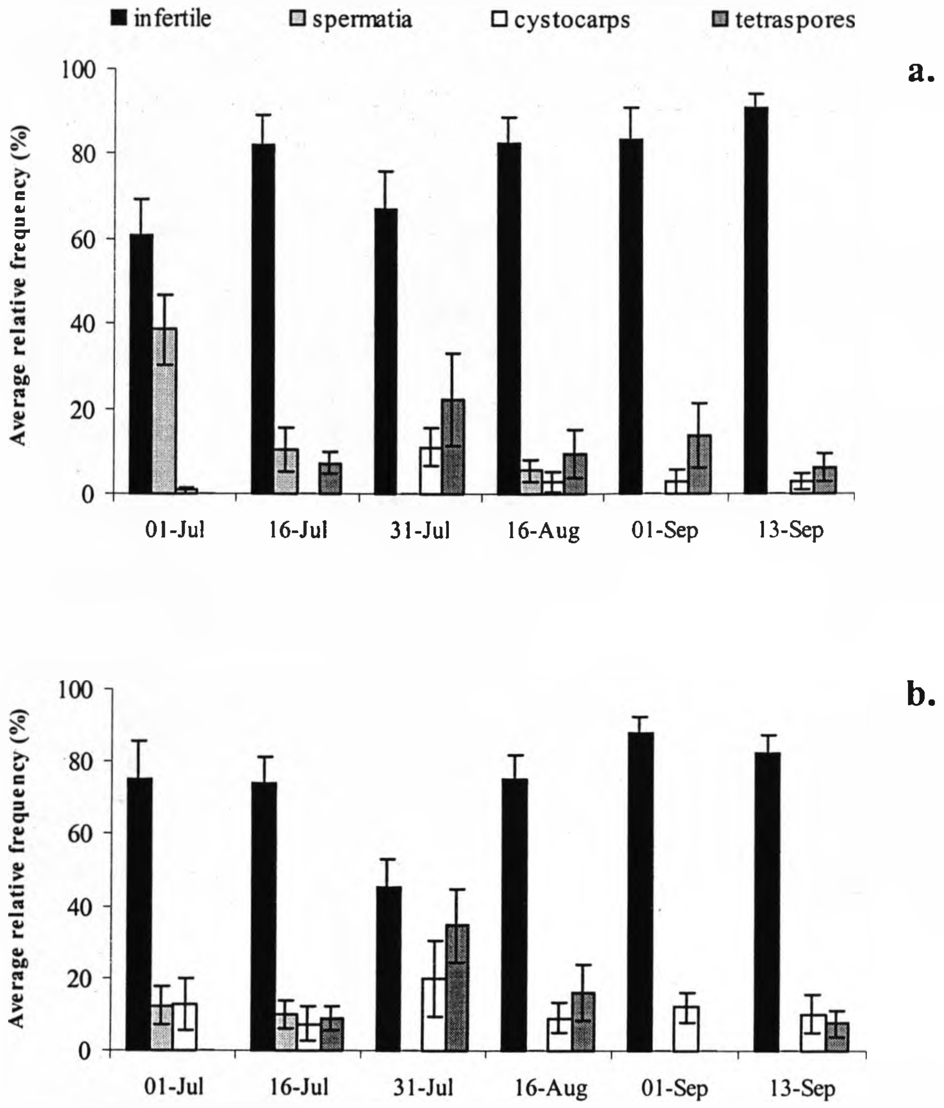


Fig 6.3 The reproductive characteristics of *Polysiphonia lanosa* at **a**. Fort Island in 2000 and **b**. at Scarlett Point in 2000. Data presented are means for counts of reproductive characteristics on 10 clumps. Error bars are± 1 Standard Error

6.3.2 Field settlement study

6.3.2.1 Preliminary Study

On the sheltered shore at Fort Island no spore settlement was observed at all. Several replicates were lost either through grazing or complete loss of the axes. On those axes that remained, the treated areas generally showed severe grazing damage and juvenile littorinid snails were commonly found in the 'wound' sites. Loss rates of laterals were very high (Table 6.2). On the exposed shore at Scarlett Point only two spores were found on the cut surfaces.

Table 6.2: The number of tagged lateral branches (out of a total of 18 per treatment) recovered at the end of the preliminary study which was terminated at the end of October 1999

Site	Control	Cut	Abrasion
Fort Island	8	5	5
Scarlett	2	6	5

6.3.2.2 Main Field Study (August 2000)

As in the preliminary study axis mortality was high at both sites. Table 6.3 shows the numbers of labelled branches recovered from each site at the end of the experiment. Survival was higher at the more exposed site of Scarlett Point than at Fort Island. Settlement of germlings was extremely low. Only three *Polysiphonia lanosa* germlings were found in the treated areas, two in epiphytised cut axes and one on a clean, cut axis.

Table 6.3: The number of experimental axes from the field study (out of a total of 30 axes per treatment) surviving at the end of the study in November 2000

Treatment	Fort I.	No of germlings	Scarlett	No of germlings
Clean, cut	15	0	10	1
Clean, control	7	0	12	0
Epiphytised, cut	6	0	7	2
Epiphytised, control	4	0	19	0

6.3.3 Laboratory settlement study

6.3.3.1 Preliminary study

In the preliminary study two types of injuries had been used: abraded and cut surfaces. The numbers of spores settling and germinating was highest on the cut surfaces and very low on both abraded and untreated surfaces. Exposure to turbulent water flows caused detachment of spores in all treatments but detachment was highest in the control and lowest on cut surfaces. Because of the generally low settlement, and because settlement was never observed on abraded surfaces in the field, abraded surfaces were not used as a treatment in the main study (Figure 6.4).

6.3.3.2 Main study

6.3.3.2.1 Experiment 1

The counts of spores and germlings of *Polysiphonia lanosa* on the host revealed considerably higher numbers of spores on control rather than treated tissue after 24 hours, but the numbers were similar after 48 and 72 hours. It was also apparent that germination of spores was more rapid in damaged areas than in the control segments. After 72 hours 50% of the spores in the treated areas had germinated compared to approximately 30% in the controls. A comparison of the total spores and germlings present at 48 and 72 hours also shows that dislodgement was significantly higher in the control segments as shown by a 3-way ANOVA (Table 6.4, Figure 6.5a).

In experiment 1 only a small number of spores became detached in treatments as well as controls as the result of a single five minute exposure to water motion (Figure 6.5b). Considered as a proportion of total spores present at the start of the disturbance, losses were highest in the controls. An analysis of variance showed a significant difference attributable to whether tissue was injured or not but no effect of disturbance, i.e. after 96 hours the *P. lanosa* germlings were attached well enough to withstand dislodgement (Table 6.5).

Table 6.4: Three-way ANOVA for the changes in spore and germling numbers over time in intact and injured A.nodosum segments

Source of variation	df	MS	*F*	p-value
Injury (1)	1	0.014	1.356	0.251
Spore/ germling (2)	1	0.228	22.049	<0.0001
Time (3)	3	0.058	5,595	0.003
1x2	1	0.023	2.230	0.143
1x3	3	0.028	2.681	0.060
2x3	3	0.232	22.396	<0.0001
1x2x3	3	0.013	1.232	0.311
Error	40	0.010		

Table 6.5: Results of a two-way ANOVA on the mean numbers of germlings per cm^2 in the first experiment testing the effect of physical disturbance on germling dislodgement

Source of variation	df	MS	*F*	p-value
Disturbance	1	0.557	1.064	0.318
Tissue type	1	9.136	17.449	0.0007
Interaction	1	0.154	0.294	0.595
Error	16			

Loss rates as the result of the disturbance were calculated separately. There were no significant differences between undamaged and damaged tissue (Paired t-test: df=25, p=0.265).

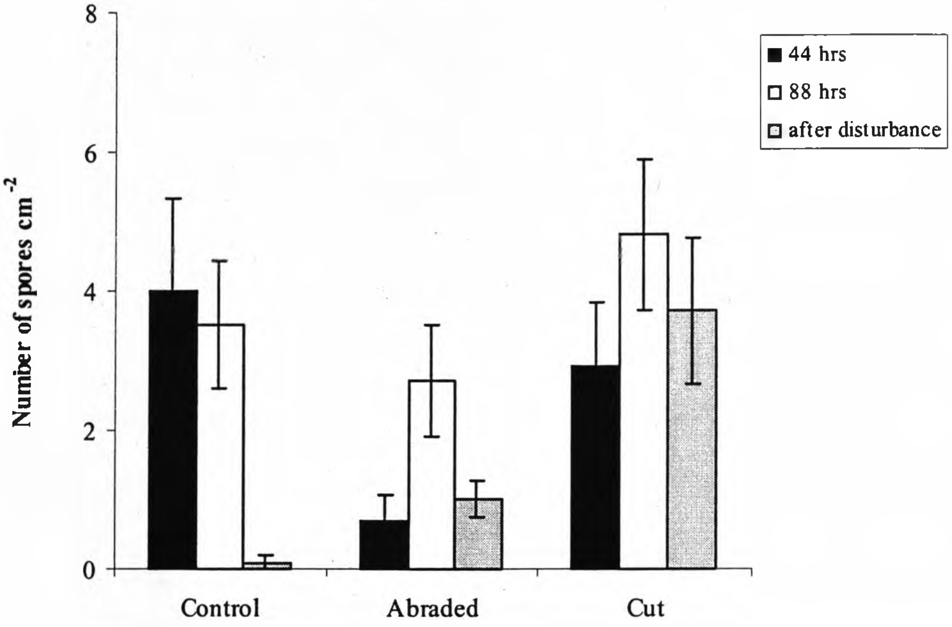
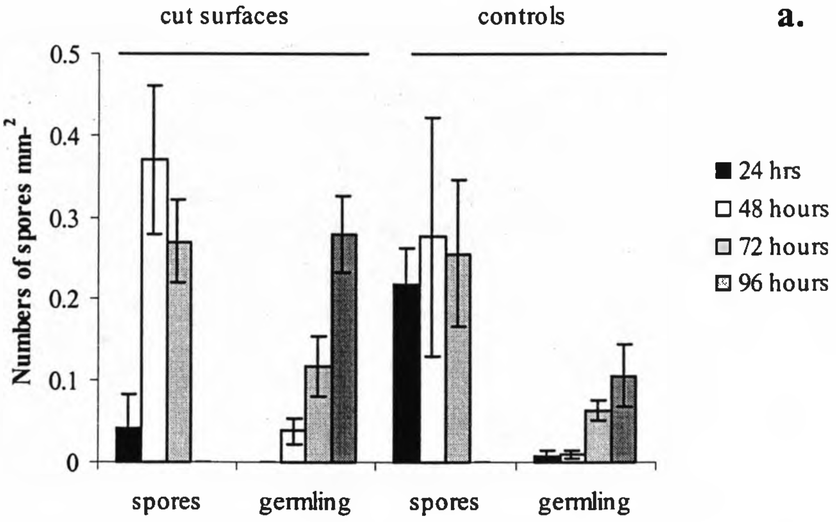


Fig 6.4 Preliminary laboratory study to test the effect of a physical disturbance in the form of water motion on the dislodgement of *P. lanosa* spores from undamaged and cut fragments of *A. nodosum,* **a.** the numbers of spores settled on the algal segments after 44 and 88 hours, b. the numbers of spores present before and after a single 5 minute exposure to water motion. Data presented are means of spores on ten *Ascophyllum* segments, error bars are ± 1 standard error.



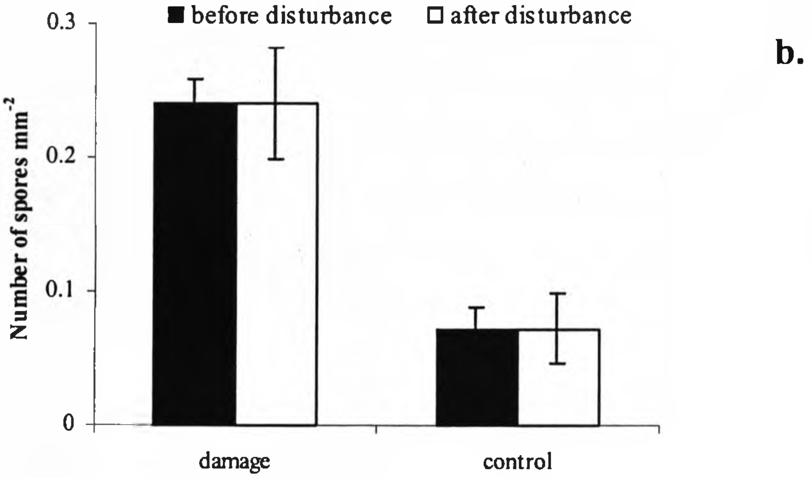


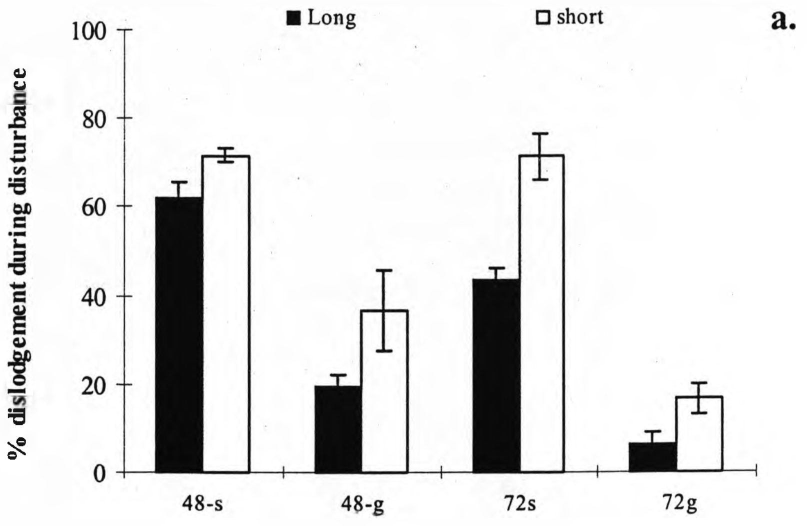
Fig 6.5: **a.** The numbers of spores and germlings of *P. lanosa* per mm^2 on segments of *A. nodosum* tissue in experiment 1 of the laboratory study, **b.** the numbers of *P. lanosa* germlings before (filled bars) and after (open bars) a physical disturbance of 5 minutes duration. Error bars are± 1 Standard Error.

6.3.3.2.2 Experiment 2

A three-way ANOVA carried out to investigate the effect of disturbance revealed a significant difference only between injured and undamaged segments, but no effect of either the length of the physical disturbance or the time of its introduction (Figure 6.6a, Table 6.6). Dislodgement and loss was generally higher in spores than germlings. There were clear differences in dislodgement of spores and germlings of *Polysiphonia lanosa* in the cut segments between long and short periods of disturbance. Whether the disturbance was introduced after 48 or 72 hours had no significant effect.

Table 6.6: Three-way ANOVA for the effect of severity of disturbance and time of introduction of the disturbance on total post settlement mortality (including germlings and spores) in experiment 2 of the laboratory study.

Source of variation	df	MS	*F*	p-level
Time of introduction (1)	1	655.602	1.341	0.258
Disturbance length (2)	1	19.507	0.399	0.844
Tissue type (3)	1	7819.201	15.992	0.001
1x2	1	506.658	1.036	0.319
1x3	1	0.364	0.0007	0.9979
2x3	1	890.683	1.822	0.190
1x2x3	1	39.218	0.080	0.779
Error	24	488.950		



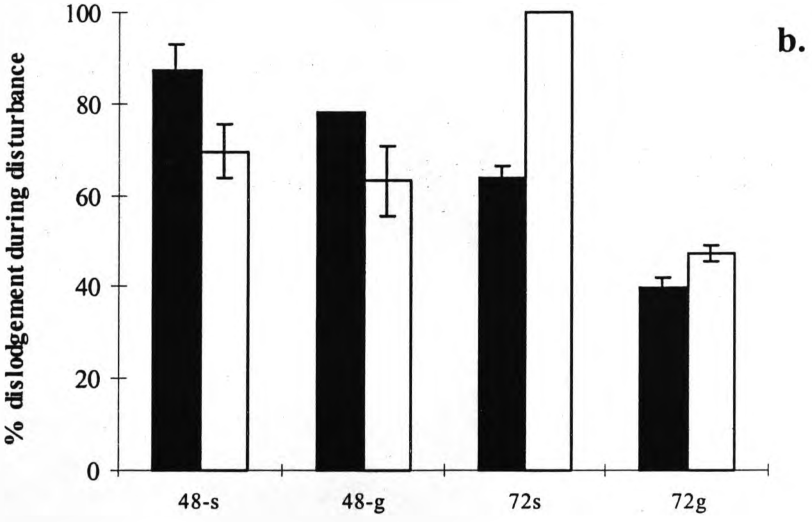


Fig 6.6 Relative loss rates of *Polysiphonia lanosa* spores (s) and germlings (g) after a five minute (solid bars) or 10 minute (open bars) physical disturbance: **a.** dislodgement from cut segments of *A. nodosum*, **b.** dislodgement from control segments (disturbances were introduced 48 or 72 hours after inoculation. Error bars are ± one standard error (n=4 per treatment)

6.3.4 Natural Settlement of P. lanosa

The numbers of *Polysiphonia lanosa* germlings were generally higher in epiphytised plants than clean ones at both sites. Germling numbers declined between November 2000 and January 2001, particularly at the exposed site where germlings were almost exclusively found on already epiphytised fronds (Figure 6.7). The densities of *P. lanosa* germlings were considerably higher on internodes that were already epiphytised than on initially clean internodes (Figure 6.7 a-d). Settlement was highest in cut surfaces and on receptacles. Settlement on vegetative tissue was mainly in the lateral pits.

In November there was significantly less settlement on vegetative tissue than expected if settlement was random and more than expected on receptacles and injured tissue. In January, similar patterns were found although for clean samples at Fort Island and epiphytised samples at Scarlett Point there were now no significant differences between observed and expected settlement (Table 6.7)

Table 6.7: Log-likelihood ratios for the natural settlement patterns of *P. lanosa* on four *Ascophyllum* tissue types at Fort Island and Scarlett Point. Settlement was recorded in November 2000 and January 2001 on clean internodes and previously epiphytised internodes. The expected frequencies in parentheses. (Significance levels: *<0.05, **<0.005, ***>0.001, ns=non-significant)

Site/date	Observed (expected) spore frquencies on different tissue types				df	G	Significance level
Fort Island	Cut	Epiphytised	Abraded	Receptacles			
November	17 (32)	5 (0.5)	0 (1.6)	34 (22)	3	30.507	***
January	8 (16.35)	1 (0.11)	0 (0.49)	20 (12.05)	3	13.250	**
Epiphytised November	41 (104.99)	1 (0.11)	0 (0.49)	180 (119.24)	3	1764.66	***
Epiphytised January	20 (48.93)	8 (0.42)	0 (2.13)	63 (39.52)	3	-6.337	ns
Scarlett							
Clean November	10 (8.95)	5 (0.18)	0 (0.06)	5 (10.81)	3	25.750	***
Clean January	0 (0.78)	0 (0.002)	0 (0.03)	2 (1.19)	3	2.076	ns
Epiphytised November	11(27.95)	14 (0.27)	0 (0.92)	35 (30.87)	3	484.09	***
Epiphytised January	1 (6.71)	3 (0.03)	0 (0.07)	15 (12.19)	3	30.05	***

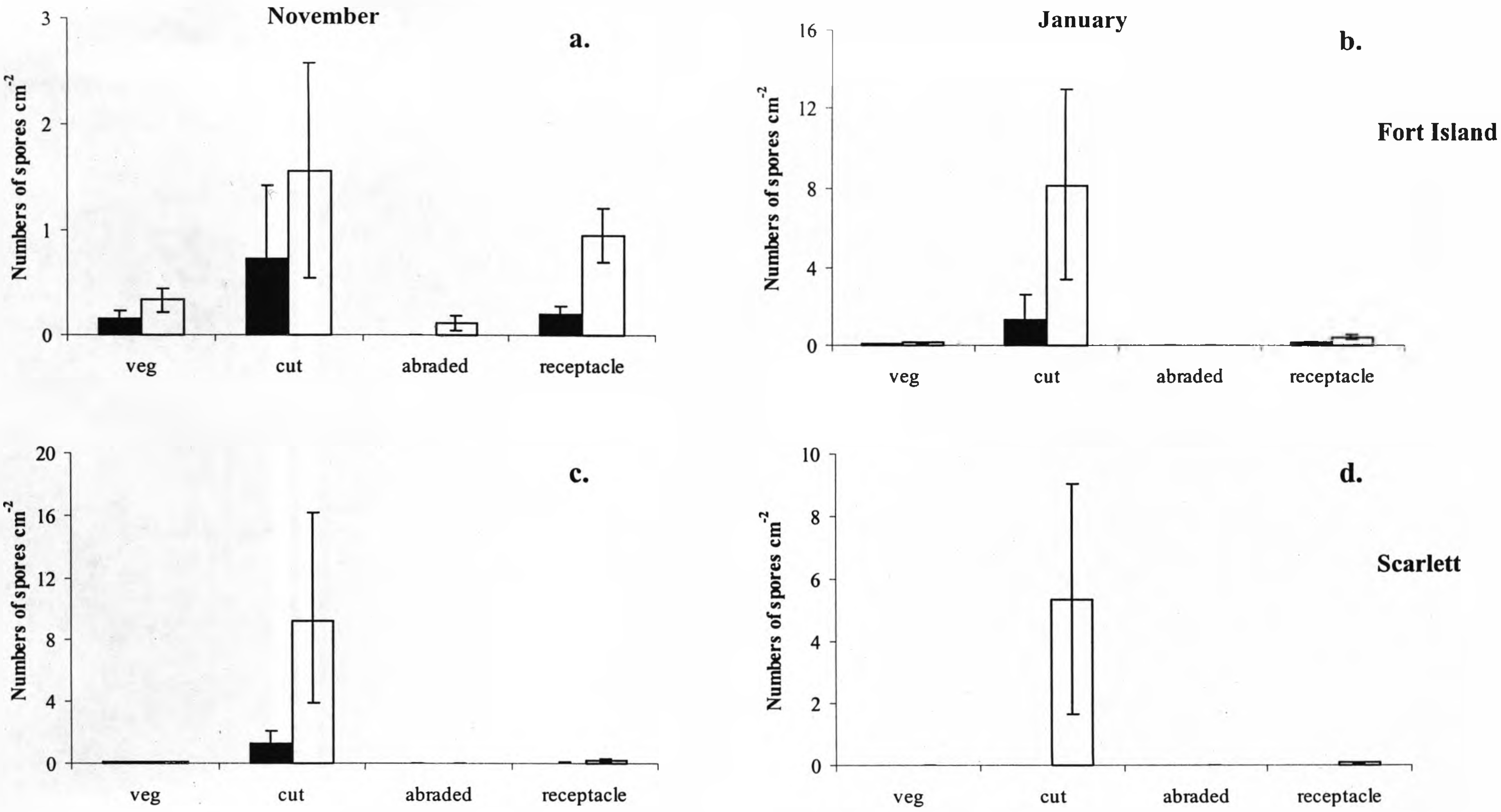


Fig 6.7 Numbers of *Polysiphonia lanosa* germlings per cm^2 of four types of tissue on 2-year old internodes of *Ascophyllum nodosum* January respectively, figures represent means for three internodes **a-b** Fort Island November and January respectively **c-d** Scarlett Point November and January respectively (note different scales on y-axis); closed bars=clean internodes, open bars=epiphytised internodes; Tissue types: veg=vegetative tissue, cut=deep tissue damage, abraded=superficial damage. Error bars are ± 1 standard error

6.4 Discussion:

The settlement behaviour of an epiphyte and its long-term survival on its host is dependent on a variety of factors. Chemical factors (Hay and Fenical 1992) as well as surface microtopography (Fletcher and Callow 1992), skin shedding by the host plant (Filion-Myklebust and Norton 1981) and grazing (Parker and Chapman 1994) have been put forward to explain the settlement dynamics of some epiphytes. One or several of these factors could be operating at any one time

Surface microtopography

Past studies have shown for a number of species that very smooth surfaces are unsuitable for the establishment of algal propagules (Norton and Fetter 1981, Steneck, *et al.* 1991, Dethier 1994, Jenning and Steinberg 1997, Tamelen *et al.* 1997, de O. Figueiredo, *et al.* 1997).)

The present study showed clear settlement patterns in the laboratory and in the field study. In both studies spore settlement occurred predominantly on damaged tissue and, in the field study, also on receptacles. There are two possibilities for the observed patterns of settlement. The non-motile spores could be 'swept' into depressions by water motion or alternatively might encounter different tissue types at the same rate but survive better in the depressions. A study by Pearson and Evans (1990), where the settlement behaviour of *Polysiphonia* on *Ascophyllum* was investigated in a flow tank, suggested that the former notion is correct. Pearson and Evans (1990) found that the water flow over the thallus was such that the spores were deposited in small depressions such as lateral pits and injuries. This indicates the importance of microtopography in facilitating epiphyte settlement. However, it is not only the presence of damage in general that is likely to be important for spore settlement, but also the type of depressions it forms. Norton and Fetter (1981) found that settlement of *Sargassum muticum* was much reduced when the depth of depressions exceeded 800µm. If the depth of the experimental cut was also important for settlement success in *P. lanosa* spores, then the depth of the depression created by cutting the thallus might explain the lack of settlement of the epiphyte in injuries in the field study. The optimal depth of depression may well depend on the size of the spores. *Polysiphonia* spores are smaller than *Sargassum muticum* zygotes and could therefore require smaller size depressions to encourage settlement. This might explain the abundance of *Polysiphonia* germlings in the conceptacles of receptacles. The absence of *P. lanosa* from other fucoid hosts might also be due to the absence of suitable sites. While grazers also consume *Fucus vesiculosus* it has much thinner fronds than *A. nodosum* and any damage by grazers would be superficial abrasions or holes rather than depressions.

Interestingly, the same type of depression that failed to encourage settlement in the field experiment, allowed settlement in the lab and, more importantly, decreased the risk of spore and germling detachment. However, in the laboratory experiments disturbances were probably of a short duration compared with the field situation and after each experiment the spores and germlings experienced a period of calm, which may have allowed survival in depressions that were suboptimal in depth.

Although, due to their small size one might expect the settled spores and germlings to be located within the calmer conditions of the boundary layer, the movement of the host plant itself is likely to create some turbulence around the settled spore. Deep injuries on the seaweed are therefore likely to provide some shelter from the high water flows germlings might be exposed to on the shore and could thus increase survival (Norton 1983). A shallow abrasion, although changing the surface texture of the thallus, would not provide any protection against wave action or possibly desiccation.

The present study has also indicated the importance of timing for successful spore settlement and subsequent survival. As spores settle onto the substratum they secrete adhesive material and then produce rhizoids to anchor to the substratum (Chamberlain and Evans 1973, McBride and Cole 1971, Norton 1983). Therefore the risk of detachment decreases with the length of time a spore has been left undisturbed after settlement (Charters *et al.* 1973, Santelices 1990). In the laboratory study spores were already invulnerable to detachment from a physical disturbance within 48 hours of settlement. This suggests that within this time rhizoid attachment and penetration of the host tissue are sufficiently advanced to securely anchor the plant to the host. Rawlence and Taylor (1970, 1972) described the mechanism for this attachment, but provided no timing for the process. However, studies by Gonzalez and Goff (1989, 1989) for two closely related red algal epiphytes of the genus *Microcladia* demonstrated that rhizoid production and anchorage to an algal substratum occurred within 2 and 4 days respectively. A period of 4 days for secure attachment is in agreement with the findings in the present study. In the turbulent intertidal environment dislodgement prior to secure anchorage is likely to lead to loss of large numbers of spores, except for those restricted to depressions on the host (Lobban and Baxter 1983).

However, even if the spores survive to the stage where they are invulnerable to dislodgement, other sources of mortality might still be effective. It is known for instance that small grazers can consume or dislodge germlings of macroalgae (Parker and Chapman 1994, Lotze *et al.* 2001). The time of peak spore settlement overlaps with that of grazer recruitment (see chapter 2) and damage caused by juvenile grazers was the reason why the first field study had to be terminated. Juvenile *Littorina* could have been responsible for at least some of the early mortality of *Polysiphonia* spores and germlings, since they were frequently observed in the settled sites. Removal of spores by juvenile grazers would negate any possible advantages gained by the provision of settlement sites resulting from the feeding action of adult grazers, especially as juvenile littorinids seem to congregate in damaged areas of the frond. As different types of damage have a different degree of effectiveness for spore settlement, it is likely that some grazers such as *Littorina obtusata*, which excavates the thallus (Norton *et al.* 1990), will have a greater effect on epiphyte settlement than *Idotea granulosa* which, although it will eventually consume all tissue presented to it will initially abrade the outer layers of the thallus (pers. obs.)

While the flow environment the spores encounter might dislodge settled spores, it could also aid dispersal (Brawley and Johnson 1992). Red algal spores are non-motile (Ngan and Price 1979, Okuda and Neushul 1981). They therefore cannot select a settlement site, and their fate might be determined by water motion to a greater degree than for other types of algae (Brawley and Johnson 1992)
However, water motion will also lead to the dilution of the existing spore and gamete densities (Kaczmarska and Dowe 1997, Engel *et al.* 1999). In the case of *Polysiphonia lanosa* this may have several consequences. As epiphyte populations are already small and patchily distributed, natural spore densities of *P. lanosa* are also likely to be small, and further dilution of gametes would be expected to reduce the incidence of fertilization. The low percentage of cystocarps found in this study and the fact that there

was little overlap between the different life-history phases seems to support this. However, fertilization success was not measured directly and the low incidence of cystocarps could have been the result of small numbers of carpogonial branches, i.e. a generally low reproductive effort. This was indicated by the number infertile plants on most sampling dates, and in a more large-scale study (Kaczmarska and Dowe 1997) also found similar proportions of non-fertile plants. The low levels of reproduction and possibly high germling mortality seem to make long distance dispersal unlikely, although it was argued by Destombe *et al.* (1992) and Engel *et al.* (1999) that even if dispersal via tetraspores is unlikely, dispersal could occur via carpospores. It might be argued that the carpospore is the main agent of dispersal. Indeed, Searles (1980) suggested that the production of thousands of diploid carpospores is a means of compensating for the inefficiency of sexual reproduction, which produces relatively few zygotes.

That dispersal ability was indeed limited in *P. lanosa* was indicated in this study by the fact that natural settlement densities were significantly higher on fronds which were already epiphytised than on those which were not, although the clean fronds were located within a distance of no more than 1m from epiphytised ones. Limited dispersal will increase the chances of settling in the vicinity of a parent plant (Destombe *et al.* 1992) and might be a further reason for the contiguous distribution of the epiphyte on the host. Increasingly high densities of the epiphyte on one frond are likely to increase the risk of frond breakage in the host plant (see chapter 5). Such frond breakage might in itself provide a dispersal mechanism for the epiphyte (Kaczmarska and Dowe 1997). Many algae can drift considerable distances (Ingólfsson 1998) and dispersal via drifting macroalgae has been described for a number of algal species (Deysher and Norton 1982, Norton and Mathieson 1983) although in these cases the drifting algae dispersed their own offspring not those of their epiphytes. There is at least one example where a red algal epiphyte was found on floating seaweed (Lin and Kraft 1996), which might have inadvertently aided in the dispersal of their epiphytes. Using the host frond as a vector would be very risky, however, since colonization of new sites would depend on the host frond drifting to a place with *Ascophyllum* plants.

It is also worth considering that *Polysiphonia lanosa* is capable of producing daughter plants via rhizoids which penetrate the host in the vicinity of the parent plant. While such a mechanism could only aid expansion over very short distances it would reduce the need for establishment of new plants from spores to maintain existing patches. In addition, if dispersal via broken *Ascophyllum* fronds is assumed to occur, the point at which epiphyte loads become high enough to cause breakage, might be brought forward by large numbers of vegetatively produced daughter clumps (Rawlence and Taylor 1970) which will concentrate growth in the vicinity of existing plants (Fletcher 1977).

Evolutionary consequences of dispersal and settlement patterns

The limited dispersal ability indicated in this study and the potential to spread vegetatively, restrict the epiphyte to small areas within the *Ascophyllum* population, which makes it unlikely that the epiphyte would exert sufficient selection pressure on the host to evolve defensive mechanisms, such as skin shedding or the production of defensive chemicals especially since costs of chemical defences could not be shown unequivocally in this and previous studies (Pavia *et al.* 1999). With respect to defensive chemicals it also has to be considered, that while grazing damage has some potential for increasing epiphyte settlement, not all damage to the host thallus will be the result of grazing. *Ascophyllum nodosum* is known to be prone to abrasion damage e.g. from brushing against rocks (Mathieson *et al.* 1982). Therefore even plants that are chemically defended against grazers would not necessarily gain an advantage from this, since chemical defences could not prevent the occurrence of damaged areas that might encourage epiphyte settlement and growth.

The present study has also indicated that a possible defence mechanism like skin shedding would have to be very rapid to be 'cost-effective' against a locally occurring epiphyte since within a few days settled spores would be invulnerable to dislodgement. Skin shedding only removes very thin layers of the outer epidermis cell walls (Moss 1982, 1984) and its effectiveness might be expected to diminish once the germling has penetrated the thallus beyond this layer (Rawlence and Taylor 1972). However, immediately following settlement of spores skin shedding is clearly an effective mechanism of germling removal not only on *Asophyllum nodosum*, but also on other seaweed species (Filion-Myklebust and Norton 1981, Russell and Veltkamp 1984).

It has to be stressed that this study was only carried out in two populations of *Ascophyllum nodosum*. *Polysiphonia* loads are known to differ between populations of the host plant and indeed are completely absent from some shores. Comparisons between populations of *Ascophyllum nodosum* with different degrees of epiphyte infestations could give quite a different picture. But within the two Manx populations studied here it seems unlikely that epiphytism alone would be able to create a sufficient selection pressure for the evolution of specific anti-epiphyte defences.

General Discussion

This study was an examination into possible phenotypic characters shaping the interactions between three species and the potential for coevolution in this association. Only phenotypic characteristics were examined in the present study as species were too long lived to observe changes in gene frequency. Several prerequisites have been identified for such reciprocal evolutionary interactions to occur between an herbivore and its 'host' plant. The most important conditions are that the herbivores affect algal fitness, exerting a selection pressure for the evolution of defences (chemical or structural) and that these defences are costly in terms of reduced growth or reproduction (Coley *et al.* 1985, Bazzaz *et al.* 1987, Briggs and Schultz 1990). These defences impose selection pressures on the herbivores, favouring those that can tolerate the chemical composition of the plant, or overcome its structural defences. The latter prerequisite implies food limitation (Spencer 1988). This is essentially an optimality approach, which assumes that a smaller reduction in fitness will be suffered by defending a structure (including the cost of the defence) than by not defending it and risking damage or loss of the structure and therefore natural selection would act to increase defences (Roff 1992, Sih and Gleeson 1995).

Costs of defence and reproduction: Evidence for trade-offs?

In the present study costs could not be established unequivocally between phlorotannins and vegetative or reproductive growth. It has been argued by some authors e.g. Yates and Peckol (1993), Peckol *et al.* (1996) and Targett and Arnold (1998) that secondary metabolites, including phlorotannins may be just a sink for carbon. They have shown that in times of nutrient shortages, when carbon cannot be invested into growth it is instead channelled into the production of secondary metabolites. In this case any deterrent effect on herbivores would be inadvertent, and while it might necessitate evolutionary change in the grazer to overcome the host's defences, a reciprocal change in the host plant would be less likely because there would be no extra costs for the production of secondary metabolites in these plants. In addition, the studies in chapter 3, which investigated the relationships between phlorotannins and reproduction, and studies by a number of previous authors suggest that there are several alternative functions for these metabolites, including phlorotannins (Brawley 1987, Brawley 1992, Clayton and Ashburner 1994, Schoenwalder and Clayton 1998). Such functions would lend further support to the hypothesis that grazers merely respond to chemicals in the host plant without actually influencing the production and concentrations of these chemicals. It would also mean that the evolution of chemical defences against specific grazers or even groups of grazers are unlikely.

With respect to reproductive costs, previous large-scale studies have shown that *A. nodosum* has a very high reproductive effort and it was usually assumed that the trade-off for a high reproductive effort is slow vegetative growth. For each individual meristem this might be the case. Growth in length is slower than in some of its competitors (Cousens 1986). However, the growth study in chapter 1 did not show any reduction in growth rate during the maturation of receptacles. Each frond of *Ascophyllum nodosum* produces a number of laterals all potentially growing simultaneously, thereby allowing considerable biomass production. Therefore the perceived growth/ reproduction trade-off might not be a real one. The only study that hinted at a possible trade-off was the growth study of canopy plants in chapter 1, where the growth of new laterals and receptacles was followed. Laterals were often of one type, i.e. either reproductive or vegetative. But even this pattern was not universal.

This study has confirmed the findings of other studies that *Ascophyllum nodosum* does not seem to fit neatly into any of the common life-history classifications, by indicating the importance of clonal growth for *Ascophyllum* (see chapter 1). Most classifications are based on sexual reproduction, and largely ignore clonal growth (Watkinson and White 1985). However, the ability to proliferate vegetatively is likely to significantly reduce the cost associated with the investment into reproductive structures and loss resulting from the mortality of offspring. *Ascophyllum nodosum* clearly has a considerable ability to produce new biomass by clonal growth and it is these vegetative units that respond to changes in environmental conditions.

In plants that habitually reproduce both sexually and clonally one might need a new approach to the measurement of reproductive costs. As in many solitary, iteroparous organisms, there is a delayed onset of reproduction in many clonal plants. However, in contrast to solitary organisms, once reproduction starts there is usually a positive correlation between growth and sexual reproduction in clonal organisms (Hall and Hughes 1996, Harvell and Grosberg 1988). Such a pattern was also found by Åberg (1996) for whole fronds of *Ascophyllum nodosum* and in the present study for *Ascophyllum* year classes. In a plant like *A. nodosum*, a possible explanation for this might lie in the fact that vegetative laterals and receptacles are initiated from the same sites (lateral pits). The receptacles are only seasonal and are shed in the spring. If the plant produced only reproductive structures early on in life, this would virtually halt any further vegetative growth and the number of gametes produced would be small because of the limited number of lateral pits. If however, vegetative growth is favoured over reproduction for a period of time, this will allow the plant to grow to a size where the production of both, vegetative and reproductive laterals can be sustained simultaneously

(Bäck *et al.* 1991). One might, therefore have to assume that sexual reproduction in this type of plant is not as costly as in solitary organisms, since changes in reproductive output would not change fitness. One possible explanation for the possible lack of costs of vegetative and reproductive growth is a lack of scaling constraints in many clonal plants. They often produce creeping rhizoids, which can theoretically grow indefinitely without placing any mechanical strains on their branches or in *Ascophyllum*, the fronds. This pattern of construction is similar to the situation in *Ascophyllum nodosum* in that it is more likely to suffer mortality from grazers and epiphytes than from hydrodynamic conditions alone. Also the primary shoots are produced in large numbers underneath the canopy and are relatively invulnerable. Without these sources of losses *Ascophyllum* could probably grow indefinitely, too.

It has been argued that vegetative spread presents a cost in itself as it prevents genetic recombination (Clayton 1990). However, somatic mutations may still arise in meristems (Bazzaz *et al.* 1987) and, like sexual reproduction, clonal proliferation provides offspring in the form of new meristems. Therefore a more important measure of 'fitness' in plants like *Ascophyllum nodosum* might be the survival of clonal and vegetative units or meristems rather than that of sexual recruits (Bonser and Aarssen 2001).

The relationship between clonal and reproductive growth might form a trade-off in its own right (Harper 1977). Rather than the production of new biomass *per se*, it could be the production of new ramets that exerts a cost. In *Ascophyllum nodosum* the new clones are produced as a large number of small shoots that show little growth until some of the overlying canopy is removed. Individual primary shoots only form a tiny part of the biomass of an *Ascophyllum* stand. However, large numbers of them have to be maintained, possibly over years, in a state, where accelerated growth is almost immediate, upon exposure to high light conditions. Losses to these primary shoots might jeopardize the stability of the whole population, since they are ultimately the source of new fronds. An allocation of resources to reproduction that results in increased mortality or decreased production of primary shoots could present a severe trade-off. Such a trade-off has been suggested by Caswell (1985). The model devised by Caswell (1985) assumes that increased allocation to clonal growth should lead to a decrease in sexual reproduction. Few studies have investigated the relationship between clonal propagation and sexual reproduction but most did not find evidence for any trade-offs (Bouzille *et al.* 1997, Lord 1998). By definition such a trade-off implies the allocation of a limited resource.

One resource that could lead to trade-offs in marine plants is a lack of nutrients, particularly nitrogen. However, a number of studies have shown that *A. nodosum* can store nutrients. Therefore, nutrients taken up when ambient nitrogen concentrations are high can be utilised during times of nutrient shortages to subsidise growth and reproduction (Chopin *et al.* 1996, Hardwick-Whitman and Mathieson 1986). Therefore, both growth and reproduction can be sustained at times of declining nitrogen concentrations. This would indicate that it is light rather than nutrients, which determines the patterns of vegetative growth and reproduction.

A possible explanation for the positive relationship between various measures of reproductive output and vegetative biomass might then be that receptacle production is simply dependent on the amount of surface area of vegetative tissue available for photosynthesis (i.e. the limit to growth and reproduction is the photosynthetic capacity of the cells). A reduction in the vegetative biomass would then not lead to an increased reproductive effort as predicted by previous studies but to a decrease (Reed 1987). Moreover, a delayed onset of reproduction would not necessarily be the result of trade-offs between vegetative and reproductive growth, but simply indicate that reproduction must follow a period of accumulation of biomass high enough to sustain reproductive growth. This is not the same as a reproductive cost. Such plants would, once they have reached a certain size, be able to sustain both growth and reproduction, as is the case in *Ascophyllum nodosum.* It also has to be taken into account that the receptacles are at least partly photosynthetic, which will further reduce the cost of their production (McLachlan and Bidwell 1978, Reed *et al.* 1988). If it were the amount of total vegetative tissue available for photosynthesis that determines reproductive effort, this would require some mechanism of transport of photosynthates from the site of production to the sites at which reproductive tissue is produced. Such long-distance transport has indeed been found in a number of kelps, some red algae and in the members of the Fucales (Diouris and Floc'h 1984). These authors found a directional transport toward growing tips in several fucoid species when they were pulsed with a radio-isotope up to 15 cm away from the apical region. Chopin *et al.* (1996) also found seasonal changes in the nitrogen content of different age classes of *Ascophyllum nodosum* and interpreted this as transport from older, slow growing internodes 4-5 years old, to actively growing meristematic tissue. *Ascophyllum nodosum* therefore seems to be able to subsidise growth at the apex from older internodes that are no longer actively growing, but are close enough to the top of the canopy to significantly contribute to the photosynthesis of the frond.

The above factors may explain why *Ascophyllum* has such a massive reproductive effort, most of it lost, and still manages to be so long lived (Åberg 1996).

In *Ascophyllum nodosum* it is local light conditions that determine whether a given initial develops into a vegetative lateral or a receptacle (Terry and Moss 1980). The abundance of receptacles might therefore be more a reflection of the number of available sites for lateral production rather than an optimal reproductive effort determined by natural selection for given environmental conditions. But reproductive effort might not be completely useless and some sexual recruits will ultimately be needed to ensure genetic recombination and therefore the ability to respond to selection pressures. Otherwise one might expect a decrease or even loss of sexual reproduction from the clonal population if there were no selection pressure for maintaining it (Eckert *et al.* 1999).

Van Noordwijk and de Jong (1986) have argued that a positive relationship between measures of vegetative growth and reproduction might be an artefact of such factors as different plant sizes. However, many of the studies in this thesis were carried out using uniform age classes and no trade-offs could be established. A number of longer-term studies have also questioned the universality of reproductive costs in both plants and animals (Tuomi *et al.* 1983, Thorén *et al.* 1996, Reznick *et al.* 2000).

Fitness consequences resulting from the association with grazers

The relationship between herbivores and their host plants has been widely studied with respect to their potential for coevolution (Ehrlich and Raven 1964, Edmunds and Alstad 1978, Futuyma 1983, Hougen-Eitzman and Rausher 1994). In marine systems, there is often a discrepancy in the amount of damage done by grazers in laboratory experiments and at natural densities in the field. Adverse effects of grazers on the food plant could often only be established when some catastrophic event elevated the grazer densities to far above their normal levels because normally the grazers are unlikely to be food limited (Fralick *et al.* 1974, Thomas and Page 1983). Food limitation is unlikely to have been a factor in the present study, even if it is assumed that different parts of *Ascophyllum nodosum* have a different nutritional quality or amounts of defensive chemicals (Pavia 1999). On sheltered shores *A. nodosum* can have standing crops of 3 kg m^{-2} (Cousens 1986). Therefore, even if all grazers were constantly feeding at their maximum rates, they would hardly be consuming more than a hundred grams of host tissue per year at the densities found in the present studies (Salemaa 1987, Pavia *et al.* 1999 and own data).

Since *Ascophyllum nodosum* relies so heavily on clonal propagation, it might be argued that grazers could still have a significant effect, if they fed predominantly on meristematic tissue. However, a typical *Ascophyllum* plant will have hundreds of meristems, so that the low densities of grazers are unlikely to affect it greatly, especially since it can produce new growing tips even from damaged sites (Van Alstyne 1989).

In the association between *Ascophyllum nodosum*, its associated grazers and the epiphyte, reciprocal interactions resulting in coevolution seem unlikely due to the apparent lack of food limitation of the grazers, possible alternative functions of the defensive phlorotannins as well as the reduced costs of sexual reproduction. All these factors mean that there can be no direct selection pressure by grazers or epiphytes for the evolution of defensive chemicals in the host plant. However, it was shown by Pavia

(1999) that *Littorina obtusata* does respond to intraspecific variations in phlorotannin concentrations in *A. nodosum* and that the plant shows phlorotannin induction as the result of attack by the snails, which indicates that there could have been some reciprocal interactions in the past. On the other hand an earlier study by Lowell *et al.* (1991) has shown in the field that mechanical strength rather than increased phlorotannin levels were induced in *Ascophyllum nodosum* as the result of frond damage. Lowell *et al* (1991) suggested that phlorotannins simply 'leaked' out of the wound sites, but added that decreases in phlorotannin concentrations were also found at some distance (sometimes in excess of 30 cm) from injured sites. They attributed this to translocation of polyphenols from more distal portions of the frond to the wound sites to be utilized in the repair process. However, it could take up to four weeks for this effect to become noticeable. Therefore this phenomenon seems unlikely to give the frond any added protection immediately after grazing, especially as they also found a higher edibility of the frond in the damaged region. It might, however, be part of the normal wound healing process.

Where the grazer exerts no clear selection pressure one would not expect the actions of host and/or epiphyte to induce any defensive response by the host and therefore no reciprocal interactions (Arrontes 1999). Consequently an alternative model is needed to explain interactions between marine grazers and their food plants.

In the absence of reciprocal interactions, the plant's chemicals might still affect the grazers and epiphytes associated with it (Tugwell and Branch 1992). However, rather than entering into a coevolutionary relationship, grazers colonizing a new plant would simply respond to its existing chemical make-up without themselves exerting any selection pressures for reciprocal changes. This process is called sequential evolution (Jermy 1984).

Such a mechanism could explain the apparent specificity of *Littorina obtusata* on *Ascophyllum nodosum*, but would not predict the plant's polyphenol levels to be a response to grazing pressures but to one or several of the alternative functions already mentioned. It might have been predation pressure in the past that determined the distribution of the snail (Smiley 1978, Williams 1992, Trussell 2000), which was followed by the evolution of adaptations to overcome the plant's defences (Bernays and Graham 1988). This concept has, however, been criticized by Thompson (1994) who stated that the fact that grazer densities are very low does not mean that they cannot exert a selection pressure as long as their effects are non-random i.e. affect different plant genotypes differentially. However, Thompson's predictions assume that host secondary metabolites are solely grazer defences, which is unlikely to be the case.

The effects of the epiphyte Polysiphonia lanosa

A number of studies have described the disadvantages for a plant of being infested with epiphytes (Sand-Jensen and Borum 1984, D'Antonio 1985). However, the present study has shown that the effect of *Polysiphonia lanosa* on the reproductive effort of *Ascophyllum nodosum* is likely to be limited except for the most heavily infested host plants. Moreover, although *P. lanosa* can lead to the dislodgement of branches by increasing drag, which might cause loss of receptacles as well as vegetative tissue, the opening up of gaps in the canopy will also allow the growth of previously shaded primary shoots to accelerate. This increases their chances of joining the 'adult' canopy and becoming reproductive themselves. Such a process has also been described for the green alga *Avrainvillea longicaulis* in which a proliferation of new blades follows the loss of old epiphytised blades and Littler and Littler (1999) interpreted this as a mechanism for controlling epiphytes. The loss of biomass as the result of epiphytism is very similar in effect to the damage caused by grazing action. How advantageous this is, would depend on the pattern of frond removal. Only if a relatively large number of fronds are removed within a small area, perhaps after a storm, might one expect this accelerated growth effect to follow.

Conclusion

While any obvious trade-offs between measures of reproduction and growth could not be demonstrated in the present study it is obvious that the plant has to strike a balance between allowing shoot loss and facilitating growth of new axes from primary shoots to maintain a stable population. This study has shown that biomass losses can be quite severe, not necessarily at the level of holdfasts, but certainly at the level of laterals. The preliminary study in chapter 1 has clearly shown that frond damage is common. The labelling study in chapter 5 also showed some losses of whole fronds. But as long as the tissue is part of an older frond that might be damaged by grazing and/or heavily epiphytised, such a loss is unlikely to lead to a decrease in fitness of the clone. In fact a strategy that allows some losses (and maximises growth where possible as shown in the primary shoot study) might be advantageous. The main factor likely to constrain frond renewal from primary shoots is access to light conditions sufficient for growth. Since *Ascophyllum* fronds could theoretically grow indefinitely, like many clonal plants, suitable conditions for the growth of new primary shoots will be rare unless some canopy fronds are removed. The present study has shown that the effects of grazers and epiphytes are a clear source of frond removal. Rather than having negative effects on the host plant, both grazers and epiphytes might play a critical role in facilitating frond renewal and the maintenance of a stable host population. Such beneficial effects have rarely been considered previously when examining the interactions between host plants and their associated grazers.

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Appendix 1

Folin Denis Reagent for Phlorotannin Analysis

Folin-Denis Reagent

1.	Na_2WO_4.H2O	100g
	$NaMoO_4$.H2O	25g
	H2O	700ml

Mix the above and add:

2. 85% H_3PO_4	50ml

Heat the mixture under reflux for 10 hours then add:

3.	$LiSO_4$	150g
	H_2O	50ml

Several drops of bromine

Boil the mixture for 50 minutes

Cool to 25°C

Dilute to 1 litre and filter.

Carbonate Reagent

Na_2CO_3	200g
NaK Tartrate	12g

Dissolve in 750ml hot water

Cool to 25oC and dilute to 1 litre

Appendix 2

Preliminary ANCOVAS for Chapter 3. On the basis of these analyses epiphytised and clean plants could be pooled as slopes and variances respectively were found to be homogeneous

A. Relationships between reproductive and vegetative tissue on 2. internodes of *A. nodosum*

1. Total receptacle dry weight vs total vegetative dry weight

	males				females			
Source of variation	df	MS	*F*	p	df	MS	*F*	p
intercept	1	0.002	0.674	0.423	1	0.002	0.751	0.405
epiphytism	1	0.006	0.187	0.671	1	0.0002	0.074	0.791
covariate	1	0.024	7.842	0.123	1	<0.0001	1.069	0.323
interaction	1	0.095	3.133	0.095	1	0.002	0.022	0.884
Error	17	0.003			11			

2. Number of receptacles vs vegetative weight

	males				females			
Source of variation	df	MS	*F*	p	df	MS	*F*	p
intercept	1	1.310	82.425	<0.0001	1	0.393	13.686	0.003
Epiphytism	1	0.048	3.029	0.100	1	0.013	0.438	0.520
Covariate	1	0.125	7.870	0.012	1	0.018	0.614	0.449
Interaction	1	0.0003	0.011	0.890	1	0.011	0.393	0.543
Error	17	0.016			12	0.028		

3. Number of receptacles per cm vs vegetative dry wt

	Males				females			
Source of variation	df	MS	*F*	p	Df	MS	*F*	p
intercept	1	1.594	117.69	<0.0001	1	0.441	19.512	0.0008
Epiphytism	1	0.051	3.765	0.069	1	0.004	0.161	0.695
Covariate	1	0.009	0.669	0.425	1	0.019	0.821	0.383
Interaction	1	0.001	0.080	0.768	1	0.003	0.128	0.727
Error	17	0.014			12	0.023		

4. Receptacle size vs vegetative dry wt

	Males				females			
Source of variation	df	MS	*F*	P	df	MS	*F*	p
intercept	1	0.0002	11.005	0.004	1	<0.0001	4.959	0.048
Epiphytism	1	<0.0001	2.079	0.167	1	<0.0001	0.159	0.697
Covariate	1	<0.0001	1.108	0.307	1	<0.0001	2.924	0.115
Interaction	1	<0.0001	2.999	0.102	1	<0.0001	0.149	0.707
Error	17	<0.0001			11	<0.0001		

5. Receptacle dry wt per g veg vs vegetative wt

	Males*				females			
Source of variation	df	MS	*F*	P	df	MS	*F*	p
intercept	1	1.306	33.11	<0.0001	1	0.003	5.456	0.039
Epiphytism	1	0.001	0.026	0.874	1	<0.0001	0.634	0.806
Covariate	1	0.007	0.167	0.686	1	0.0002	0.181	0.679
Interaction	1	0.848	2.149	0.161	1	<0.0001	0.010	0.921
Error	17	0.039			11			

*data are log not (log+1) transformed

B. Relationships between Total Phlorotannin concentrations and reproductive/ vegetative tissue mass

1. Phlorotannin concentration (dependent variable) and number of receptacles per g vegetative tissue (covariate)

	Males				females			
Source of variation	df	MS	F	P	df	MS	F	p
No of rec per g	1	0.379	0.269	0.611	1	0.875	3.427	0.091
epiphytism	1	0.0034	0.024	0.879	1	0.996	0.390	0.544
interaction	1	0.0000	0.0001	0.992	1	0.072	0.282	0.606
residual	15	0.1405			10	0.255		

2. Total phlorotannin concentration concentrations in clean and epiphytised plants (dependent variable) total receptacle dry weight (covariate)

	males				females			
Source of variation	Df	MS	F	p	df	MS	F	p
Total rec. dry wt.	1	0.012	0.01	0.92	1	1.246	5.33	0.044
epiphytism	1	0.126	1.06	0.319	1	0.568	2.43	0.150
interaction	1	0.193	1.63	0.222	1	0.552	2.36	0.155
residual	15	0.119			10			

3. Total phlorotannin concentration (dependent variable) and receptacle dry weight per gram vegetative weight (covariate)

	Males				females			
Source of variation	df	MS	F	P	df	MS	F	p
rec dwt per g veg	1	0.089	0.7	0.415	1	0.638	2.386	0.129
epiphytism	1	0.085	0.67	0.425	1	0.558	2.734	0.153
interaction	1	0.250	1.98	0.180	1	0.464	1.986	0.189
residual	15				10	0.234		

4. Total phlorotannin concentration in epiphytised and clean plants (dependent variable) and receptacle size (covariate)

	males				females			
Source of variation	Df	MS	F	p	df	MS	F	p
Receptacle size	1	0.004	0.040	0.843	1	0.112	0.342	0.572
epiphytism	1	0.280	2.56	0.131	1	0.575	1.750	0.215
interaction	1	0.5	4.554	0.049	1	0.472	1.438	0.258
residual	15				10	0.329		