The Role of Shallow Seagrass Meadows as Habitat for Fish

by

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Summary

Shallow seagrass meadows are considered important as habitat for small fish, including juveniles of economically important species. Surveys of eelgrass (*Zostera*) and unvegetated patches in the Barker Inlet region, a shallow, sheltered, marine-dominated estuary in South Australia, showed that there were more fish over eelgrass than over adjacent unvegetated habitat. Currently the most strongly supported model explaining such patterns is that fish are able to detect and preferentially select one habitat over another. An alternative explanation for this pattern in fish distributions is that fish simply swim until they find food, eat it, then swim again until finding more food. Under this scenario, fish will spend more time where there is more food. King George whiting (*Sillaginodes punctata* (Cuvier & Valenciennes)) is the most economically valuable fish species in South Australia. Like most fish living in shallow, sheltered embayments, juveniles were shown to be carnivorous, feeding on the small, motile invertebrates associated with seagrass leaves and sediment surface (epifauna). Food availability (epifaunal production) was much greater in eelgrass than in adjacent unvegetated patches in the Barker Inlet region.

The importance of above-ground vegetation to fish was tested in a field experiment comparing fish catches from replicate 30 m² patches of four habitats: eelgrass, eelgrass canopy removed, eelgrass intact but with simulated removal, and unvegetated. The disturbance concomitant with canopy removal had little effect on fish. Fish numbers in plots from which eelgrass had been removed were lower than in control eelgrass plots but did not decline to levels found over unvegetated habitat. Epifaunal production also declined upon removal of canopy cover in this experiment and in an experiment in the Mediterranean Sea involving partial and total removal of canopy but, again, was not reduced to levels found in unvegetated habitat. Fish numbers matched levels of food availability more closely than levels of eelgrass cover. This was taken as necessary but not sufficient evidence for the simple feeding model.

Predictions from the two models, habitat selection and simple feeding, were tested in a laboratory experiment in which juvenile *Sillaginodes punctata* could choose between eelgrass and unvegetated habitat. When food was offered in unvegetated habitat only, the distribution of fish initially supported the simple feeding model, but once fish were satiated their distribution supported neither model. In the absence of food, fish distribution supported the habitat selection model. The failure of this test to distinguish conclusively between the two models may lie in the reduced habitat patch size compared with field experiments and surveys, but the models are not necessarily mutually exclusive.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Signed:

Date: 26th August 1994

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Chapter 1

Introduction

1.1 Overview

Seagrass meadows are renowned as important habitats for small fish, and usually have greater species richness and higher fish abundances than adjacent unvegetated areas. The role of seagrass meadows as habitat for small fish, however, has not yet been adequately explained. The importance of seagrass meadows may differ depending on location, depth and type of vegetation, and the emphasis of this thesis is on shallow, near-shore meadows consisting predominantly of fine-leaved vegetation such as the eelgrass *Zostera*. Most of my work has been done in South Australia in the Barker Inlet region (see Fig. 3.1), which is a shallow, marine-dominated estuary consisting of a patchwork of eelgrass and unvegetated areas. The Barker Inlet region is considered the prime nursery area in Gulf St Vincent for juveniles of the most important commercial and recreational species in South Australia, King George whiting (*Sillaginodes punctata*), and special attention is paid to this species.

The most frequently invoked explanation for the importance of seagrass to small fish is that the vegetation provides a shelter from predators such as larger fish. By implication, a simple model explaining higher fish abundances associated with seagrass is that fish are more abundant in seagrass meadows because they are taken by predators less frequently there than over unvegetated areas. Much of the work on seagrass - fish ecology has involved surveys showing associations between seagrass presence or density and fish numbers, but the most powerful tests between competing explanatory models use manipulative experiments. Field tests by other researchers in which seagrass density and height were altered in experimental plots showed that the exclusion of predators .

(larger fish) did not affect the difference in abundances resulting from alteration of seagrass cover. These results were taken to suggest that fish are more common in denser seagrass not directly because of differential predation rates but because they select habitat. The ultimate agent resulting in the evolution of habitat selection by fish may still have been the increased likelihood of being eaten in areas with less seagrass cover. An alternative to the habitat selection model is that fish simply swim until they find food, eat it, then swim again until finding more food. Under this scenario, fish will spend more time where there is more food. This model is worth considering because there is usually more food in seagrass than in adjacent unvegetated areas.

This thesis sheds light on the significance of seagrass to small fish in a series of surveys and experiments presented as separate chapters. The materials and methods used are explained in the materials and methods sections of individual chapters, although to avoid repetition techniques are explained in full only in the chapter in which they are first encountered. In Chapter 2 a pop net designed specifically for collecting fish in the experiment described in Chapter 6 is compared with a seine net used during survey work described in Chapter 3. The survey of small fish from eelgrass and unvegetated areas of the Barker Inlet region (Chapter 3) confirms that the oft-quoted differences between the fish fauna of eelgrass and adjacent unvegetated areas hold for this region. This is the first demonstration of such a pattern in South Australian waters. The diet of *Sillaginodes punctata* is reported in Chapter 4. The small, motile invertebrates associated with eelgrass leaves and the sediment surface (epifauna) are the predominant food of *S. punctata* and of most fish species living in shallow, sheltered embayments. Epifaunal abundance, biomass and production from eelgrass and unvegetated areas of the Barker Inlet region are compared in Chapter 5. Having established not only that there are more fish in eelgrass areas but also that there is more food available there, a seagrass removal experiment was done to test the importance of the presence of seagrass canopy to small fish (Chapter 6). Contrary to expectations from a model in which fish select seagrass habitat based on the presence of above-ground vegetation, fish numbers in plots

from which eelgrass had been removed did not decline to levels found over unvegetated patches. The amount of available food (epifauna) was also measured in the same experiment (Chapter 7), and matched fish numbers more closely than levels of eelgrass cover. Experiments in *Cymodocea* meadows in shallow embayments of the Mediterranean Sea (Chapter 8) made distinctions between the effects on epifauna of total canopy removal and partial reduction in height of canopy. Predictions from the two competing models, habitat selection and food availability, were tested using juvenile *S. punctata* in a laboratory experiment in which fish could choose between eelgrass and unvegetated habitat either in the absence of food or with food present only in unvegetated habitat (Chapter 9). Results of all surveys and experiments are discussed in Chapter 10.

The contents of several chapters have been published, are in press, or have been submitted for publication as separate scientific papers. The papers differ from the chapters of this thesis in that the content of the Introduction, Materials and Methods, and Discussion sections overlap from one paper to the next because of the need for each paper to stand alone. I am the sole author of all papers except that corresponding to Chapter 8. All work has been done by me during my candidature except for voluntary help in the field as acknowledged in the papers. I am the main author of the paper corresponding to Chapter 8; Dr Alan Butler's role was to provide the administrative and logistic framework for the field experiments in France and to assist in writing the paper. The papers are bound as an appendix in support of this thesis, together with a manuscript concerning statistical power whose history is explained there.

1.2 Literature review

Seagrasses are important in shallow near-shore marine environments for several reasons. They can reduce water flow (Fonseca *et al.* 1982), stabilise sediments (Bulthuis *et al.* 1984), sustain high levels of primary production (Hillman *et al.* 1989), provide attachment sites for diverse epiphytic algal assemblages (Harlin 1975), and are important in nutrient cycling (Hemminga *et al.* 1991). Perhaps most importantly, seagrass areas are recognised around the world (Kikuchi 1980, Pollard 1984) and in Australia (Middleton *et al.* 1984, Pollard 1984, Bell & Pollard 1989) as an important habitat for fisheries production and especially as nursery areas for juveniles of commercially important species. It has been shown in many places that the fish fauna of seagrass is characterised by higher numbers of species and individuals and different types of fish than unvegetated areas (Beckley 1983, Weinstein & Brooks 1983, Sogard & Able 1991, and reviewed by Bell & Pollard 1989). Assemblages associated with seagrass consist mainly of small, inconspicuous species and juveniles of larger species, whereas the fauna of unvegetated areas is characterised by adults of large, mobile fish and species protected by schooling behaviour or camouflage against sediments (Bell & Pollard 1989). In an estuary in Western Australia, total fish abundance and biomass of fish, but not species richness, were greater in vegetated than in unvegetated areas (Humphries *et al.* 1992). Only in two studies reviewed by Bell & Pollard (1989) were the numbers of fish not shown to be greater over vegetated than over unvegetated habitat. In a South African study, Hanekom & Baird (1984) suggested that the failure to catch more fish over seagrass was due to very high turbidity levels, and consequently lower predation rates, diminishing the importance of seagrass. In a study in Chesapeake Bay, USA, differences in numbers of fish from seagrass and unvegetated areas were obscured by large numbers of one species found over unvegetated areas (Heck & Thoman 1984). Ferrell & Bell (1991) also found that although many more species and individuals were found over seagrass (*Zostera capricorni* Aschers.) than over bare sand greater than 100 metres from seagrass, the differences were either not as pronounced or not evident at all when fish

from seagrass were compared with those from bare sand within 10 metres of seagrass. Fish assemblages have also been shown to vary with position within an estuary (Bell *et al.* 1988, McNeill *et al.* 1992).

Seagrass meadows are declining in area due to pollution and land reclamation in Australia (Shepherd *et al.* 1989, Walker & McComb 1992) and elsewhere (Walker & McComb 1992). The loss of seagrass cover has been correlated with declining fish catches, for example of King George whiting, *Sillaginodes punctata*, in Victoria (Bell & Pollard 1989).

The importance of seagrass to small fish may lie in the protection it offers from predators (larger fish). This aspect of the ecology of seagrasses has received the most attention over the last 15 years (e.g. Heck & Orth 1980, and review by Bell & Pollard 1989), primarily because of some early experiments on invertebrates, especially macroinvertebrates, associated with seagrass suggesting that predation rates are limited by denser seagrass cover (reviewed by Orth *et al.* 1984). There are typically more epifaunal invertebrates associated with seagrass than with unvegetated areas (Orth *et al.* 1984, Howard *et al.* 1989, Edgar & Shaw 1993), and the same types of explanations for these higher abundances in seagrass have been used to explain patterns in fish abundance. Less work has been done using fish than using invertebrates, and studies using invertebrates have more often been experimental, whereas studies on fish ecology have tended to be mensurative. The reason for this may be the small size, higher densities and in some cases smaller scale movements of invertebrates compared with fish. Surveys showing associations of fish faunas with environmental variables are useful in suggesting possible explanatory models but ultimately cannot determine whether seagrass *per se* or another factor associated with seagrass (e.g. a water quality variable) is important to fish.

When a canopy of *Zostera capricorni* was shortened in experimental plots, total fish abundance declined, as did the abundance of many individual species. Thinning the canopy also caused several species to become less abundant, although three species increased in abundance (Bell & Westoby 1986a). Bell & Westoby (1986c) manipulated seagrass densities in field experiments and used predator exclusion cages to show that small fish were more common in denser seagrass regardless of the presence or absence of predators. They showed convincingly that low fish numbers in patches with less dense seagrass cover were not due to increased predation, and concluded that small fish select habitat. As Bell & Westoby (1986b) point out, their results may be explained in other ways. Fish might, for example, be attracted to more abundant food in denser seagrass. Food abundance was not measured by Bell & Westoby (1986a, 1986b, 1986c).

Experiments investigating the importance of seagrass to invertebrates as a source of protection from predators have tested between the two factors, protection from predators and habitat selection. There is some evidence that amphipods can detect differences in canopy density, and that species that are more vulnerable to predation by fish show a greater preference for dense seagrass (Stoner 1980). The behavioural mechanism of habitat selection is assumed to be a response to the habitat itself, but the underlying advantage might be in terms of any of several factors, including increased living space and food availability (Leber 1985). Movement to find food is not the same thing as habitat selection, but it may result in occupation of habitat that supports more food. The selection of habitat and trade-offs between foraging and remaining safe from predators have also been studied in animals from non-marine systems. This work was traditionally aimed at elucidating rules governing decision-making in animals, and to a large extent is now based on optimality modelling (Krebs & Kacelnik 1991, Orzack & Sober 1994).

Small fish from shallow, soft-substratum habitats are predominantly carnivorous, and epifaunal invertebrates form the major part of their diet (Casabianca & Kiener 1969, Robertson 1977, Duka 1978, Bell & Harmelin-Vivien 1983, Khoury 1984, Pihl 1985, Lubbers *et al.* 1990, Scholz *et al.* 1991, Webb 1991, Shaw & Jenkins 1992, Edgar *et al.* 1993). Crustaceans are the most important prey (Watson *et al.* 1984, Robertson 1984, Ryer & Orth 1987, Heck & Weinstein 1989, Klumpp *et al.* 1989, Edgar *et al.* 1993), and epifaunal, but not infaunal, polychaetes and molluscs are also taken (Klumpp *et al.* 1989).

Placement of artificial seagrass structures in areas devoid of but near to natural seagrass can distinguish between the importance of above-ground "vegetation" and other factors such as measures of water quality. Artificial seagrass structures attract a similar fish fauna to that in natural seagrass, and support much higher numbers of small fish than adjacent unvegetated areas (Bell *et al.* 1985, Bell *et al.* 1987, Sogard 1989). According to the model of Bell & Westoby (1986b), completely unvegetated areas have different fish assemblages and fewer fish as a result of habitat selection; fish choose to settle in seagrass beds in preference to adjacent unvegetated areas. Artificial seagrass structures, however, also attract a similar invertebrate fauna to that in adjacent seagrass meadows, with both macrofaunal (Virnstein & Curran 1986) and meiofaunal (Bell & Hicks 1991) numbers being higher in artificial seagrass than in unvegetated areas. The amount of food associated with artificial seagrass would therefore also be higher than in adjacent unvegetated areas.

The impetus for the work in this thesis was provided by the following model, developed by Bell & Westoby (1986b) to explain comprehensively the distribution of small fish in shallow, sheltered embayments: 1) fish larvae are distributed patchily when ready to settle, 2) fish select seagrass of any density rather than unvegetated areas when settling to a benthic existence, and 3) fish redistribute themselves within a bed by selecting denser seagrass but do not, at least in the short term, move across large, unvegetated expanses to other beds. Predictions from this model are that more fish will be found where seagrass is present, or, within a bed, where seagrass is denser. The observations leading to points 2 and 3 above, however, may also be consistent with the alternative model in which fish simply swim until they find food, pause to eat it, then swim again. Under this model, referred to below as the simple feeding model, abundances of small fish are predicted to match prey availability.

Alternatives to the two models, habitat selection and simple feeding, described above are (as listed by Lewis (1984), apropos of invertebrates): 1) the presence of physical structure usable as living space, 2) dampened hydrodynamic forces, 3) increased number of microhabitats, and 4) greater stabilisation and deposition of sediment.

This thesis aims to determine the importance of shallow eelgrass meadows to small fish, in particular to juvenile *Sillaginodes punctata*. The work focuses on the two models, habitat selection and simple feeding, although other possible explanations for the importance of seagrass to fish are discussed where results bear upon these. The underlying rationale for the work is the loss of and continuing threat to shallow seagrass habitat around the world (Walker & McComb 1992).

Chapter 2

Comparison of fish catches from a buoyant pop net and a beach seine net

2.1 Introduction

Most ecological studies of seagrass fauna include estimates of fish densities. Methods used to count fish include netting, poisoning and visual surveys. The composition of fish assemblages reported depends on the method of collection. Assemblages collected from the same meadow using two different methods (poisoning and trawling) can be more different than assemblages collected from different meadows with the same method (Pollard 1984, Gray & Bell 1986). Even the catches from different types of beam trawl from within one seagrass meadow vary in number of species and number of individuals (McNeill & Bell 1992). Visual surveys can be used in seagrass habitats where, for conservation reasons, trawling is not permitted; these two methods give quite different results, however, when used in the same place (Harmelin-Vivien & Francour 1992).

A small, fine-mesh beach seine was used to collect fish in Barker Inlet (Chapter 3). Data from seine netting is more informative when an estimate can be made of the catching efficiency of the net (Parsley *et al.* 1989), and to do this, a buoyant pop net was designed with the aim of making a more complete catch. The seine net is also unwieldy when fish need to be collected from small, defined areas. The pop net permits collection of fish from such areas, for example from experimental plots in which habitat has been manipulated (Chapter 6).

Buoyant pop nets usually consist of four mesh walls, depressed prior to release, and a mesh floor. Such traps can be lifted clear of the water to collect ensnared fish. Small,

floorless pop nets (area 5.6 m²) have been used in vegetated backwaters of a river (Dewey *et al.* 1989) while a larger net (14.5 m²) was tested for estimating fish abundances associated with artificial structures in lakes (Larson *et al.* 1986). In the present study, a floorless pop net was designed with the same intention as the small, floorless net of Dewey *et al.* (1989); viz., to avoid the problem that any floor of mesh fine enough to catch the target fish (10 - 100 mm length in the present study) would alter the nature of the sea bed and might disrupt the feeding behaviour of benthic fish. The pop net is an alternative to the floorless lift net designed to collect fish from littoral marshes (Rozas 1992). The pop net design nets four times the area of the lift net and avoids the need for above-ground structures used to raise the net. This absence of above-ground structures may be especially important in more open, less densely vegetated habitats.

This chapter compares fish catches from the pop net and seine net in shallow eelgrass meadows to determine the relative catching efficiencies of the two methods.

The contents of this chapter are substantially the same as those in Connolly (1994b) entitled "Comparison of fish catches from a buoyant pop net and a beach seine net in a shallow seagrass habitat", which is included as Appendix A.1.

2.2 Materials and Methods

The experiment was done in November 1991 on Torrens Island in the Barker Inlet - Port River estuary, South Australia (see description and map in Chapter 3). Netting was done in eelgrass (*Zostera muelleri* Irsmisch ex Aschers.) beds during the day on an incoming tide, at water depths between 40 - 100 cm. Sites were positioned at random along a 1 km stretch of coast and at each site both a pop and seine net were used simultaneously within 40 m of one another. The order of netting was chosen randomly. The aim was to

compare the catch of the two methods. The assumption was made that with this experimental design, different catch rates would reflect different catching efficiencies of the net types.

The seine net used was 5 m long by 2 m high, of 1.4 mm diameter fibreglass mesh, and was weighted along the bottom with floats at the top. The net was pulled by two people, one at either end, for (a pre-measured) 20 m; the actual area netted was calculated over ten pulls to be 84 m² (s.e. 1.19).

The pop net consisted of four walls of the same material and mesh size used in the seine net, and was 5 m long by 1.4 m high (Fig. 2.1). The top of the mesh was sewn around lengths of 25 mm diameter PVC pipe, sealed at the ends for buoyancy. The bottom was weighted, and also pegged to the sea bed. The net was set at low tide when the sea bed was exposed. The netting was folded and sandwiched between the sea bed and the PVC pipe, which was pushed down so as to make the whole apparatus as nearly as possible flush with the sea bed. The top of the net was weighted with eight flat concrete blocks of 10 kg each, so that when the net was covered with water it did not move until released. Each concrete block was attached by wire along the sea bed to one of two remote points, at least 10 m from the net. The net remained in place for one day, being released on the following day's incoming tide. At release, one person moved slowly to each of the two remote points, and all blocks were simultaneously pulled away from the net. The top of the net surfaced within two seconds of release in water up to 1 m deep. The area netted was 25 m².

A solid-framed collecting net (Fig. 2.1), of the same mesh, was used to collect fish from within the pop net. The collecting net, which fits neatly inside the pop net, was pulled three times through the area with the pop net walls being held against the ends of the collecting net at all times. Fish were collected separately from each pull. This method removes fish immediately after net release, unlike the method of Rozas (1992) in which

fish are collected in a pit on the receding tide. Although predation within nets was not reported as a problem in the densely vegetated marshes in which Rozas (1992) worked, predation by decapods, birds or other fish could result in losses on open eelgrass flats, and collecting fish immediately avoids this potential problem. The pursing together of the mesh sides to collect fish (Dewey *et al.* 1989), whilst useful for small nets, is not manageable over 25 m^2.

All fish were identified and the first 50 of each species from each pull of the seine net or the collecting net were measured. Lengths of individuals of common species were compared from the two net types by calculating a mean length for individuals within a net and testing differences over all nets using a paired *t*-test.

Recovery efficiency within pop nets was estimated using the method of Kneib (1991). Numbers from the three pulls of the collecting net tended to describe an exponential decay function, and a linear regression through the three points (using $\log_{10}$ transformed values of number of fish and number of pulls) predicted the number of pulls needed to remove all fish from the pop net. The total number of fish within the pop net was estimated by summing the predicted number of fish from all pulls. Efficiency was calculated as the proportion of the estimated total number of fish actually caught in each of the three pulls, counted cumulatively. For total fish numbers and *Favonigobius lateralis* (Macleay) (long-finned goby) numbers this procedure was done separately for each pop net. Individuals of *Sillaginodes punctata* (King George whiting) and *Atherinosoma microstoma* Günther (small-mouthed hardyhead) were uncommon in the second and third pulls of collecting nets, and Kneib's (1991) method was used on data averaged over all pop nets. The precision of the recovery efficiency value could therefore not be estimated for these two species. When the method was used on total fish and *F. lateralis* numbers summed over all pop nets, however, estimates of recovery efficiency were similar to the means of values from separate pop nets. This suggests that the recovery efficiencies estimated for *S. punctata* and *A. microstoma* are reliable. No

A. microstoma were caught in the third pull of collecting nets in any pop net, so the regression for this species was calculated on $\log_{10}(x + 0.1)$ data. This procedure would tend to underestimate recovery efficiency.

Prior to the main experiment, four paired nettings were done on the same stretch of coast. Results (total fish numbers per m²) were as follows: pop net - mean 3.80; seine net - mean 1.70; differences (within pairs) - mean 2.10, standard deviation 0.80. The estimate of the variability (standard deviation) of differences between pop net and seine net catches was used to estimate the sample size required to attain desired probabilities of type I and II statistical errors for a specified effect size. I wanted a good chance of detecting a difference when seine net catches differed from pop net catches by more than 20%. The consequences of the two error types were considered equally serious, implying $\alpha = \beta$. Taking into account the cost and effort involved in using pop nets and the high variability of fish densities, I believe that $\alpha = \beta = 0.05$ is appropriate. For an effect size of 0.76 fish/m² (i.e., 20% of 3.80, the mean catch of the pop net, above), using Equation 8.8 in Zar (1984) for a paired *t*-test, the required number of pairs is 17 or more.

Seventeen pairs were therefore used in the main comparison. After checking the normality of the distribution of differences using Lilliefors' (1967) test, data were analysed using a paired *t*-test. Although it was considered unlikely that the catch rate of the pop net would be lower than that of the seine net, a two-tailed test was used so as not to preclude the possibility of testing the significance of any departure in that direction.

To determine why the effectiveness of the two net types might be different, underwater observations were made of fish behaviour before and after pop net release and from behind the seine net during seine netting.

2.3 Results

A total of 4991 fish of 15 species were counted during the study (Table 2.1). The number of fish caught in pop nets was significantly greater than that from seine nets (Table 2.2). Analysis of the more common species shows that the higher number of fish in pop nets reflects catches of the most common species, *Favonigobius lateralis*, which was caught much more often in pop nets (Table 2.2). Numbers of *Sillaginodes punctata* and *Atherinosoma microstoma* from the two net types were not shown to be different. In the case of these two species, the statistical power of the tests was low. The chance of detecting a difference in seine and pop net catches of $\geq 20\%$ of the mean pop net catch was 12% for *S. punctata* and 7% for *A. microstoma*.

Numbers of *Spratelloides robustus* Ogilby (blue sprat) provide no meaningful comparison of net types because all fish were caught in one seine pull. A total of only ten *Arenigobius bifrenatus* Kner. (bridled goby) were caught during the study, but all of these were from pop nets, with individuals found at four different sites. The lack of any *A. bifrenatus* in seine catches, despite the area netted by seine nets being more than three times that netted by pop nets, is notable.

No significant differences were found in the lengths of fish from pop and seine nets for any of the three common species, *S. punctata*, *F. lateralis* and *A. microstoma* (Table 2.3). Numerous spat of *A. microstoma* (length 7 - 20 mm) were collected in several pop nets but were rarer in seine nets.

The proportion of fish in pop nets caught by each successive pull of the collecting net declined rapidly for all species combined and for the common species (Table 2.4). Note, however, that the decline in the proportion of *F. lateralis* caught in successive nets was less steep than for *S. punctata* and *A. microstoma*. Recovery efficiencies were very high for *S. punctata* and *A. microstoma*, with virtually all *S. punctata* and all *A. microstoma*

recovered by the third pull. Recovery efficiencies for *F. lateralis*, and since this was the most abundant species therefore also for all species combined, were lower (Table 2.4).

Underwater observations of pop nets gave no evidence of fish being more common around the net or blocks prior to release. *F. lateralis* and juvenile *S. punctata* usually swam alone but sometimes showed weak schooling behaviour. *F. lateralis* always remained on the sea bed, while juvenile *S. punctata* tended to swim just over the top of the eelgrass canopy. *A. microstoma* schooled strongly and swam near the water surface.

After net release, but prior to use of the collecting net, individuals of the above three species behaved as if they were not aware of being trapped, and swam within the 25 m² confine. Larger fish, such as adult *Torquigener glaber* (Freminville) (smooth toadfish, up to 70 mm long), >1 year old *Aldrichetta forsteri* (Valenciennes) (yelloweye mullet, to 80 mm), and > 1 year old *Hyporhamphus melanochir* (Valenciennes) (sea garfish, to 130 mm) were uncommon, but sometimes swam around the edge of the enclosure.

When trapped in a small volume of water at the end of a collecting net pull, *Sillaginodes punctata* and *Atherinosoma microstoma* were observed swimming near the surface, whereas *Favonigobius lateralis* remained near the sea bed.

Observers of seine nets saw some larger, fast swimming fish (unidentified) swim out of the ends of the net. Smaller schooling fish such as *A. microstoma* sometimes swam along in front of the net, but were caught as the net-pullers moved together at the end of a run, closing off any escape. Observations of fish in vegetated habitats in shallow water are, of course, not quantitative, but no fish were seen escaping under the net.

2.4 Discussion

The pop net caught more fish than the seine net, mainly because nine times more *Favonigobius lateralis* were caught in the pop net. *F. lateralis* individuals remained on the sea bed at all times, and not only avoided capture by the seine but also had a lower recovery efficiency within the pop net than other common species. Although no *F. lateralis* were seen escaping under the seine, this is the most likely place of escape, as small fish could fit into the shallow depressions in the sea bed over which the bottom rope of the seine would pass. Observations of the seine being used in meadows of seagrass species having a more erect habit (e.g. *Posidonia australis* Hook.) have clearly shown that small, bottom-dwelling fish do escape under the net, which glides through the seagrass but not actually along the sea bed. During the testing of a beach seine net in a reservoir, fish species known to be associated with the bottom escaped more often over coarse sediment, presumably by getting under the net via irregularities in the bottom (Parsley *et al.* 1989). The possibility remains that *F. lateralis* is actually attracted to the pop net area, perhaps towards extra feeding opportunities provided by the disturbance of sediment whilst setting the net, violating the assumption that approximately equal numbers of fish would be in the vicinity of both net types. However, the evidence against this possibility is that observers did not detect any increased abundance of *F. lateralis* near the net prior to release, and that *F. lateralis*, even when trapped within the pop net, avoided the collecting net more often than the other common species. *Arenigobius bifrenatus*, which is also intimately associated with the sea bed and at times even burrows in the sediment, also seemed to avoid capture by the seine net.

Sillaginodes punctata, which swims mid water, seems to be caught equally well by both net types. Furthermore, most of the individuals of this species were caught in the first pull of the collecting net within the pop net. The numbers of *Atherinosoma microstoma*, which swims near the water surface, were also not shown to be different from the two net types, and very nearly all of the individuals of this species were caught in the first

pull of the collecting net. Although no significant difference was shown in the mean length of *A. microstoma* from pop and seine nets, observations indicate that the seine net may catch *A. microstoma* spat less effectively than the pop net.

For general survey work in shallow embayments the seine net, which is much faster and cheaper to use than the pop net, can be considered a relatively accurate method for collecting small fish other than species that remain intimately associated with the sea bed (and possibly also spat less than 20 mm long). The seine net would also be useful for comparing numbers of a single species, such as *Sillaginodes punctata*, from different locations.

Most areas of eelgrass in South Australia are emergent or nearly so at low tide, permitting use of the pop net. Where there is particular interest in species that are not well caught by the seine, or where accurate collection of fish from relatively small, defined areas is required, the pop net described in this chapter is a useful new design. The pop net, as presented here, would also be useful subtidally for the collection of fish species that remain within the seagrass canopy when disturbed. The relatively unsophisticated release mechanism was designed to be robust in fast flowing water which sometimes carries large quantities of drift algae. It also overcomes most of the problems caused by anglers (Larson *et al.* 1986), and during this study all net releases were successful. The range of recovery efficiencies within pop nets from 65 % for *Favonigobius lateralis* to 100% for *Atherinosoma microstoma* approximately matches that for fish in Rozas' (1992) lift net. The main advantage of the pop net presented here is that it collects fish from larger areas than most other designs without losing portability. Kneib's (1991) flume weir collects fish reliably from an even larger area (100 m²) but is best used for repeated sampling of the same site. The method of fish retrieval from the pop net worked well in eelgrass, but would be less effective in taller, more robust vegetation, where the collecting pits of Rozas (1992) would be more effective.

Table 2.1 Number of individuals of main species caught during study

Pop net and seine net combined. * all caught in one seine net.

	Number of fish	% of total
Favonigobius lateralis	3209	64.3
Sillaginodes punctata	1233	24.7
Atherinosoma microstoma	217	4.4
Spratelloides robustus	167*	3.3
Other species	165	3.3
All species total	4991	100

Table 2.2 Comparisons of pop net and seine net catches

Numbers in first 3 columns are means of 17 sites (fish/m²). For differences, pop net catch is greater than seine net except where difference is negative. 17 pairs used in *t*-tests.

	Pop net	Seine net	Paired differences	Difference as % of pop	Probability from *t*-test
All species	6.318	1.615	4.703	74	< 0.001
Favonigobius lateralis	5.414	0.636	4.778	88	< 0.001
Sillaginodes punctata	0.584	0.690	-0.106	- 18	0.462
Atherinosoma microstoma	0.125	0.080	0.045	36	0.423

Table 2.3 Comparison of lengths of fish from pop net and seine net

Numbers are means (in mm) from all nets of the mean length of fish within a net.
Differences were tested with a paired *t*-test, using only pairs of nets in which at least one
individual of the species was caught in both net types (this figure in column "Number of
pairs").

	Pop net	Seine net	Number of pairs	Probability from *t*-test
Favonigobius lateralis	28.58	29.54	17	0.109
Sillaginodes punctata	30.56	30.55	16	0.993
Atherinosoma microstoma	32.85	41.64	12	0.178

Table 2.4 Comparisons of catches from three pulls of collecting net in pop nets

E% = recovery efficiency, calculated as described in text (numbers are means, with standard errors in parentheses; no estimates of precision are possible for *S. punctata* and *A. microstoma*). C% = percentage of total pop net catch caught in collecting net (calculated as % of total catch of individual pop net; means shown).

	Pull 1		Pull 2		Pull 3	
	E%	C%	E%	C%	E%	C%
All species	50.1 (7.6)	61.1	61.8 (7.9)	24.9	67.8 (7.6)	14.0
Favonigobius lateralis	50.0 (9.0)	56.8	60.3 (9.2)	27.4	65.3 (9.0)	15.8
Sillaginodes punctata	83.3	88.6	95.2	5.9	98.8	5.5
Atherinosoma microstoma	99.1	97.9	100	2.1	100	0

Fig. 2.1 Pop net design: a) Prior to release (diagram is representative only -- actual number of blocks used was eight, four from each of the two stakes, which were situated diagonally to the net so that all sides received two blocks), b) After release, with collecting net

A Net set (tide out)

stake

Wires along sea bed

5m

10m

B Net released (tide in)

collecting net

buoyant pipe attached to mesh

collecting net lowered into pop net

water surface

pop net

pegged at bottom
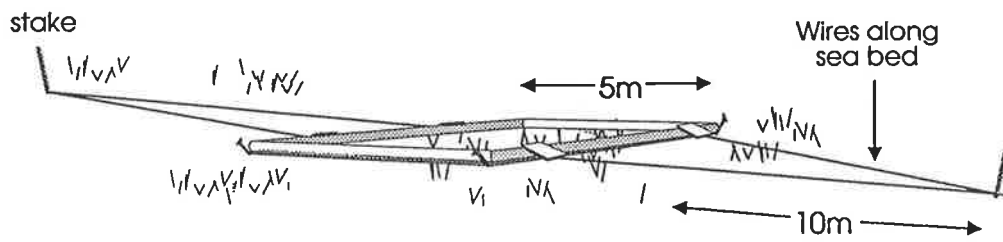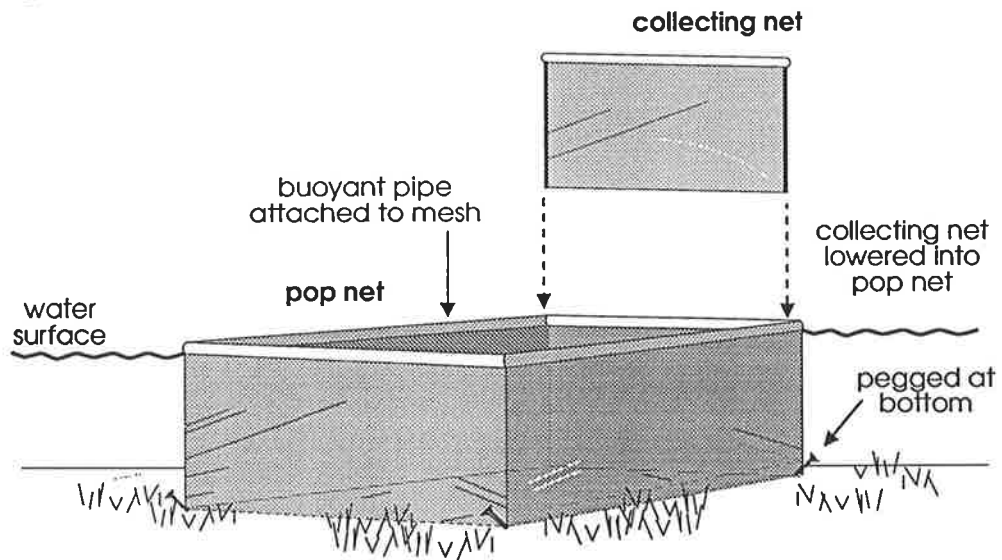
Chapter 3

Comparison of fish assemblages from seagrass and unvegetated areas

3.1 Introduction

Seagrass meadows in many parts of the world support large numbers of juvenile fish and provide a nursery habitat for many commercially important species (Pollard 1984). Unvegetated areas adjacent to seagrass meadows have different fish assemblages, usually with fewer fish and fewer species (Bell & Pollard 1989). Assemblages associated with seagrass consist mainly of small, inconspicuous species and juveniles of larger species, whereas the fauna of unvegetated areas is characterised by adults of large, mobile fish and species protected by schooling behaviour or camouflage against sediments (Bell & Pollard 1989).

The Barker Inlet/Port River region is a sheltered, marine-dominated estuary comprising extensive intertidal areas with either eelgrass (*Zostera, Heterozostera*) cover or no vegetation. The estuary has very high abundances of juveniles of commercially important fish species (Jones 1984) and mainly for this reason has been declared an aquatic reserve.

The estuary is almost surrounded by the city of Adelaide (population > one million people), and is consequently subjected to many types of pollution and human impacts, viz. : treated sewage, stormwater, agricultural and horticultural run-off, spilt oil, thermal effluent, shipping, altered flow regimes and fishing. Further extensive urban development along the foreshore is planned (OPUD 1992). Toxic dinoflagellate blooms (Hallegraeff *et al.* 1988), loss of mangroves (*Avicennia marina* (Forsk.)) to clearing (Talbot 1982), and build-up of the macroalga *Ulva* sp. (Connolly 1986) have all been recorded, but changes in eelgrass cover within the estuary are not documented. Since

the installation of Adelaide's main sewage outfall just north of the Barker Inlet/Port River estuary, six hundred hectares of intertidal eelgrass have been lost adjacent to the outfall (Shepherd *et al.* 1989).

Within the estuary itself, various sized patches with and without eelgrass occur. Although the factors contributing to the presence or absence of eelgrass are unknown, it is very likely that eelgrass cover has been and will continue to be altered by human activities. The aim of this survey was to compare assemblages of small fish from eelgrass and unvegetated areas as a preliminary step in determining the importance of eelgrass to small fish.

The contents of this chapter are substantially the same as those of Connolly (1994c) entitled "A comparison of fish assemblages from seagrass and unvegetated areas of a southern Australian estuary", which is included as Appendix A.2. Slightly more emphasis is given here than in the paper to the patterns of size and abundance of *S. punctata* because this species is the focus of experiments described in later chapters.

3.2 Materials and Methods

Site selection

Unvegetated and eelgrass areas (mostly *Zostera muelleri*, occasionally *Heterozostera tasmanica* (Martens ex Aschers.)) of the Barker Inlet - Port River region (138° 30' E, 34° 45' S) were mapped in January 1990 (Fig. 3.1). Excluding the region south of Inner Beacon (because it is grossly affected by warm water effluent from a power station (Jones *et al.* unpubl. MS)), there were seven eelgrass and eight unvegetated areas. At each of five sampling periods between January 1990 and February 1991, 20 sites were selected for sampling, ten in each habitat. In some sampling periods, the actual number

of sites sampled was less than the 20 selected because of the limited time of correct tidal height to sample (see sample sizes in Table 3.2).

The spatial distribution of areas of the two habitats was predetermined by the state of the estuary. There was, however, some interspersion of habitats, so that the situation of having all or most of one type of habitat at one end of the estuary, for example, was avoided. Within the patches of habitat, choice of sites was random subject to the restrictions that 1) each of the designated areas of both habitats received at least one sample and 2) within a patch of habitat, no two sampling sites were less than 200 m apart. The order in which sites were sampled was randomised, with the proviso that at least two sites of each habitat were sampled on each day. Where sites were covered with the macroalga *Ulva* sp., sampling was abandoned.

The above-ground biomass was measured at each eelgrass site in January 1990 only. At each site, three squares of 625 cm² were harvested and dried at 60° C for two days. The mean above-ground biomass for all eelgrass sites was 146 g dry weight/ m² (s.e. = 20.7, n = 9 sites).

Fish Sampling

Fish were sampled at all sites using two different sized beach seine nets which were operated sequentially, 30 m apart, pulled perpendicular to and towards the shore for (a pre-measured) 20 m. The small net was 5 m long with mesh size 1.4 mm (as described in Chapter 2), and the large net was 22 m long with mesh size 6 mm. The actual area netted was calculated over 10 pulls to be 84 m² (s.e. 1.19) for the small net and 347 m² (s.e. 7.80) for the large net. All fish were identified and counted, and *Sillaginodes punctata* were also measured. Numbers from the two nets were combined, as both nets together constitute the sampling unit.

All netting was done at or just after the daytime low tide. All sites could not be sampled on one date, since it was important to take all nettings at a similar tidal state. Sampling was therefore spread over four consecutive days chosen for similarity of tides. The actual day on which sites were sampled was randomised, and all samples were treated as temporally equivalent. That is, variation between days within sampling periods was not analysed.

In June and October 1991, the small seine net was used to catch *S. punctata* as part of a dietary study (Chapter 4). The same sampling procedure was followed as described above, and the number of sites is shown in Section 3.3. At these two periods, a limited amount of sampling was also done at night. The results from these nettings are reported so as to strengthen the seasonal pattern of size of *S. punctata* juveniles and as an indication of diel activity. This sampling was done at or just after low tide between 2300 and 0100 hrs.

The comparison of fish catches using the small seine net with catches using the pop net (Chapter 2) showed that the former probably underestimated abundances of species such as *F. lateralis* that remain in contact with the sediment when disturbed. Numbers of *F. lateralis* in the seine were only one ninth of those in the pop net. The comparison of netting methods was done in eelgrass, but not in unvegetated areas. Results from an experiment, however, in which fish numbers were compared from, *inter alia*, eelgrass and unvegetated habitat using the pop net (Chapter 6) provide some evidence that the seine net correctly represents the *relative* abundance of fish from the two habitats. The same patterns of fish abundance from eelgrass and unvegetated habitat shown in the present chapter for *S. punctata* and *F. lateralis* were found using the pop net.

Water temperature and salinity

Water salinity and especially temperature (given the warm water discharges in the south of the estuary) could influence fish distributions and could also be associated with the presence of eelgrass. Water temperature was measured at each site at the time of netting, at 30 cm depth in water 60 cm deep. Water samples for salinity analysis were taken at the same time and place. The Practical Salinity Scale of 1978 (PSS 78) has been used.

Data Analysis

Comparisons between unvegetated and eelgrass areas of total abundances and abundances of the key species are straightforward applications of the Mann-Whitney U-test. The number of fish at eelgrass sites in January 1990 was tested for association with the above-ground biomass estimate of each site using Spearman's rank test. A comparison of assemblages (all species together) from eelgrass and unvegetated sites suggests a multivariate analysis of variance (MANOVA). The assumption of multivariate normality, however, is likely to be grossly violated by present data, the fish samples being characterised by small numbers with many zeros. A non-parametric analogue with no assumption of normality is the analysis of similarities (ANOSIM), which has the added advantage over MANOVA of being able to detect differences between groups without any need for assumptions of constant spread within each group (Clarke 1993). ANOSIM compares ranked similarities between and within groups selected *a priori* (here eelgrass and unvegetated habitats) using a randomisation test for significance. At each sampling period, assemblages from the two waterways, Barker Inlet and Port River, were also compared using a two-way crossed ANOSIM with habitat (eelgrass or unvegetated) as the second factor. This analysis determines whether assemblages from the two waterways differ after accounting for habitat differences. All

ANOSIM tests involved 5000 simulations using the PRIMER package from Plymouth Marine Laboratory, U.K.

The relationships amongst assemblages from each site are graphically represented using non-metric multidimensional scaling (MDS), which is an ordination technique that uses the same matrix of ranked similarities as ANOSIM. MDS displays samples in low (usually two) dimensional space while retaining as nearly as possible the similarity rankings between samples.

For comparisons of fish assemblages between unvegetated and eelgrass sites, raw counts were transformed using $x^{0.25}$ to emphasise the distribution of less common species in the analysis. The transformation $x^{0.25}$ gives slightly more emphasis to less common species than log x in cases such as this where counts are small (Clarke 1993). The Bray-Curtis similarity coefficient is used throughout, as a meaningful and robust measure (Clarke 1993). Analysis of the similarity matrix used in MDS and ANOSIM has also been used to highlight the species making the largest contribution to between-group differences (Clarke 1993).

The association of environmental variables with patterns in biotic data can be measured by correlating the ranked similarity matrices of the environmental and biotic data (Clarke & Ainsworth 1993). At each sampling period, the association between fish assemblages and the environmental variables 1) water temperature, 2) salinity, and 3) distance to open water were measured using the weighted Spearman's coefficient recommended by Clarke & Ainsworth (1993). Distance to open water was measured as the shortest distance by sea from sites to gulf waters unprotected by islands or shoals. Distances ranged from 1.0 to 9.1 km.

All comparisons (univariate and multivariate) have been done for each period separately, because the fauna changes over time within both habitats as juveniles of larger species

move to deeper water or grow too large to be caught by the nets. An MDS ordination has also been performed on data from all periods combined, and a two-way crossed ANOSIM was used to test differences between periods and habitats.

3.3 Results

Number of Species

A total of 36 species were caught during the survey. More species were caught at eelgrass sites than unvegetated sites at all sampling dates (Fig. 3.2).

Number of individuals

A total of 13871 fish were caught, 9866 (71%) from eelgrass sites and 4005 (29%) from unvegetated sites.

The number of individuals caught at eelgrass and unvegetated sites is shown for the ten most common species from both eelgrass and unvegetated sites in Table 3.1. Comparisons of catches at eelgrass and unvegetated sites are shown (Fig. 3.3) as mean number per site (small and large net combined), separately at each sampling date, for a) total catch (all species), b) *Sillaginodes punctata* c) *Atherinosoma microstoma* and d) all species except *A. microstoma* and *Spratelloides robustus*. Results of Mann-Whitney U-tests for differences between catches from eelgrass and unvegetated sites are listed in Table 3.2 for all dates.

There were more fish at eelgrass sites than at unvegetated sites at all dates (Fig. 3.3 a), and only at August 1990 was the difference not significant (Table 3.2). In February 1991 the mean numbers of fish at both eelgrass and unvegetated sites were very high because

of an extraordinarily large number of *Pelates octolineatus* (Jenyns) (striped perch) at eelgrass sites and *A. microstoma* at eelgrass and unvegetated sites. The above-ground biomass of eelgrass at eelgrass sites was not correlated with total fish numbers in January 1990 (Spearman's rank test: p > 0.1).

Sillaginodes punctata

The number of *S. punctata* at eelgrass sites was significantly greater than at unvegetated sites at all of the main sampling dates (Table 3.2, Fig. 3.3 b). More fish were caught over eelgrass than unvegetated habitat in June 1991 (eelgrass: mean = 10.0 fish, n = 6 sites; unvegetated: mean = 1.0, n = 7), although this difference was not significant when analysed with a Mann-Whitney U-test of medians ($p = 0.141$). Significantly more fish were caught over eelgrass in October 1991 (eelgrass: mean = 17.4 fish, n = 9 sites; unvegetated: mean = 2.9, n = 8; Mann-Whitney U-test of medians: $p = 0.017$). Nearly all fish were caught in large nets in the first half of 1990 and 1991, and in small nets in the second half of 1990. This highlights not only the different size selectivity of the two nets used, but also, in conjunction with length frequency data of *S. punctata* (Fig. 3.4), shows the growth of year classes. *S. punctata* spawns in April in South Australia (Jones *et al.* 1990), is first caught in the estuary about mid-year, and after another year is too large to be caught by the nets used in this study.

At night in June 1991 there were significantly more *S. punctata* per site over eelgrass (mean = 6.8 fish, n = 9 sites) than over unvegetated areas (mean = 0.8 fish, n = 5 sites) (Mann-Whitney U-test of medians: $p = 0.03$). At night in October 1991, one *S. punctata* was caught over unvegetated habitat (n = 1 site) compared with 28 from eelgrass sites (n = 3 sites).

Median lengths of *S. punctata* from eelgrass and unvegetated sites have been compared at dates where enough fish were caught at unvegetated sites to permit a reasonable

comparison (Table 3.3). Even at these dates sample sizes were much larger at eelgrass sites, however the variance and degree of skewness and kurtosis were similar across habitats, so test results can be regarded, cautiously, as meaningful. *S. punctata* were longer in the unvegetated habitat at all dates, significantly so at January 1990 and February and June 1991.

Atherinosoma microstoma

A. microstoma was the most abundant species at both eelgrass and unvegetated sites (all dates combined). Only at two dates were significantly more caught in eelgrass, while similar numbers were caught at eelgrass and unvegetated sites on three dates (Table 3.2 and Fig. 3.3 c).

All species except *A. microstoma* and *Spratelloides robustus*

If *A. microstoma*, which is numerically dominant and at some dates does not show a strong pattern of greater abundance over eelgrass, is excluded and the numbers of all other species are combined, then numbers are greater at eelgrass sites than at unvegetated sites at all dates (Table 3.2, Fig. 3.3 d). *Spratelloides robustus* occurred infrequently but was also excluded because it is similar in size and behaviour to *A. microstoma*.

Stigmatopora nigra Kaup (wide-bodied pipefish)

166 individuals of *S. nigra* were caught over the survey period. 13 of these were caught in the large net and can be excluded because this species can swim easily through the mesh of the large net and is only caught when it is entrapped by algal fronds. Of the 155 fish caught in small nets, 153 were over eelgrass and two were over the unvegetated habitat.

Rhombosolea tapirina Güenther (greenback flounder)

This species, of which only juveniles were caught, was found mainly at unvegetated sites. They are effectively caught in both small and large nets, and of the total of 53 individuals taken over the survey period, 12 (23%) were from eelgrass sites and 41 (77%) were from unvegetated sites.

Multivariate analysis

The differences between eelgrass and unvegetated sites in species richness and abundances of several common species suggest differences between assemblages of these habitats. Two-dimensional ordination plots show, at every period, strong grouping of eelgrass and unvegetated sites (Fig. 3.5). Assemblages of the two habitats were significantly different at all periods as judged by the ANOSIM results (Table 3.4). Of the species contributing most to differences in assemblages associated with the eelgrass and unvegetated habitats (Table 3.4), *Sillaginodes punctata* predominated. No differences were found at any period between assemblages of the two waterways, Barker Inlet and Port River.

Favonigobius lateralis was caught almost exclusively in the small net during the present survey. If the number of *F. lateralis* is multiplied by nine to correct for the underestimation of this species in small net catches (Chapter 2), ordination plots look the same and the resultant slight changes in ANOSIM results do not alter interpretation of statistical significance. However, *F. lateralis* is more prominent under this correction as a species discriminating between the two habitats.

Water temperature and salinity did not match the biotic data. At all periods, the distance from sites to open water was the only environmental variable having any importance in

matching the patterns in fish assemblages. Nor did any combination of the three environmental variables match the biotic data better than distance to open water alone. However, even distance to open water was only weakly matched, with correlations between matrices of fish assemblages and distance ranging from 0.10 to 0.34. There is currently no test for significance of these correlations but the values are low (Clarke & Ainsworth 1993). A simple overlay of distance to open water onto MDS plots of fish assemblages (Fig. 3.6) reveals that for all periods except August 1990 one site, with the smallest distance to open water, is far from any other site. In all cases, this site is from the unvegetated patch at the extreme north-western end of the sampling region. In August 1990, no fish were caught at the site in this unvegetated patch and the sample was therefore excluded from the MDS ordination. When the site is included, the correlation between similarity matrices of fish assemblages and distance to open water is 0.21. After excluding the site (as in the plots in Figs 3.5 & 3.6), the correlation drops to - 0.01. This evidence, taken together with the overlays at other periods, suggests that the weak correlation of distance to open water and fish assemblages is due mainly to this one unvegetated patch which is near open water and has a peculiar fish assemblage, persistent over time. This patch with greatest exposure to open water is characterised by having very low fish catches, always including at least one *Rhombosolea tapirina*. Any importance of distance to open water in determining fish assemblages seems to be in separating the patch most exposed to open water from other areas rather than causing a gradual change along the length of the estuary.

When all periods are combined, the distinction between eelgrass and unvegetated sites remains the overwhelming difference (Fig. 3.7). After accounting for differences between habitats, sampling periods are, however, also different (Two-way ANOSIM, factor "period", $p < 0.001$). Differences over time are due to either the sporadic occurrence of large numbers of one or two species or fluctuations in the number of individuals of species present in the estuary at all periods. The relative position of sites over time is different within the two habitats (Fig. 3.7). Within the unvegetated habitat,

periods are quite evenly spaced. Within the eelgrass habitat, January 1990, April 1990 and February 1991 are separated from August 1990 and October 1990. This is mainly the result of large catches of *Pelates octolineatus* at the first three dates.

Water temperature and salinity

Water temperatures and salinities differed among dates, with both showing marked seasonality. Mean temperatures and salinities were, however, very similar at eelgrass and unvegetated sites (Table 3.5). No significant differences were found in either temperature or salinity between the two habitats using Mann-Whitney U-tests.

3.4 Discussion

The fish assemblages of eelgrass and unvegetated areas were distinctly different, and these differences persisted over time. Differences between habitats were as found in other researchers' comparisons (Bell & Pollard 1989, and references therein). The eelgrass habitat was typified by the syngnathid *Stigmatopora nigra*, a small species with cryptic habit, and juveniles of *Sillaginodes punctata*, while *Rhombosolea tapirina*, a flounder with extreme modifications for camouflage against a sand/mud background, was found mostly in unvegetated areas. *Atherinosoma microstoma* is a small species that schools, and might have been expected to be mostly over unvegetated habitat. However, this fish feeds near the surface of the water, at least during the day, and has little to do with the sediment, vegetated or not; this may explain why large numbers of this species were caught over both habitats.

Changes in fish assemblages over the duration of the study were due to seasonal fluctuations in abundances of several species. The common fish of the estuary are either

permanent or temporary residents (see definitions in Bell & Pollard 1989). Species which are small as adults such as *A. microstoma*, *F. lateralis*, *S. nigra* and *Kaupus costatus* (Waite & Hale) are permanent residents. Seasonal fluctuations in abundance of these species result from short-term (often annual) recruitment and mortality. The temporary residents are larger species that recruit seasonally and move elsewhere later. *S. punctata*, for example, spawns offshore, and planktonic larvae settle to a benthic existence in the estuary from June to August. Fish move out of the estuary to deeper waters after two to three years (Jones *et al.* 1990). The changing size of species over time reflects the growth of these fish. In this study, the largest sized *S. punctata* captured was 140 mm (about 1 year old) in April 1990. Failure to catch larger fish in this study, however, can be attributed to the smallness of the two nets, rather than to the departure of larger *S. punctata* to deeper waters.

Different parts of an estuary can have different fish assemblages regardless of vegetation (Bell *et al.* 1988, McNeill *et al.* 1992), which could lead to spurious associations of faunas with vegetation types. Fortunately thorough interspersion of the two habitats was possible in this survey, limiting the likelihood that the different assemblages were simply a result of different locations. Differences in fish assemblages between eelgrass and unvegetated habitat can also depend on how far unvegetated sites are from eelgrass (Ferrell & Bell 1991). The differences shown in the present study are clear even though the distance of unvegetated sites from eelgrass varied widely. Distance to open water explained some of the pattern in assemblages, especially the distinctive assemblage of the most exposed patch. Since this patch was unvegetated, the comparison of habitats would have been influenced by the peculiarity of the assemblage there. However, the difference between eelgrass and unvegetated sites evident in the ordination plots was consistent across the estuary as a whole at all periods. No difference was detected between fish assemblages of the two waterways, Barker Inlet and Port River. This is despite Port River being a major shipping lane and having been modified by dredging, wharf building and reclamation of its shores. The fauna of Port River may not have been

greatly affected by human activities because eelgrass, which is a relatively fast growing, colonising plant, has persisted. The similarities of the fauna of the two waterways could also be taken to imply that both waterways have been affected by human activities.

Eelgrass presence was not associated with water temperature or salinity, so these factors are unlikely to be the cause of the different fish assemblages. Even secondarily to habitat differences, temperature and salinity were not associated with any faunal differences. This may simply be a result of the extent of the survey region, which included only relatively open, well-mixed waters. Temperatures in this study were similar to those measured by Jones *et al.* (unpubl. MS) outside the region influenced by hot water effluent entering the southern part of the estuary, and the comparison presented here should be considered representative only of the areas north of the southern limit to sampling shown in Fig. 3.1.

This comparison of habitats is a necessary step in confirming that eelgrass in the Barker Inlet/Port River estuary is important as habitat for small fish and in particular for juveniles of commercially important species. Absence of eelgrass may be correlated with one or more other factors, not measured in this survey, which cause the fish distributions reported here; experimentally manipulating eelgrass densities can distinguish between absence of eelgrass and other factors. Manipulative experiments have been done in the estuary to clarify the role of eelgrass as habitat for small fish (Chapter 6).

Of most significance to fisheries management, especially in Gulf St Vincent, is the close association of *Sillaginodes punctata* with eelgrass. *S. punctata* accounts for nearly half the value of inshore scalefish landings in South Australia (Anon. 1992). Robertson (1977) found that small *S. punctata* in Westernport, Victoria, used unvegetated areas at some times of the year; there is no evidence of that in the Barker Inlet/Port River region, at least during the day. Some species have very different distributions at night compared to day (Robertson 1980, Bell & Pollard 1989) but, apart from a small amount of data for June and October 1991, this study does not address that possibility. Sampling in June 1991 showed that *S. punctata* were over eelgrass more than unvegetated patches at night also. The greater median length of *S. punctata* at unvegetated sites at several times of the year raises the possibility of size-selective mortality, at one or more times of year. Many other alternative explanations exist, however, and this presents a line for further investigation.

Table 3.1 Number of individuals of the ten most common species (all dates combined) in eelgrass and unvegetated habitat

First ten species were most common at eelgrass sites. Final four species are those in the most common ten species at unvegetated sites not included in the first ten species. Numbers in parentheses are percentages of total number in that habitat.

Species name	Eelgrass	Eelgrass rank	Unvegetated	Unvegetated rank
Atherinosoma microstoma Small-mouthed hardyhead	4046 (41.0)	1	2333 (58.3)	1
Sillaginodes punctata King George whiting	2291 (23.2)	2	87 (2.2)	5
Pelates octolineatus Striped perch	1823 (18.5)	3	10 (0.2)	10
Favonigobius lateralis Long-finned goby	559 (5.7)	4	417 (10.4)	3
Aldrichetta forsteri Yelloweye mullet	435 (4.4)	5	909 (22.7)	2
Stigmatopora nigra Wide-bodied pipefish	166 (1.7)	6	2 (0.0)	14.5
Hyporhamphus melanochir Sea garfish	125 (1.3)	7	40 (1.0)	7
Kaupus costatus Deep-bodied pipefish	78 (0.8)	8	5 (0.1)	12.5
Haletta semifasciata Blue weedy whiting	58 (0.6)	9.5	0	9.5
Heteroclinus perspicillatus Common weedfish	58 (0.6)	9.5	0	9.5
Spratelloides robustus Blue sprat	3 (0.0)	25.5	100 (2.5)	4
Rhombosolea tapirina Greenback flounder	12 (0.1)	15.5	41 (1.0)	6
Sillago schomburgkii Yellowfin whiting	12 (0.1)	15.5	27 (0.7)	8
Arripis georgianus Tommy rough	2 (0.0)	27.5	12 (0.3)	9
Other species	198 (2.0)		22 (0.5)	
Total	9866		4005	

Table 3.2 Results of Mann-Whitney U-tests for differences in abundance between eelgrass and unvegetated sites

Results are probabilities: significance criterion = 0.05. Non-significant comparisons marked "ns".

Sampling period	Number of sites eelgr : unveg	All species	*Sillaginodes punctata* (King George whiting)	*Atherinosoma microstoma* (Hardyhead)	All species except *A.microstoma, S.robustus*
Jan 90	9:11	0.037	0.001	0.183 ns	0.004
Apr 90	10:10	0.003	0.001	0.041	0.019
Aug 90	8:7	0.298 ns	0.001	0.601 ns	0.015
Oct 90	8:7	0.003	0.008	0.024	0.005
Feb 91	10:9	0.050	0.019	0.205 ns	0.003

Table 3.3 Comparisons of median length of *Sillaginodes punctata* at eelgrass and unvegetated sites

Habitats: e = eelgrass, u = unvegetated. Mann-Whitney U-test probabilities: symbols as for Table 3.2.

Period	Habitat	Number of fish	Median length (mm)	Mann-Whitney U-test result
Jan 90	e	310	80	< 0.001 ***
	u	13	110	
Apr 90	e	210	110	not tested
	u	4	130	
Aug 90	e	202	24.5	not tested
	u	2	25.5	
Oct 90	e	221	32	0.060 ns
	u	19	35	
Feb 91	e	169	90	0.002 **
	u	19	110	
Jun 91	e	48	21.5	0.003 **
	u	7	24	
Oct 91	e	106	25	0.095 ns
	u	23	29	

Table 3.4 Multivariate comparisons of eelgrass and unvegetated assemblages

ANOSIM probabilities: all comparisons are significant at 0.05 level. Contributing species: two main species only. A.f = *Aldrichetta forsteri*, A.m = *Atherinosoma microstoma*, H.m = *Hyporhamphus melanochir*, P.s = *Pelates octolineatus*, S.n = *Stigmatopora nigra*, S.p = *Sillaginodes punctata*.

Sampling period	Probability from ANOSIM test	Main contributing species
Jan 90	< 0.001	S.p, A.m
Apr 90	< 0.001	S.p, H.m
Aug 90	< 0.001	S.n, S.p
Oct 90	0.008	S.p, A.f
Feb 91	< 0.001	S.p, P.s

Table 3.5 Water temperature and salinity at eelgrass and unvegetated sites separately at each sampling period

Habitats: e = eelgrass, u = unvegetated. Temperature measured in degrees Celsius. Salinity measured using the Practical Salinity Scale of 1978 (PSS 1978). Numbers are means with standard errors in parentheses.

Sampling period	Habitat	Temperature	Salinity
Jan 90	e	22.7 (0.46)	38.5 (0.22)
	u	22.8 (0.44)	38.3 (0.37)
Apr 90	e	21.9 (0.39)	38.1 (0.15)
	u	22.8 (0.40)	38.3 (0.18)
Aug 90	e	15.2 (0.61)	35.3 (0.18)
	u	14.6 (0.86)	35.3 (0.14)
Oct 90	e	19.1 (0.80)	36.1 (0.31)
	u	18.5 (0.63)	36.5 (0.19)
Feb 91	e	27.7 (0.40)	37.8 (0.16)
	u	27.4 (0.23)	38.0 (0.17)

Fig. 3.1 Map of survey region and habitat locations

a

AUSTRALIA

b

Survey area

Adelaide

Gulf
St Vincent

c

Beacon

Saint Kilda

Outer Beacon

Section Bank

Barker Inlet

Outer Harbour

Inner Beacon

Gulf
St Vincent

Torrens Island

Southern
Edge of
Survey
Area

Power
Station

Garden
Island

Angas Inlet

Port
River

North
Arm

Legend:

0   1
km

N

Shallow sand flat
Unvegetated

Land

Shallow sand flat with
eelgrass cover

Shallow flats: not
surveyed (including
mangrove forest and
samphire marsh)

Fig. 3.2 Total number of species from eelgrass and unvegetated habitats caught at each sampling period

Fig. 3.3 Mean number of fish per site from eelgrass and unvegetated habitats: (a) all species, (b) *Sillaginodes punctata*, (c) *Atherinosoma microstoma*, (d) all species except *A. microstoma* and *Spratelloides robustus*. Lines = standard error (of mean per site, small and large net combined)

(a)

Fish/site

350
300
250
200
150
100
50
0

360

Jan 90 Apr 90 Aug 90 Oct 90 Feb 91

(c)

140
120
100
80
60
40
20
0

183   151

Jan 90 Apr 90 Aug 90 Oct 90 Feb 91

(b)

Fish/site

70
60
50
40
30
20
10
0

85   78

Jan 90 Apr 90 Aug 90 Oct 90 Feb 91

Sampling Period

(d)

250
225
200
175
150
125
100
75
50
25
0

254

Jan 90 Apr 90 Aug 90 Oct 90 Feb 91
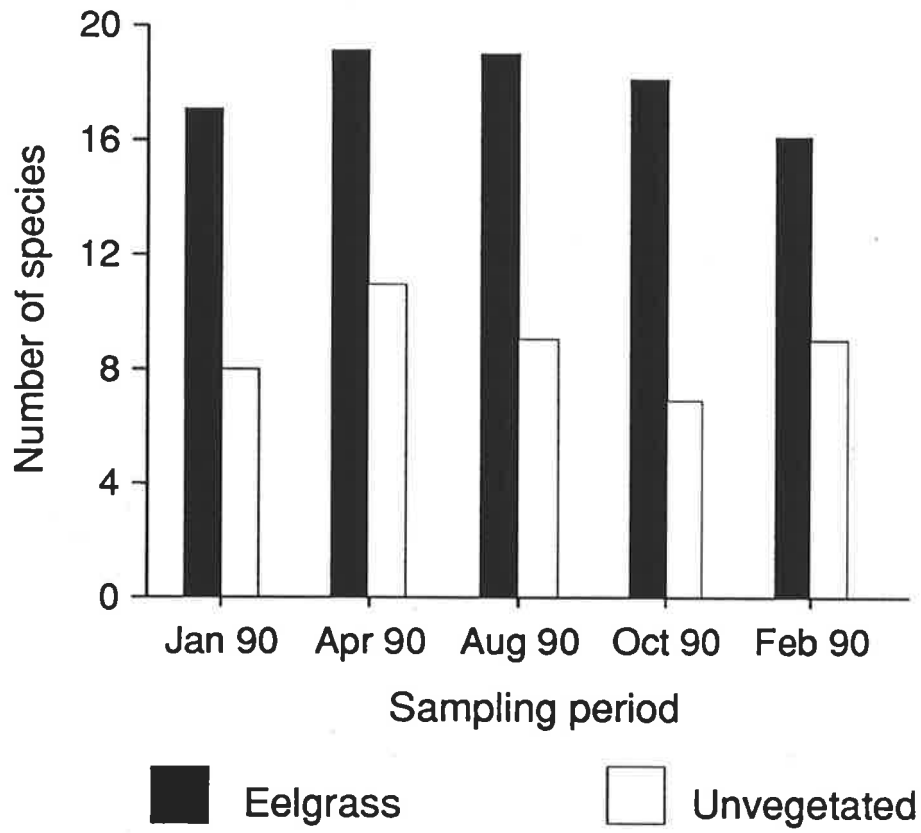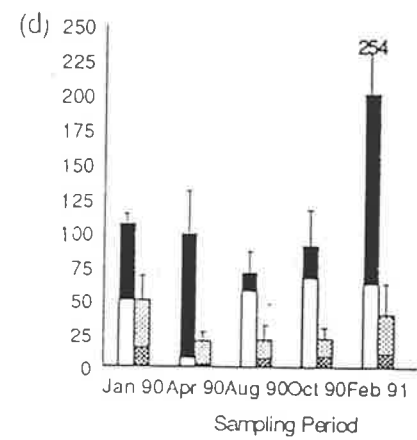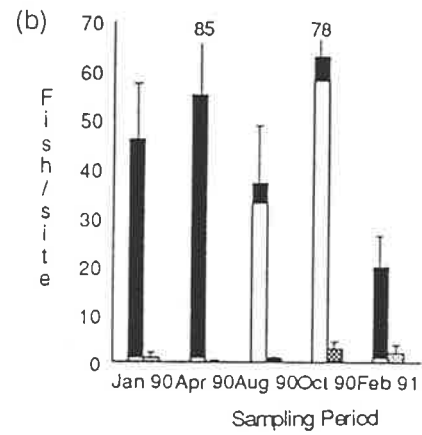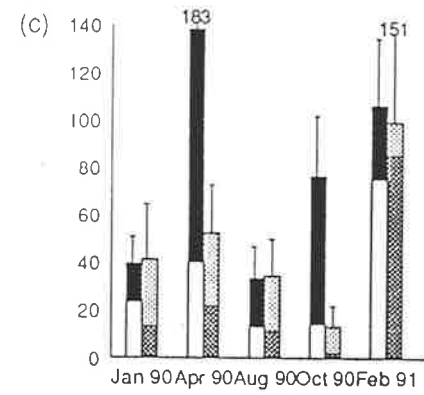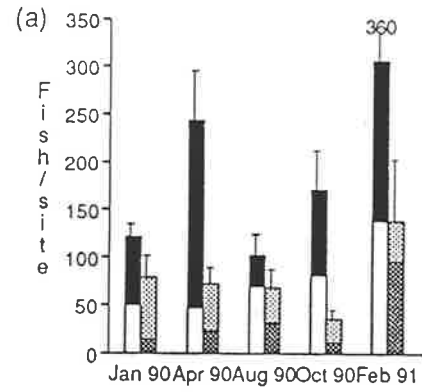
Sampling Period

☐ Eelgrass- small seine
■ Eelgrass- large seine

▨ Unvegetated- small seine
▨ Unvegetated- large seine

Fig. 3.4 Length - frequency of *Sillaginodes punctata* at each sampling period

Frequency histograms by length (mm) for January 90, February 91, April 90, June 91, August 90, October 91, and October 90.
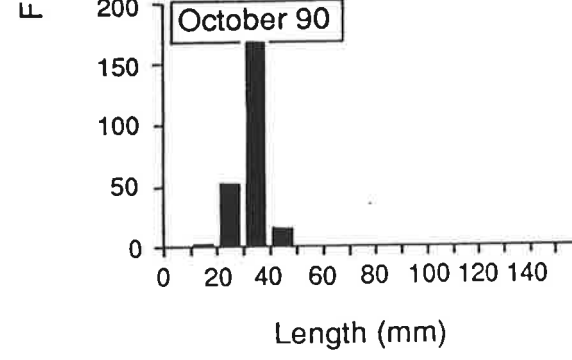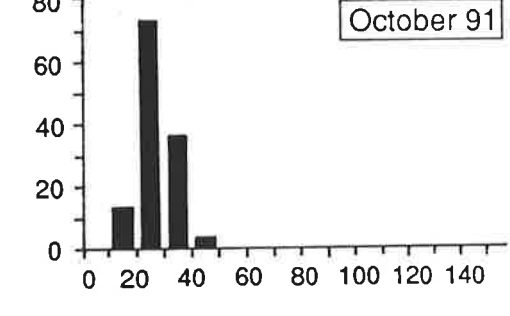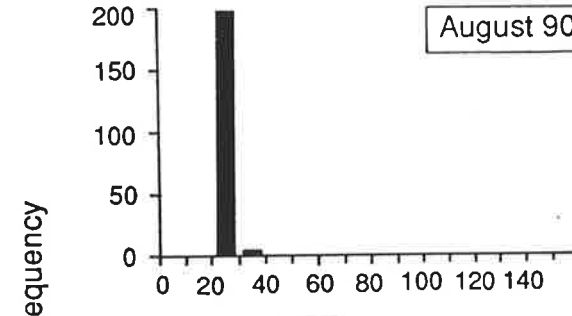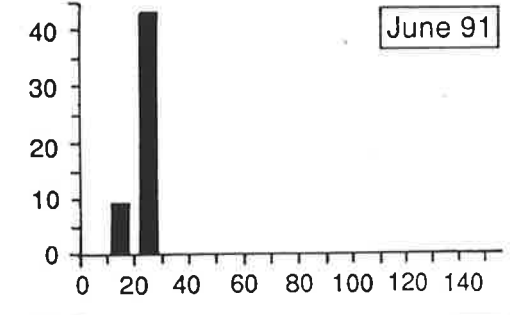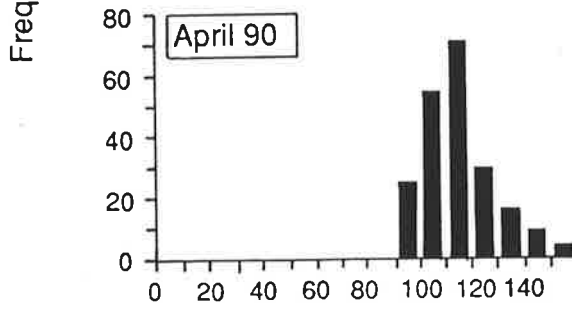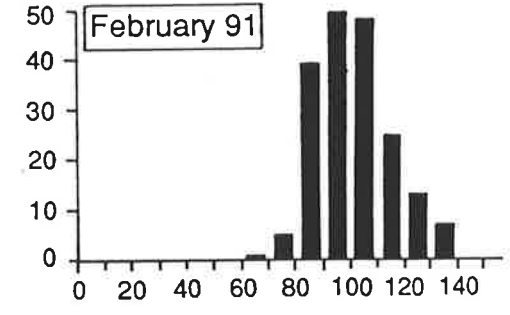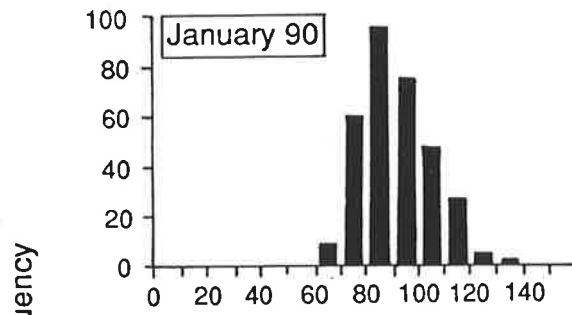
Fig. 3.5 Two-dimensional MDS ordination plots of fish assemblages showing habitat differences: (a) January 1990 (stress value (Kruskal's formula 1) = 0.088), (b) April 1990 (0.104), (c) August 1990 (0.098) - one unvegetated site at which no fish were caught is excluded, (d) October 1990 (0.129), (e) February 1991 (0.195)
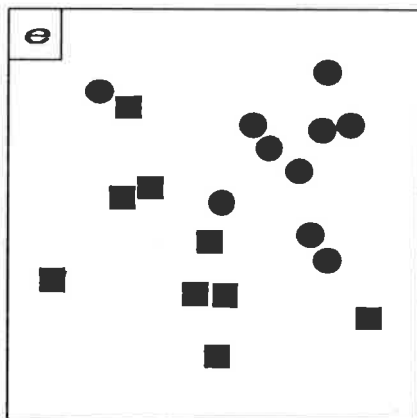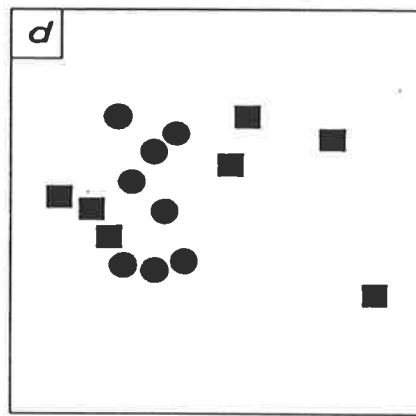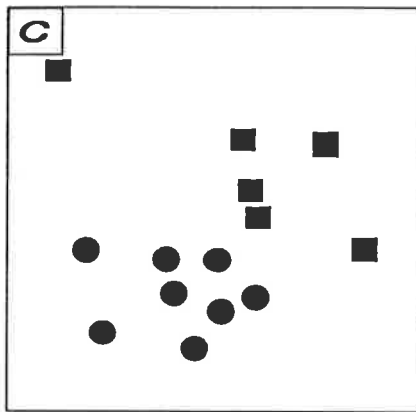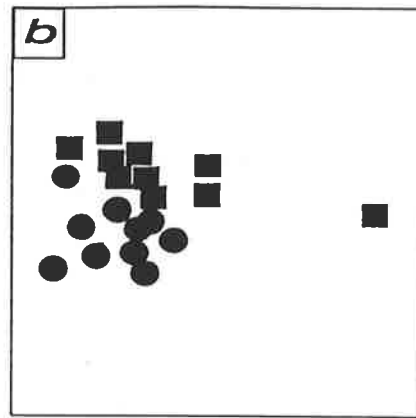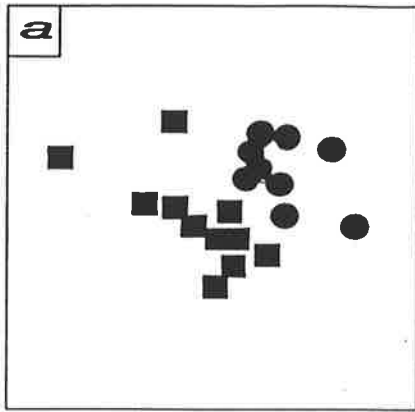
Fig. 3.6 Overlay of distance from site to open water onto MDS ordination plots of Fig. 3.5. Diameter of circle is proportional to distance. Smallest circle in any plot = 1.0 km, largest = 9.1 km
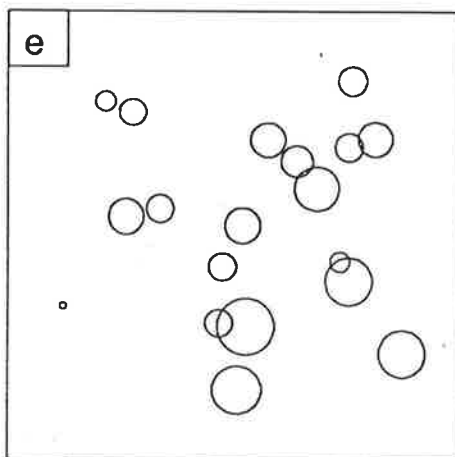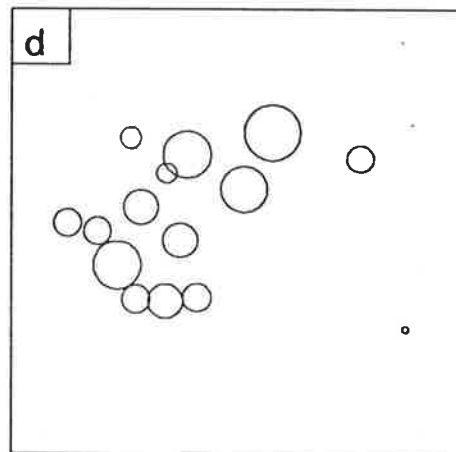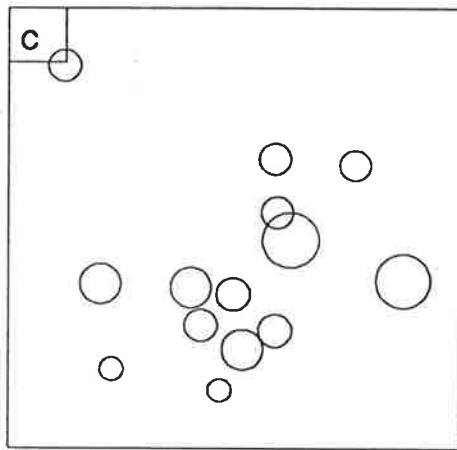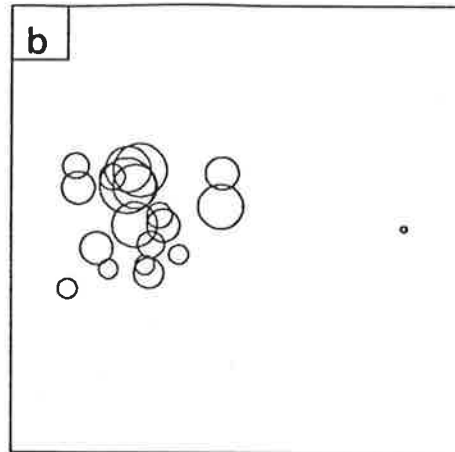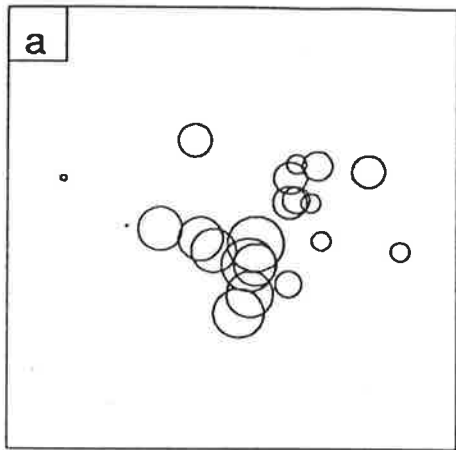
Fig. 3.7 Two-dimensional MDS ordination plot of fish assemblages over all periods. Stress = 0.197. Sites of a given habitat (eelgrass or unvegetated) at a given period have been combined and plotted at their centroid. Periods are indicated by numbers: 1 = January 1990, 2 = April 1990, 3 = August 1990, 4 = October 1990, 5 = February 1991
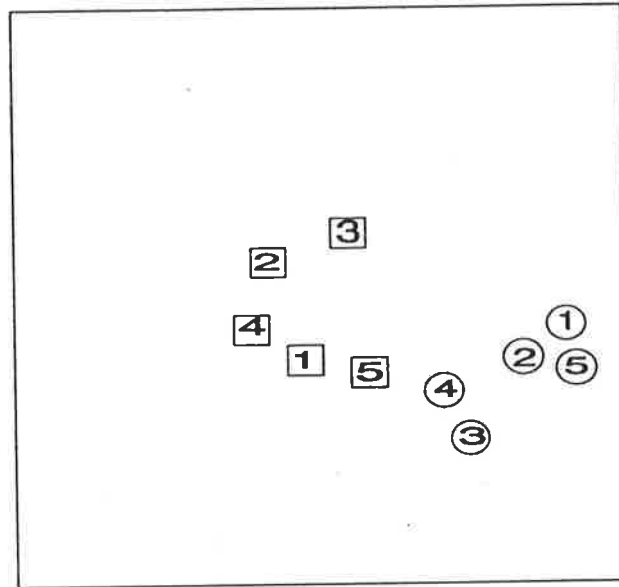
○ Eelgrass

□ Unvegetated

Chapter 4

Diet of King George whiting, *Sillaginodes punctata*

4.1 Introduction

Most fish from shallow, soft-substratum habitats are carnivorous. Despite the high levels of primary production sustained by shallow seagrass meadows (Hillman *et al.* 1989), few fish actually consume seagrass in temperate waters (see review in Klumpp *et al.* 1989), although this seems to be more common in tropical waters (McRoy & Helfferich 1980). A notable exception in southern Australian waters is garfish (*Hyporhamphus melanochir*), which eats eelgrass during the day and crustaceans at night (Robertson & Klumpp 1983). The diet of some species includes algae growing on seagrass (Bell *et al.* 1978, Klumpp *et al.* 1989, Edgar *et al.* 1993).

Small motile invertebrates are the main prey of fish in shallow, soft-substratum habitats (Robertson 1977, Duka 1978, Bell & Harmelin-Vivien 1983, Pihl 1985, Lubbers *et al.* 1990, Scholz *et al.* 1991, Webb 1991, Shaw & Jenkins 1992, Edgar *et al.* 1993, Humphries & Potter 1993). Invertebrates associated with the seagrass canopy or sediment surface (epifauna) are more important than invertebrates from within sediment (infauna) (Klumpp *et al.* 1989). Several dietary studies of small fish from seagrass beds and adjacent sandflats have shown that crustaceans are the predominant food item of small fish (Burchmore *et al.* 1984, Robertson 1984, Watson *et al.* 1984, Ryer & Orth 1987, Heck & Weinstein 1989, Klumpp *et al.* 1989, Steffe *et al.* 1989, Edgar *et al.* 1993). Epifaunal polychaetes and molluscs are also eaten (Klumpp *et al.* 1989).

The diets of small fish from seagrass habitats in South Australia have not been reported. The diets of fish in Western Port, Victoria, an enclosed waterway with vegetation similar to that in the Barker Inlet region, have been studied by Robertson (1984) and Edgar *et al.* (1993), who confirm the importance of epifaunal invertebrates, especially crustaceans. The diet of juvenile *S. punctata* in Western Port is described in Robertson (1977). Fish of this species fed on crustaceans (harpacticoid copepods, mysids and amphipods) after settling from a planktonic larval stage into eelgrass beds. Larger juveniles (> 40 mm length) fed upon ghost prawn (*Callianassa*) larvae and polychaetes, primarily in unvegetated patches adjacent to eelgrass.

The diet of most fish species changes ontogenetically, and is often related to mouth gape (Klumpp *et al.* 1989). Smaller fish eat smaller prey. Individuals of most fish species from Western Port feed on progressively larger prey, beginning with copepods, then amphipods, isopods and mysids, and later on crabs and shrimps (Edgar *et al.* 1993). Edgar *et al.* (1993) showed a linear relationship between the weight of fish and the weight of prey items consumed.

The primary aim of the present study was to record the diet of juvenile *S. punctata* in the Barker Inlet region, because this species is emphasised in later chapters in tests between the habitat selection and simple feeding models. A secondary aim was to compare diets of fish from eelgrass and unvegetated habitats. A wide variety of measures and indices have been used in attempts to quantify the relative importance of food categories to fish using gut analyses (Berg 1979, Hyslop 1980). The frequency of occurrence (what proportion of fish contained the food category), abundance (proportion of all items in a fish by number), weight (as for abundance, but by weight, either wet or dry) and volume (as for weight) have all been used. Measures of abundance overestimate the importance of prey occurring as numerous, small items, so weights and volumes are considered more informative (Berg 1979). Indices combining the basic measures listed above in various ways have also been used

(e.g. Pinkas *et al.* 1971) but no index is advantageous in all situations. Different food categories gain prominence depending on the weighting given to the different variables in the index. Berg (1979) recommends that where an index combining abundance, weight or volume, and perhaps frequency of occurrence, is used, values for the separate variables should also be shown. I consider that it may be as informative to forego the index, given that it is influenced by the weighting given to each variable, and simply present results based on, for example, abundances and weights. A further problem arises when comparing the importance of discrete food categories such as motile invertebrates or fish, with categories such as algae which have no identifiable unit.

Decisions about the importance of food categories to fish are best based not on the weight or volume of prey remaining in the gut but on the weight or volume of prey ingested. The ideal way of calculating weight or volume of food intake for prey such as motile invertebrates is to determine the weight - size (e.g. length) relationship for all prey categories using whole animals, and then to estimate the weight or volume of ingested prey based on the number and size of individual items found in fish. Edgar *et al.* (1993) describe an approximate method for estimating weights in which prey items are allocated to a size-class known to represent the range of sizes retained on a particular sieve mesh size within a stack of hierarchically arranged sieves. The size of invertebrates is then used to estimate their weight (Edgar 1990a). It is commonly considered that, ideally, the calorific content of prey items should be determined, although this is rarely done in practice.

When using gut contents to indicate the relative importance of different food types, differential gut passage rates for different food categories result in biased estimates of importance (Berg 1979, Hyslop 1980). Food types that are either quickly digested or that pass through the gut quickly are underestimated.

In calculating the relative importance of food types by dividing the number, weight or volume of a food type by the number, weight or volume of all food in a particular fish, no distinction is made between a fish having in its stomach one harpacticoid copepod and one calanoid copepod and a fish having 50 harpacticoids and 50 calanoids. Many studies therefore include some estimate of gut fullness. The most common method has been to assign guts to one of several subjective categories of fullness (Berg 1979). A measure more easily able to be repeated by other workers is *l' indice de repletion* of Hureau (1969, described in Berg 1979) in which the weight of ingested food is presented as a proportion of the total weight of the fish.

4.2 Materials and Methods

The diet of *Sillaginodes punctata* collected during surveys of the Barker Inlet - Port River region as described in Chapter 3 was examined. At each period, the stomachs and oesophagi of all fish (or of 10 randomly selected fish where more than 10 fish were caught) from each site were removed and the contents examined. For the purposes of determining what fish eat, the whole alimentary tract can be examined, but there are three advantages to examining items only from the anterior part of the tract. Firstly, food items from the oesophagus and stomach are more likely to be intact and are more easily recognised than items further along the gut. Secondly, by examining only items newly introduced to the gut, the bias caused by differential gut passage rates or digestion rates of different food items is likely to be reduced (Berg 1979). Thirdly, items towards the anterior end of the tract give a more reliable guide to the diet of fish just prior to capture. This is an advantage for the secondary aim of this study, that of comparing the diet of fish caught over eelgrass and unvegetated habitat. The tract of *S. punctata* less than 25 mm has no stomach and is a simple, uncoiled tube, narrowing posteriorly; contents of these smaller fish were examined from the section anterior to the narrowing.

The number of sites and total number of fish examined at each period are shown in Table 4.1. Individual items were mostly either intact or nearly so, and were identified to major taxa and counted. Animals were measured using a graticule in the microscope eye-piece, and were assigned to a sieve mesh size-class, so that weights of ingested prey could be estimated using the length - weight relationship described by Edgar (1990a). The majority of prey items were crustaceans, and where individuals were not whole, sizes were estimated by roughly piecing together parts of an animal (in the case of large crustaceans such as amphipods and mysids) or by using other individuals of the same taxon as a guide (for copepods). The only taxa recorded other than crustacea were polychaetes and chironomid larvae. Chironomid larvae were rare and were always whole. Although worms were often whole, sometimes they were in pieces; estimating sizes of polychaetes chopped into pieces was the most problematic part of this method. In these cases the number of anterior ends were counted and lengths were estimated to try to take account of the general size of individuals.

Each prey item was assigned to a size-category relating to the range of lengths of that taxon retained on different mesh sizes. These size ranges were determined by measuring the length of numerous specimens of each taxon collected during the survey of the invertebrates of the Barker Inlet region described in Chapter 5. For each taxon, relative length - frequency histograms were plotted for each mesh size, and a range of lengths was chosen as representative of a mesh size by selecting upper and lower limits where histograms from adjacent mesh sizes crossed. Size ranges for each taxon are shown in Table 4.2. The mean ash-free dry weight of invertebrates can be related to sieve mesh size using Edgar's (1990a) equation, $\log B = a + b \log S$ (where $B = $ AFDW (mg), $S = $ sieve size (mm) and a and b vary depending on broad taxonomic category). This method was used in the survey of invertebrates and is described in more detail in Chapter 5. For the purposes of determining size ranges in this diet study, a sieve with mesh size 4 mm was used in addition to those listed in Chapter 5.

For each fish, the percentage abundance of each food category was calculated as n/N, where n is the number of individuals of the food category and N is the total number of individuals of all categories in that fish. The same calculation was made for each category based on estimated weight (AFDW). The average percentage abundance and weight of each food category were calculated for each site. The average percentage abundance and weight at each sampling period (with night collections in June and October 1991 treated as sampling periods) were then calculated separately for eelgrass and unvegetated sites.

The total estimated weight (AFDW, mg) of the stomach contents of a fish was recorded as a proportion of the estimated total weight of the fish (dry weight, g). This proportion gives the same information as Hureau's (1969, described in Berg 1979) *indice de repletion*, although Hureau's index used the same units in numerator and denominator and is reported as a percentage. By using mg as the unit for the numerator, the ratio (fullness index) used here minimises the occurrence of numbers less than one. The weight of the whole fish, including stomach contents, was estimated using the relationship between dry weight and fish length. Fifty *S. punctata* collected from different periods and ranging from 18 to 133 mm total length were weighed after being dried to constant weight (at least 48 hrs) at 60°C. The dry weight of a fish is best estimated by its length using the relationship, $\log W = 3.261 \log L - 6.396$, where W is dry weight (g) and L is total length (mm). This relationship is shown in Fig. 4.1 ($r^2 = 0.997$) and 4.2.

Robertson (1977) shows two regressions of wet weight on fish length, with a slightly different relationship for fish shorter or longer than about 45 mm (termed 7 months of age in Robertson 1977)). This reflected a noticeable change in shape of fish at about this size (Robertson 1977). Prior to oven-drying, I determined wet weights after leaving fish to air-dry in an exhaust cabinet for 1 hr. A slight change in slope is evident in the plot of logs of wet weight against lengths (Fig. 4.3) between log length 1.6 and

1.8 (length approximately 40 and 60 mm), and although the line of best fit

(log WW = 3.703 log L - 6.682, where WW is wet weight (g)) has an extremely high r^2

value (0.992), a plot of residuals provides further evidence of a change in slope

(Fig. 4.4). I have not shown the separate relationships for the two sizes of fish because

for the purposes of estimating fish weights, only the regression of dry weight on length

is needed. It is worth noting that the slope of the regression line for all fish (wet

weight) is similar to that for smaller fish in Robertson (1977). Robertson's line has

greater elevation, probably as a result of his use of length to caudal fork rather than

total length, and perhaps also because of his different method of removing excess water

(using tissue paper). No change in slope is evident in the plot of dry weight against

length, and the plot of residuals also gives no hint of it (Fig. 4.2). Robertson does not

report dry weights of fish.

An alternative measure of stomach fullness is the weight of stomach contents as a

percentage of the greatest contents weight of any fish collected in a particular size-class

(Lubbers et al. 1990). This method is a useful alternative to the one used in the present

study as long as small size-classes are used. The method rests on the assumption,

however, that within each size-class enough fish are caught from a variety of times and

places to include fish with full stomachs.

Fullness indices of fish from eelgrass and unvegetated sites were compared using

Mann-Whitney U-tests at periods when fish were collected from enough unvegetated

sites to make meaningful comparisons. Indices were also compared for fish from

eelgrass sites between day and night samplings at June and October 1991. The

Mann-Whitney U-test is less powerful than a t-test if data meet the assumptions of

normality and homoscedasticity but, in cases such as these where sample sizes are very

small and tests of normality are impossible, it is a more reliable method of testing

differences in central tendencies (here, medians).

4.3 Results

The diet of juvenile *Sillaginodes punctata* consisted entirely of invertebrates. Thirteen categories were recorded, as shown in Fig. 4.5. Prey were either crustaceans or polychaetes, except for a small number of chironomid larvae taken in October 1990. Porcellid harpacticoids and caprellid amphipods were counted separately from their general taxa (harpacticoids and amphipods, respectively) because of their different form. Porcellid harpacticoids have a wide, flattened, shield-like shape and caprellids are extremely long and thin compared to gammarid amphipods. The prominence of small items such as copepods, especially harpacticoids, was greater when based on abundance than when based on weight. The prominence of larger items such as amphipods and polychaetes was, conversely, more obvious when based on weights. Notwithstanding these different emphases, the change in diet of *S. punctata* as fish grew larger is shown clearly in Figs 4.5 - 4.13. The median length of fish at each period is reported in Table 3.3.

At sampling periods in the second half of the year (August, October 1990; June, October 1991) when fish were small, harpacticoid copepods were the most conspicuous prey by abundance, and amphipods along with harpacticoids were dominant by weight. The abundance and weight of amphipods were noticeably lower in June 1991 than at later periods, and this may be attributable to the smaller size of fish at this period. Cyclopoid and calanoid copepods, which are typically more planktonic than harpacticoids, were taken consistently at these periods but were small contributors to diet by abundance or weight. Ostracods, caprellid amphipods, mysids, tanaids and polychaetes occurred occasionally but were not important by abundance or weight. Cumaceans were recorded in small numbers in October 1991.

At sampling periods in the first half of the year (January, April 1990; February 1991) when larger fish were examined, polychaetes were the main food category by

abundance and weight. Because polychaetes are grouped as one taxon in Figs 4.5 -
4.13, differences in size of polychaetes taken at different periods cannot be shown.
There was, however, a clear difference in the size of polychaetes taken by fish in
periods in the second half of the year compared with the first half of the year.
Polychaetes taken in June 1991 were small, and ranged from less than 1 mm to 3 mm
long (although the larger of these are large relative to other prey). Polychaetes taken
in October 1990 and 1991 were between 2 - 10 mm. Polychaetes in fish from periods
in the first half of the year ranged from 7 - 50 mm in length. Amphipods were the
second most important category by abundance, although by weight amphipods were no
more prominent than the other two frequently recorded categories, mysids and tanaids.
Harpacticoids were found in a small number of fish in January 1990. Fish in which
harpacticoids were found had no other categories of prey present, so that although the
harpacticoids were not numerous and were small, they comprised 100 % of the food in
those fish, based on abundance or weight. Caridean shrimps were recorded
infrequently in February 1991.

The percentage of fish having empty stomachs is shown in Table 4.3. Very few of the
fish caught during the day had empty stomachs at any sampling period, and no
difference is evident between sites from eelgrass and unvegetated habitat. The weight
of stomach contents, as a proportion of total fish weight, varied markedly from site to
site but did not seem to vary consistently with season (Table 4.3). In periods when fish
were caught at enough unvegetated sites to make a reasonable comparison possible,
the weight of stomach contents did not differ between eelgrass and unvegetated
habitats (Mann-Whitney U-test results: October 1990, $p = 0.234$; February 1991,
$p = 0.734$; June 1991, $p = 0.773$; October 1991, $p = 0.174$). Results of tests were
identical for all periods whether or not fish with empty stomachs were included.
Although it is not possible to calculate the statistical power of a Mann-Whitney U-test,
the small numbers of sites would suggest that, no matter what test is used, the chance
of demonstrating any difference is low.

More than half the fish caught at night in June and October 1991 had empty stomachs. In fish caught at night with food in their stomachs, the types of food were similar to those in fish caught during the day. The quantity of food in fish caught at night was significantly less than in fish caught during the day at the same period when fish with empty stomachs were included (Mann-Whitney U-test results: June 1991, $p = 0.047$; October 1991, $p = 0.014$), but was not significantly different when fish with empty stomachs were excluded (June 1991, $p = 0.186$; October 1991, $p = 0.221$).

Comparisons of the diet of fish caught over eelgrass and unvegetated habitat are limited by the small number of *S. punctata* caught over unvegetated habitat and the small number of unvegetated sites at which fish were caught. Over all periods, there seemed to be a greater prominence of polychaetes in fish from unvegetated habitat. In October 1990 and 1991, when fish were caught at 4 unvegetated sites giving a stronger chance that the data are representative of the habitat more generally, only fish from unvegetated sites had taken polychaetes. Fish from eelgrass sites tended to have a greater range of crustaceans. Caprellid amphipods, for example, were recorded only from fish caught over eelgrass at both periods.

4.4 Discussion

The diet of *Sillaginodes punctata* fits within the typical diet for fish from shallow, soft-substratum habitats. Stomach contents at the periods sampled give no indication of feeding on anything other than motile invertebrates. Juvenile *S. punctata* caught at periods in the first half of the year were large enough to be able to take small individuals of other fish species but there was no evidence of this. Although gastropods are eaten by some fish species, none was found in the present study. I examined the stomach contents of a small number of juvenile *S. punctata* collected in October 1990 from eelgrass beds in Coobowie Bay, almost directly west from Barker

Inlet on the opposite side of Gulf St Vincent, and found, amongst numerous harpacticoids and amphipods, one gastropod larva in each of two fish. Either the range of animals eaten by fish from Coobowie Bay is different to that for fish from Barker Inlet or gastropods are eaten by fish in Barker Inlet but were not found during the present study. The prominence of harpacticoids and amphipods in the diet of smaller juveniles and an increased prominence of polychaetes in older juveniles matches the pattern in *S. punctata* from Western Port, Victoria (Robertson 1977). Another feature of the diet of fish longer than about 45 mm in Western Port was the appearance of ghost prawn larvae, which were taken in unvegetated areas.

Results suggest that fish feed on a narrower range of prey and include more polychaetes in their diet when over unvegetated habitat. Lubbers *et al.* (1990) have also reported that for juveniles of several species of fish from an estuary in Chesapeake Bay, USA, diets of fish collected from unvegetated areas included a much greater proportion of polychaetes than diets of fish collected from vegetated areas. Evidence from the present study is, however, obtained from only a small number of fish from very few sites. The small number of fish examined from unvegetated sites could account for the failure to find food types such as caridean shrimps recorded infrequently in fish from eelgrass sites.

Evidence from the two night sampling periods suggests that juvenile *S. punctata* feed mainly during the day. The stomachs of fish collected at night were often either empty or contained only a small quantity of food. Either fish feed in a limited way at night or food in the stomach of fish collected at night remained from feeding during daylight hours. The time between sundown and collection of fish at night ranged from four to seven hours. The rate at which food is evacuated by juvenile carnivorous, marine fish of a similar size to the fish studied here have been shown to range from 2.7 to 4.8 hrs (Rosenthal & Paffenhofer 1972), 6 hrs (Archambault & Feller 1991) and 10 to 30 hrs (Ryer & Boehlert 1983). These laboratory estimates of gut evacuation times, however,

tend to be overestimates (Lockwood 1980). Food is presumably clear of the stomach before it is fully evacuated from the gut, so stomach emptying times could be shorter than those mentioned above. On the other hand, gut passage rates are much slower in colder water (Durbin *et al.* 1983, Ryer & Boehlert 1983) and, in the evening water temperatures of June and October 1991 of around 14^0C, food may have remained in guts much longer. It is therefore impossible to distinguish between the possibilities of limited nocturnal feeding and food remaining in stomachs from daytime feeding.

The ratio of ingested food to total fish weight did not seem to vary consistently with the size of fish taken at different periods. This contrasts with the study of silver hake (*Merluccius bilinearis*) and Atlantic cod (*Gadus morhua*) by Durbin *et al.* (1983), using the same measure, in which it was found that the ratio was greater in larger fish. Durbin *et al.* (1983) were using a much larger range of sizes, however, including juvenile and adult fish. Differences in the ratio may be evident for *S. punctata* if larger fish were to be examined.

Any differences in gut passage rates or rates of digestion for different food types could have affected the relative importance of food types. These biases were not determined during the present study, but should have been limited by examining food only from the oesophagus and stomach of fish. Differential digestion rates tend to underestimate the importance of soft-bodied invertebrates (Scholz *et al.* 1991), and for juvenile *S. punctata* this means that polychaetes are the taxon most likely to be underestimated.

Table 4.1 *Sillaginodes punctata* examined for stomach contents: number of sites at which *S. punctata* were caught, and number of fish examined, separately for each habitat at each sampling period

All fish were collected during the daytime except those marked **Night**.
Habitats: E = eelgrass, U = Unvegetated.

Sampling period	Habitat	Number of sites	Number of fish examined
January 1990	E	8	66
	U	2	11
April 1990	E	9	68
	U	1	4
August 1990	E	8	65
	U	2	2
October 1990	E	8	68
	U	4	17
February 1991	E	8	71
	U	4	15
June 1991	E	4	34
	U	4	7
June 1991 **Night**	E	7	54
	U	1	4
October 1991	E	8	67
	U	4	17
October 1991 **Night**	E	3	28
	U	1	1

Table 4.2 Size ranges (mm) of prey types matching mesh sizes

Blank cells indicate that prey type was not found on that mesh.

Prey type	Mesh size (mm)						
	4	2	1	0.5	0.25	0.125	0.075
Harp				>0.8	>0.68-0.8	0.55-0.68	<0.55
Porc				>0.6	≤0.6		
Cycl					>0.65	0.55-0.65	<0.55
Cala				>1	0.7-1	<0.7	
Ostr			>1.6	>0.8-1.6	0.5-0.8	<0.5	
Amph	>10	>5-10	>2-5	1-2	<1		
Capr		>7	3.8-7	<3.8			
Mysi		>5	3-5	<3			
Tana		>5	>2.6-5	1.2-2.6	<1.2		
Cuma		>6.5	4-6.5	<4			
Cari	>9.5	6-9.5	<6				
Poly	>11	>5-11	>2.9-5	>1.3-2.9	0.6-1.3	<0.6	
Chir		>4.8	>2.6-4.8	1.2-2.6	<1.2		

Table 4.3 Numbers of fish with no food in stomach, and fullness indices, separately for each habitat at each sampling period

Habitats: E = eelgrass, U = Unvegetated. The number of fish with empty stomachs is shown firstly by number (n) and secondly as a percentage (%), but percentages should be interpreted cautiously for unvegetated habitat where total fish numbers are very small. Fullness index is weight of stomach contents (mg AFDW) as a proportion of weight of whole fish (g dry weight). Fullness indices are shown as means of site means, with standard errors in parentheses (n/a = fish caught at one site only, therefore no s.e. available). (a) = excluding fish with empty stomachs, (b) = including fish with empty stomachs (and fullness index of zero).

Sampling period	Habitat	Empty (n)	Empty (%)	Fullness index (a)	Fullness index (b)
January 1990	E	3	5	4.18 (0.51)	4.01 (0.50)
	U	0	0	4.80 (1.84)	4.80 (1.84)
April 1990	E	0	0	2.94 (0.49)	
	U	0	0	2.04 (n/a)	
August 1990	E	0	0	4.07 (0.81)	
	U	0	0	2.55 (0.26)	
October 1990	E	2	3	4.29 (0.71)	4.20 (0.69)
	U	0	0	2.60 (1.36)	2.60 (1.36)
February 1991	E	7	10	4.23 (1.23)	4.06 (1.26)
	U	0	0	3.60 (0.63)	3.60 (0.63)
June 1991	E	0	0	2.52 (0.73)	2.52 (0.73)
	U	1	14	3.33 (0.89)	3.11 (1.04)
June 1991 **Night**	E	33	61	1.14 (0.33)	0.52 (0.14)
	U	2	50	0.96 (n/a)	0.48 (n/a)
October 1991	E	3	5	4.94 (0.84)	4.72 (0.79)
	U	0	0	3.35 (0.76)	3.35 (0.76)
October 1991 **Night**	E	18	64	2.99 (0.91)	1.00 (0.21)
	U	0	0	0.98 (n/a)	0.98 (n/a)

Fig. 4.1 Length - dry weight relationship for juvenile *Sillaginodes punctata*. Regression equation: $\log W = 3.261 \log L - 6.396$, where W is dry weight (g) and L is total length (mm). $r^2 = 0.997$

Length - Dry weight
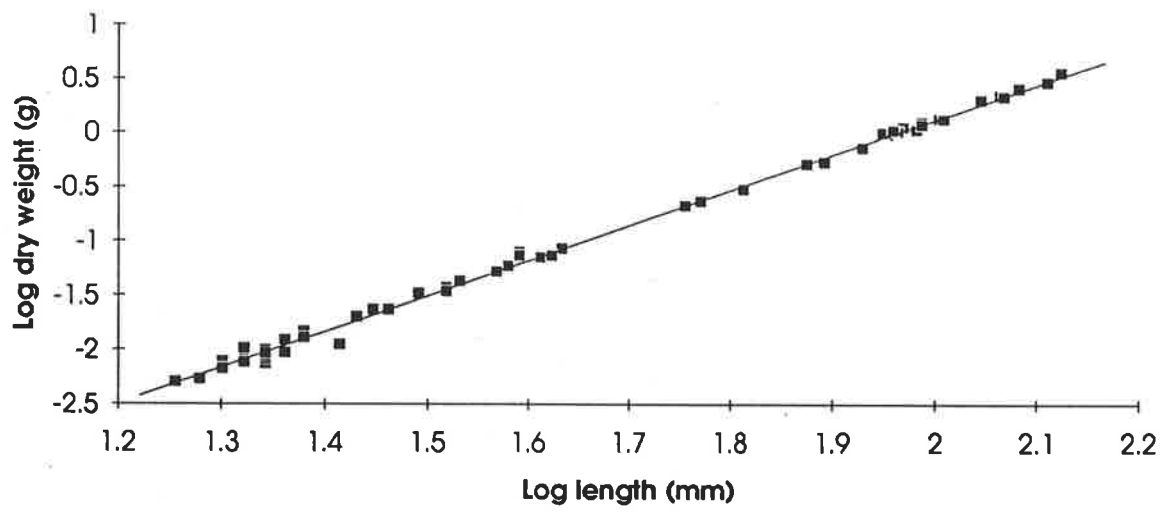
Fig 4.2 Plot of residuals of linear regression for length - dry weight of *Sillaginodes*
punctata shown in Fig. 4.1

Residuals: Length - Dry weight
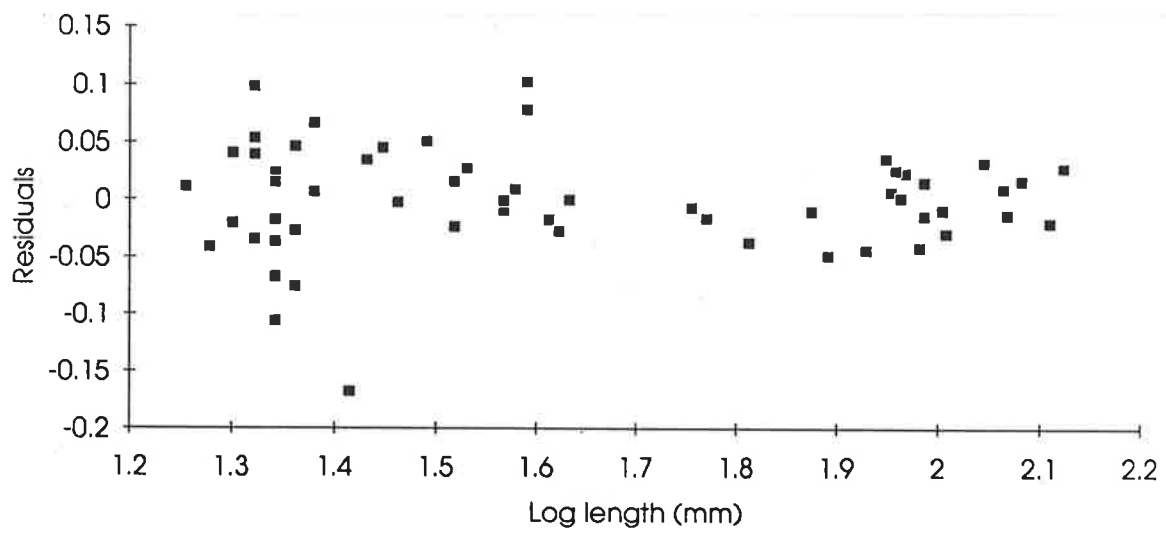
Fig. 4.3 Length - wet weight relationship for juvenile *Sillaginodes punctata*. Regression equation: log WW = 3.703 log L - 6.682, where WW is wet weight (g) and L is total length (mm). $r^2 = 0.992$

Length - Wet weight
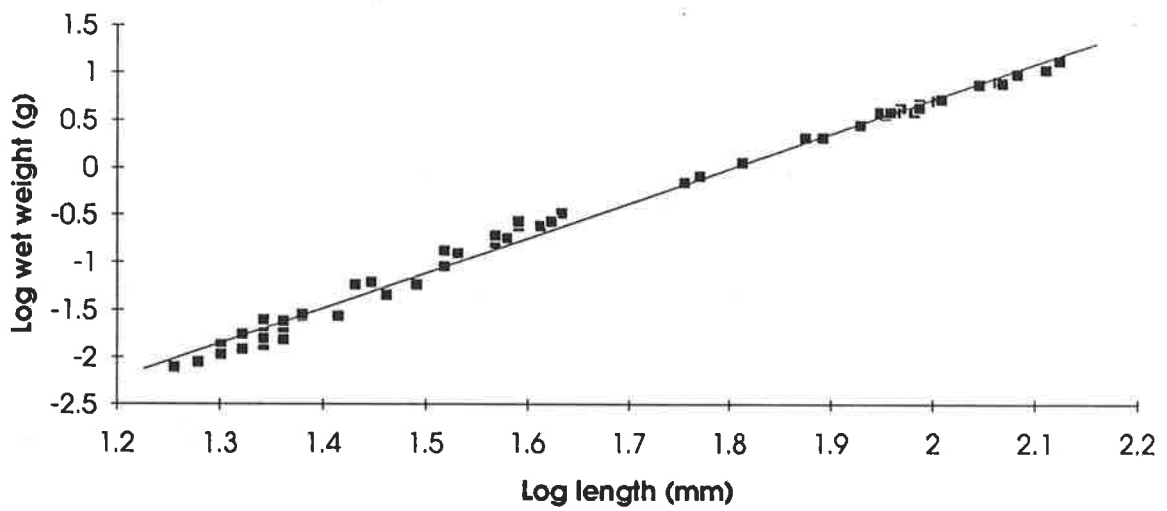
Fig 4.4 Plot of residuals of linear regression for length - wet weight of *Sillaginodes* *punctata* shown in Fig. 4.3

Residuals: Length - Wet Weight regression
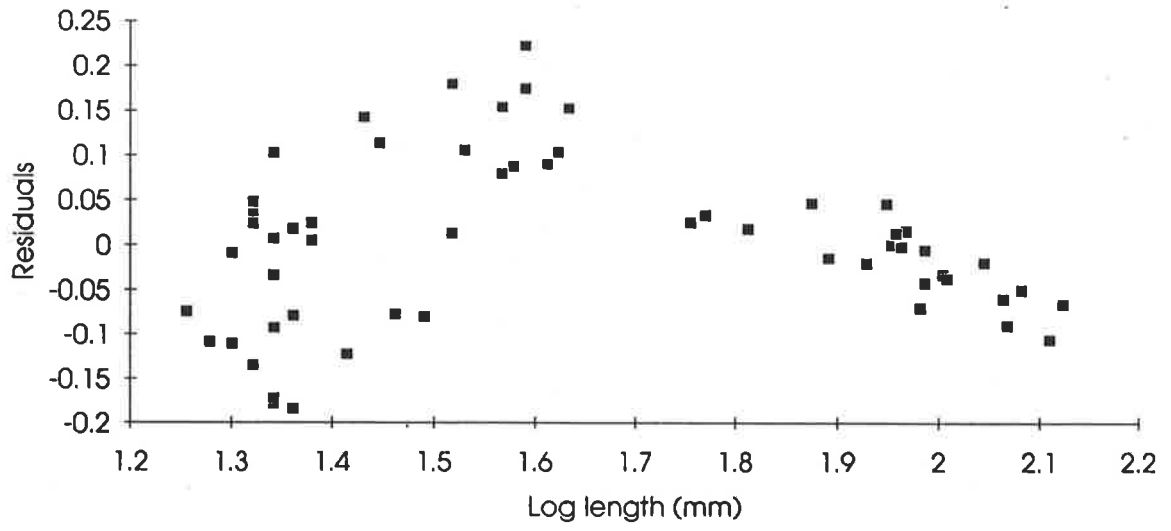
Fig. 4.5 Stomach contents of *Sillaginodes punctata* in January 1990 by habitat, based on a) abundance, and b) weight. Height of column represents mean percentage of food category from all sites, and lines are standard errors. The number of sites from which fish were examined is shown in Table 4.1. Food category abbreviations are as follows:

Harp   Copepoda - Harpacticoida

Porc   Copepoda - Harpacticoida - Porcellidiidae

Cycl   Copepoda - Cyclopoida

Cala   Copepoda - Calanoida

Ostr   Ostracoda

Amph   Amphipoda - Gammaroidea

Capr   Amphipoda - Caprellidae

Mysi   Mysidacea

Tana   Tanaidacea

Cuma   Cumacea

Cari   Caridea

Poly   Polychaeta

Chir   Chironomidae, larvae

a

b
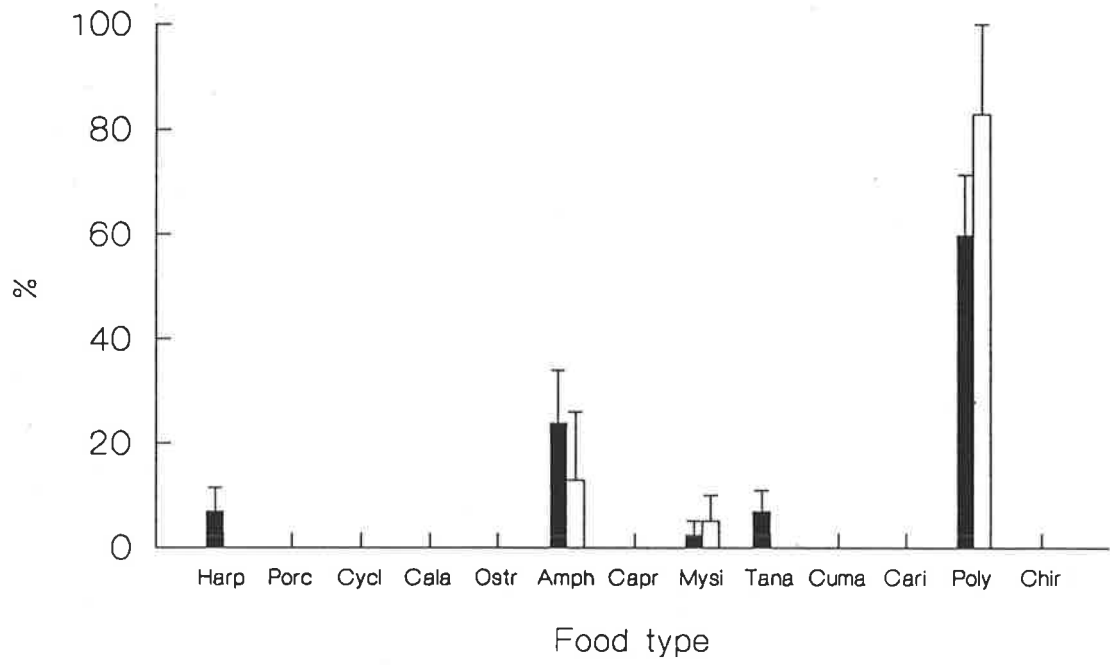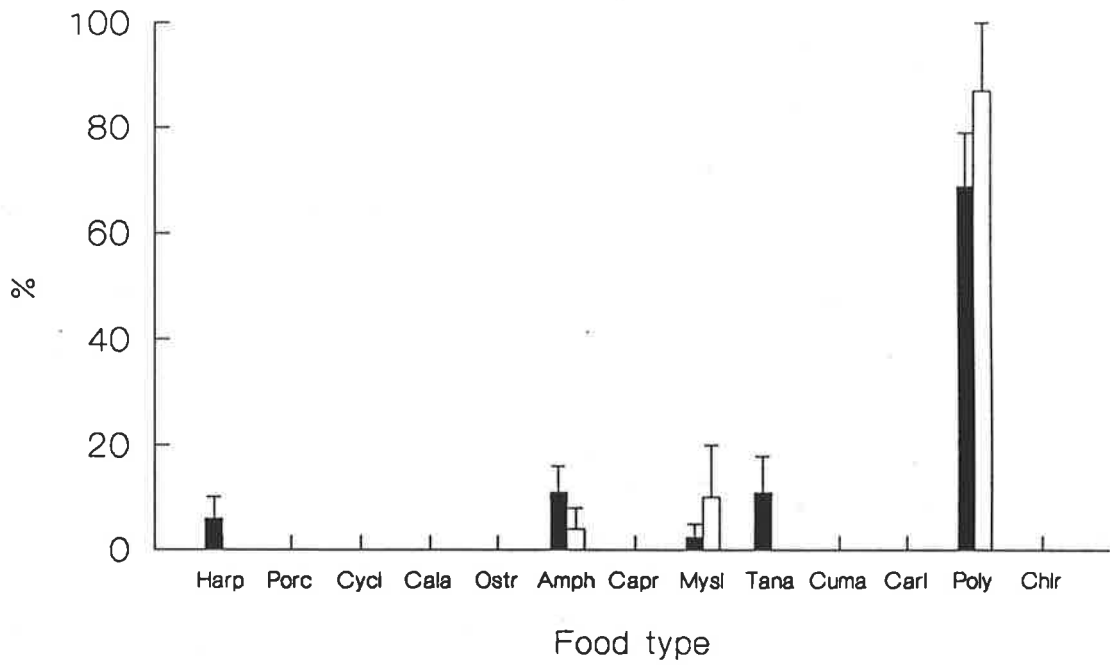
□ Unvegetated
■ Eelgrass

Fig. 4.6 Stomach contents of *Sillaginodes punctata* in April 1990 by habitat, based on a) abundance, and b) weight. Details as for Fig. 4.1. Fish were caught at only one unvegetated site, so no standard error is shown

a

b
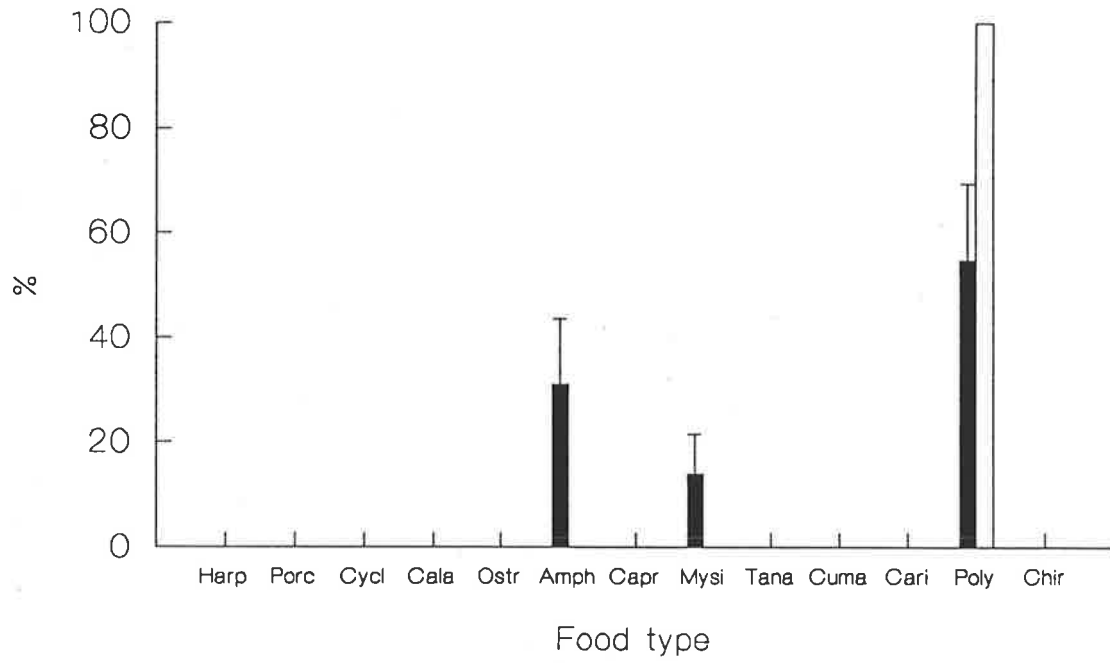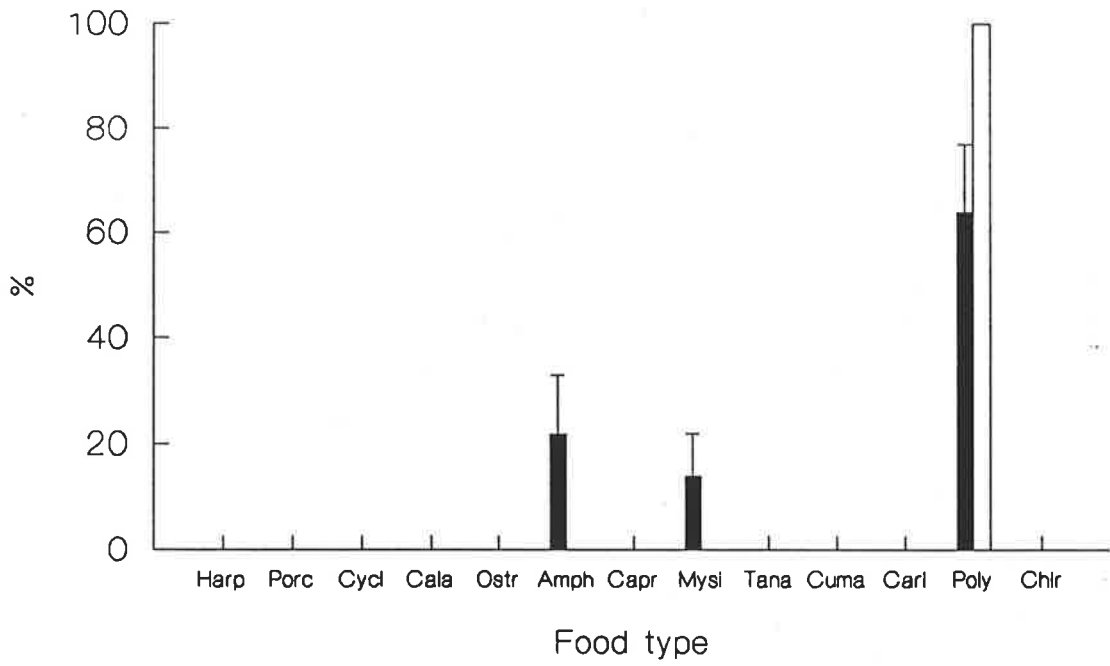
□ Unvegetated
■ Eelgrass

Fig. 4.7 Stomach contents of *Sillaginodes punctata* in August 1990 by habitat, based on a) abundance, and b) weight. Details as for Fig. 4.1

a

b
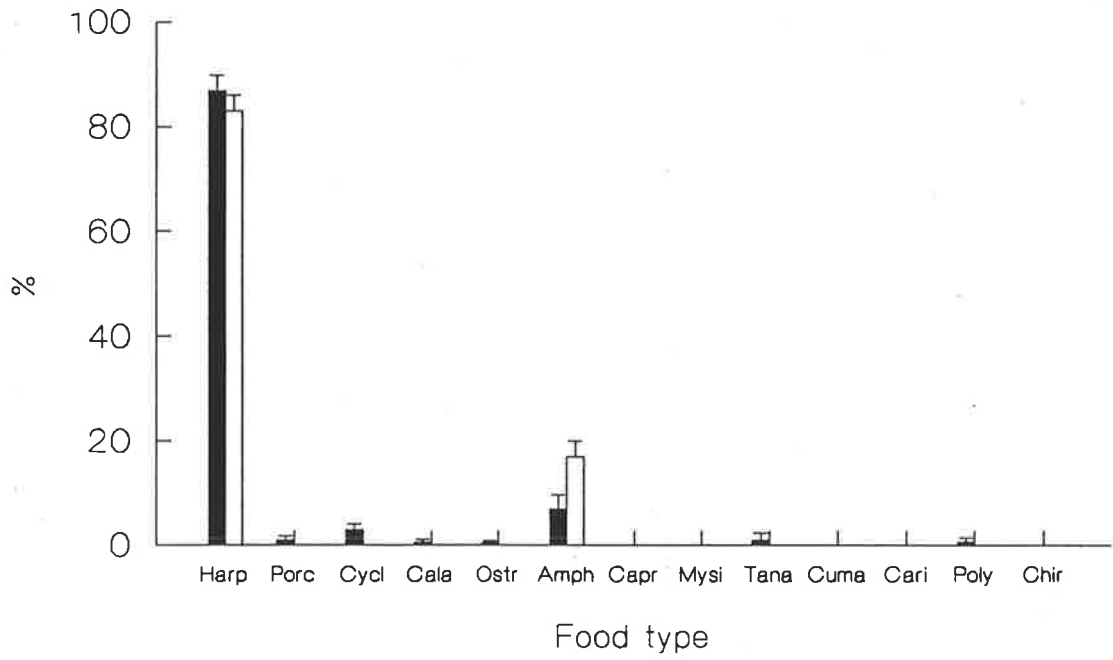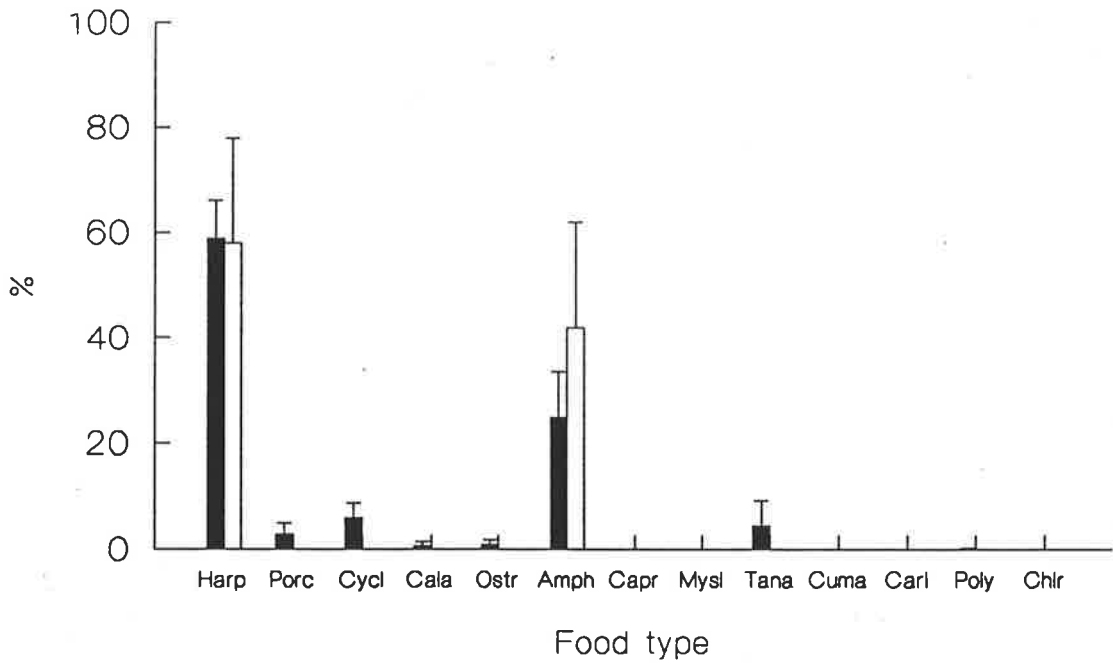
□ Unvegetated
■ Eelgrass

Fig. 4.8 Stomach contents of *Sillaginodes punctata* in October 1990 by habitat, based on a) abundance, and b) weight. Details as for Fig. 4.1

a

b
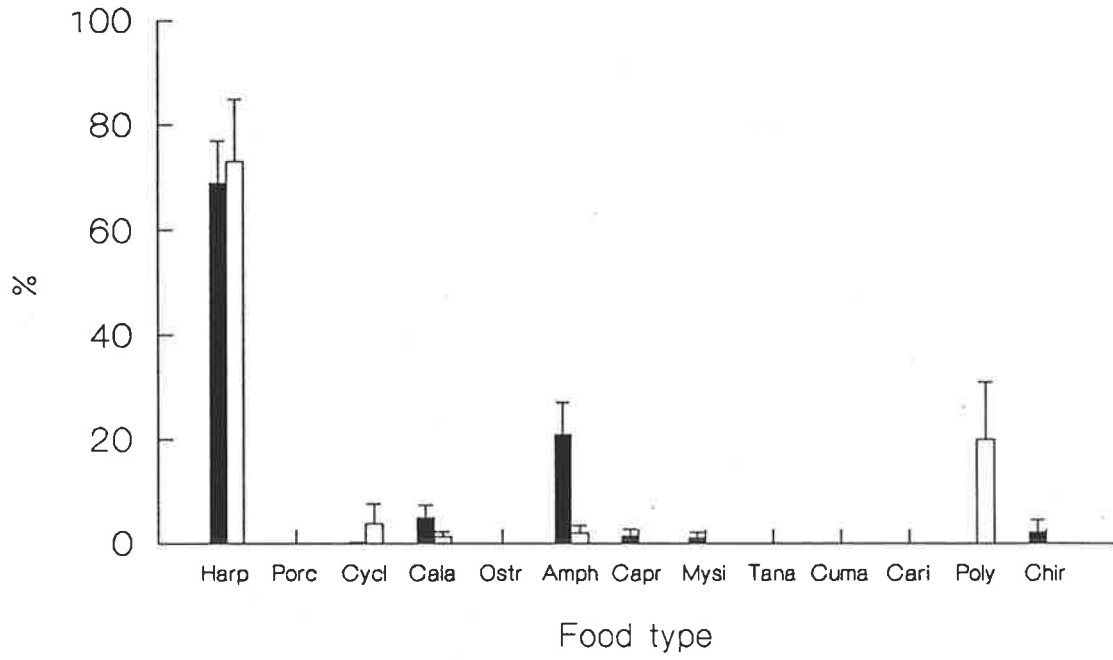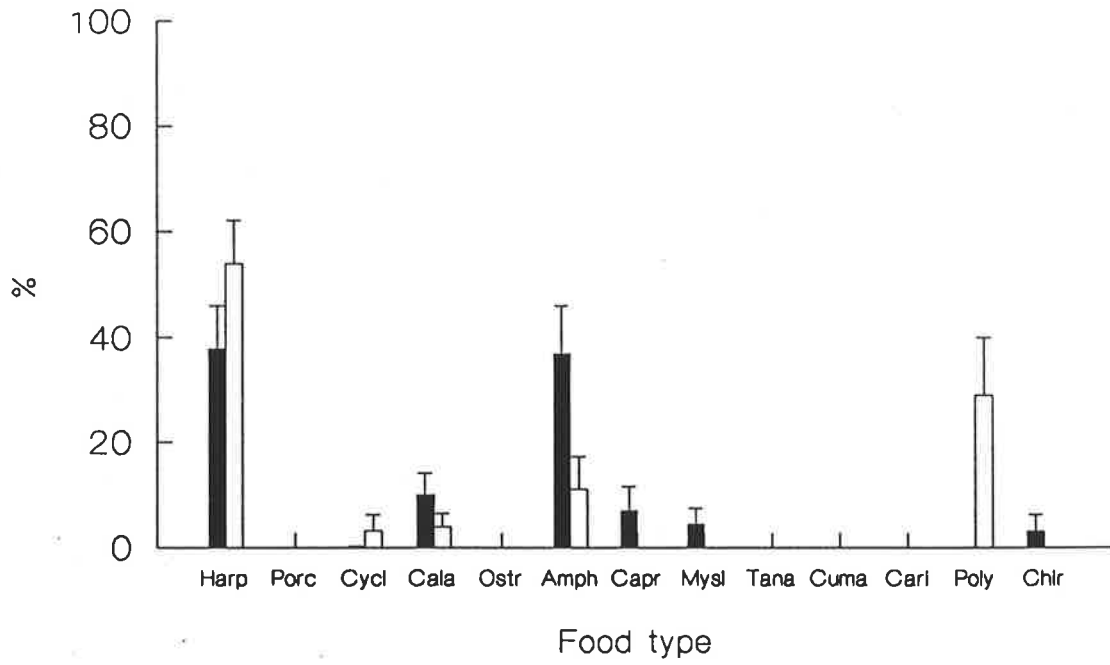
Unvegetated
Eelgrass

Fig. 4.9 Stomach contents of *Sillaginodes punctata* in February 1991 by habitat, based on a) abundance, and b) weight. Details as for Fig. 4.1. Only polychaetes were recorded from fish at unvegetated sites, and no standard error has been shown

a

b
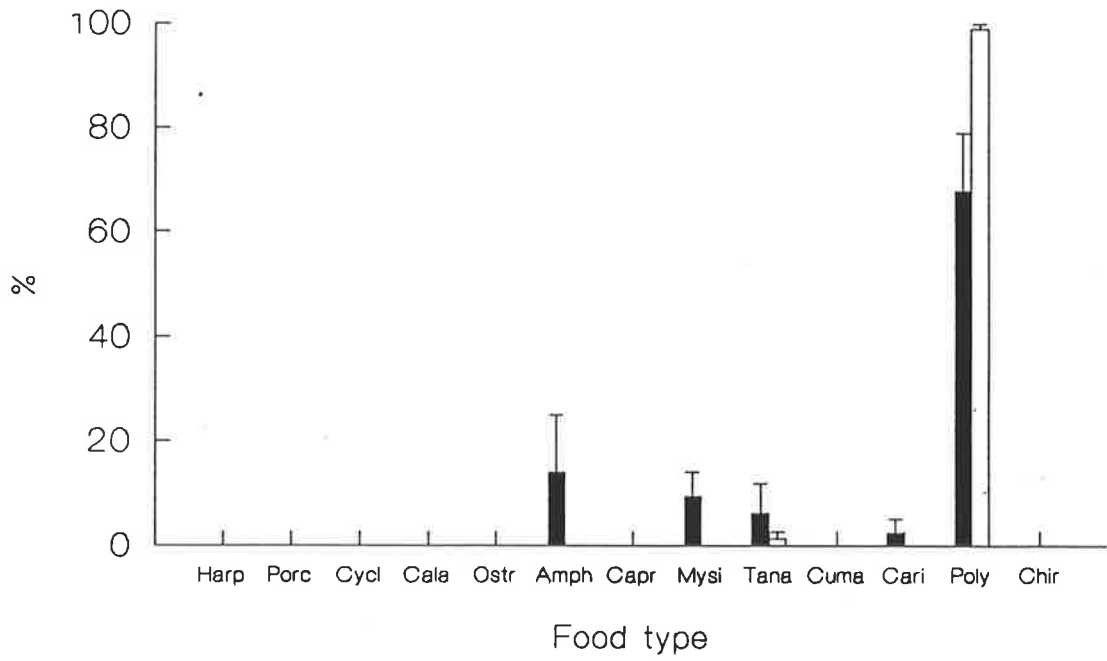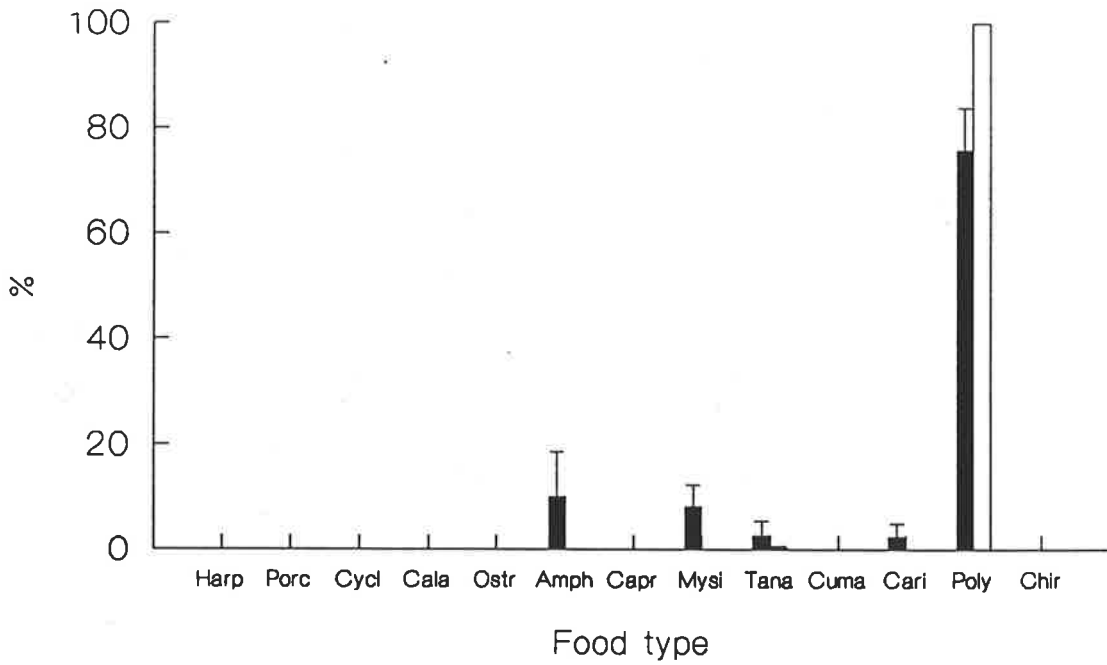
□ Unvegetated
■ Eelgrass

Fig. 4.10 Stomach contents of *Sillaginodes punctata* in June 1991 by habitat, based on a) abundance, and b) weight. Details as for Fig. 4.1

a

b
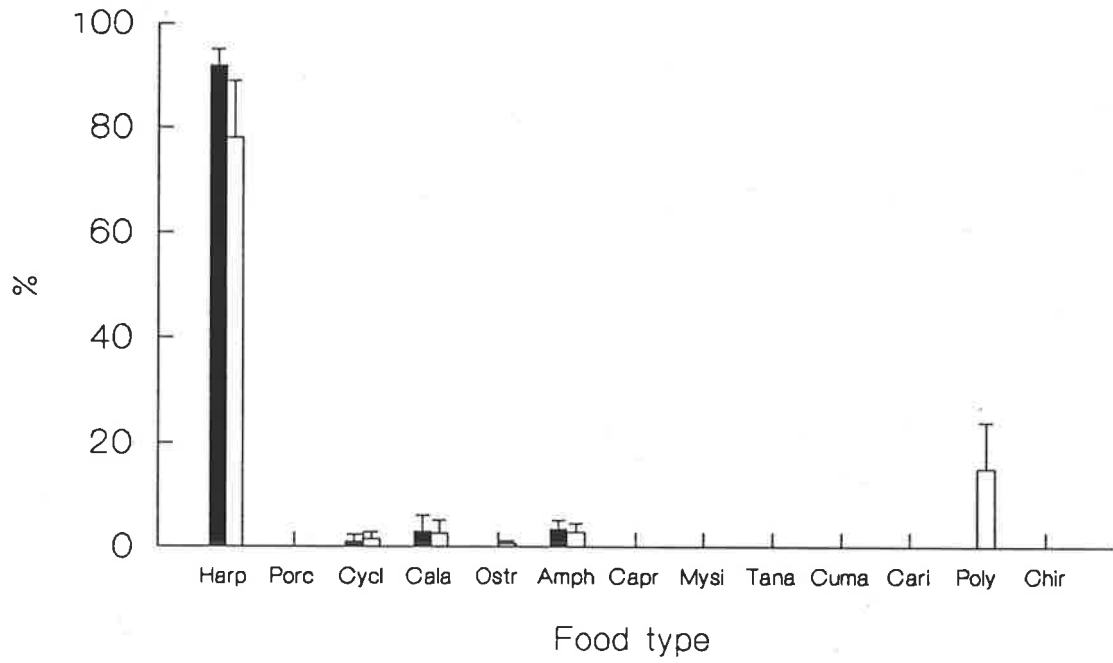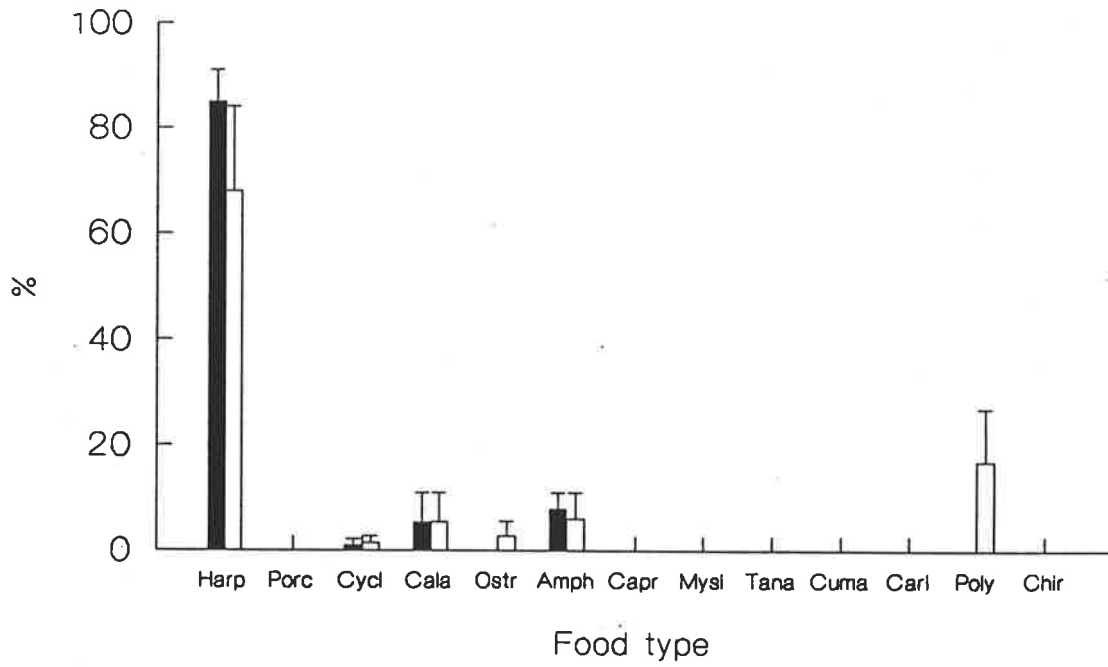
☐ Unvegetated
■ Eelgrass

Fig. 4.11 Stomach contents of *Sillaginodes punctata* collected at night in June 1991 by habitat, based on a) abundance, and b) weight. Details as for Fig. 4.1. Fish were caught at only one unvegetated site, so no standard error is shown

a

b
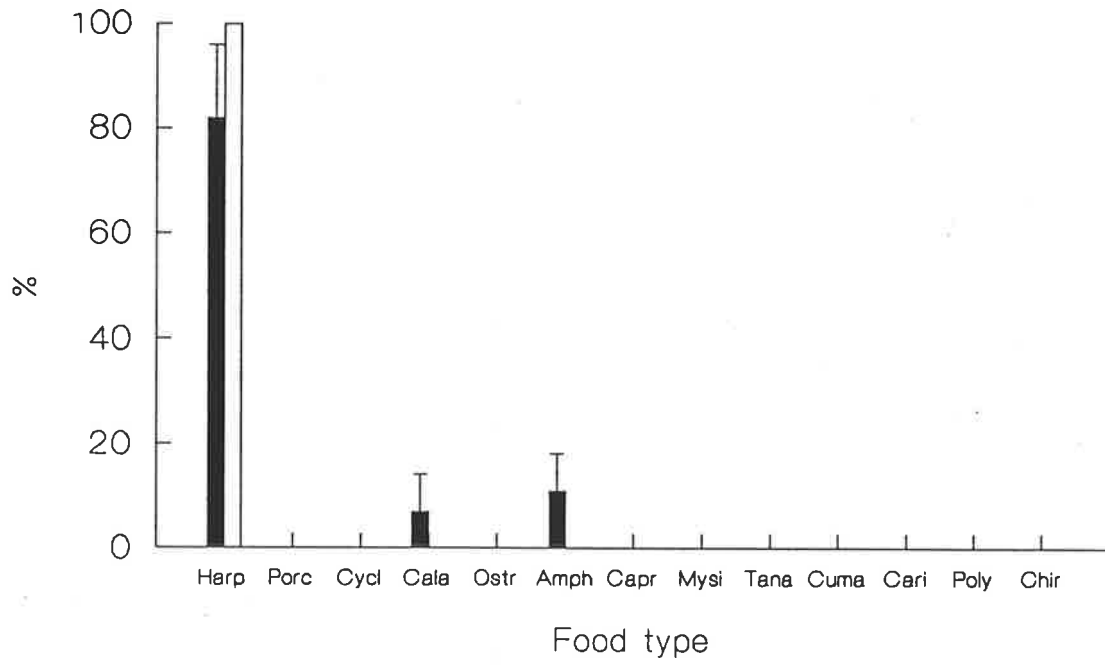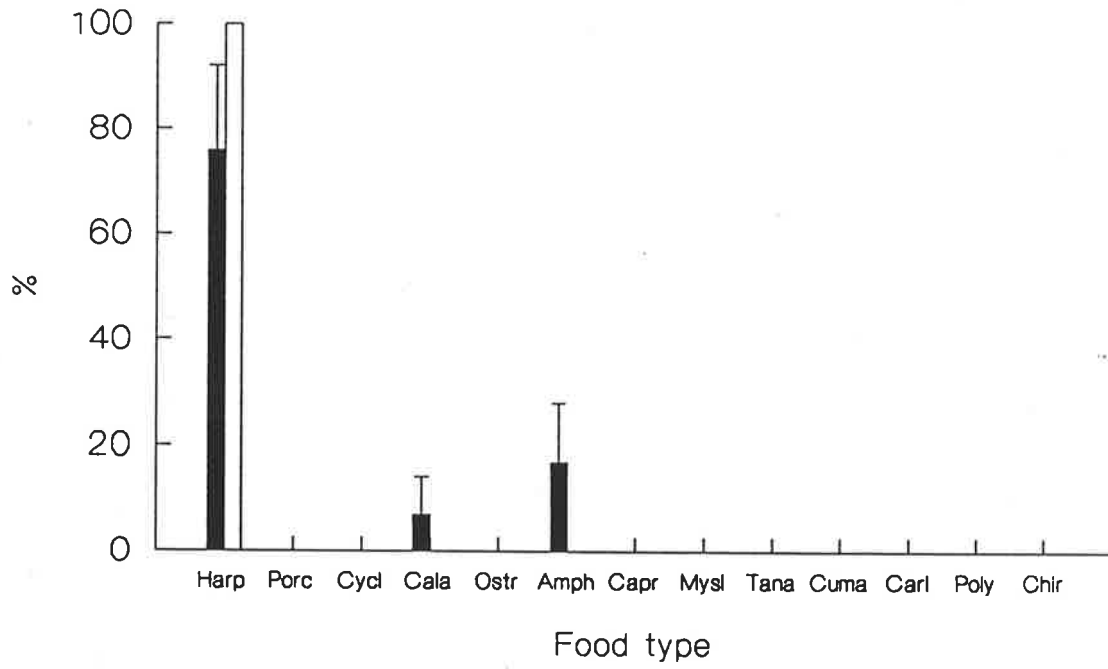
□ Unvegetated
■ Eelgrass

Fig. 4.12 Stomach contents of *Sillaginodes punctata* in October 1991 by habitat, based on a) abundance, and b) weight. Details as for Fig. 4.1

a

b
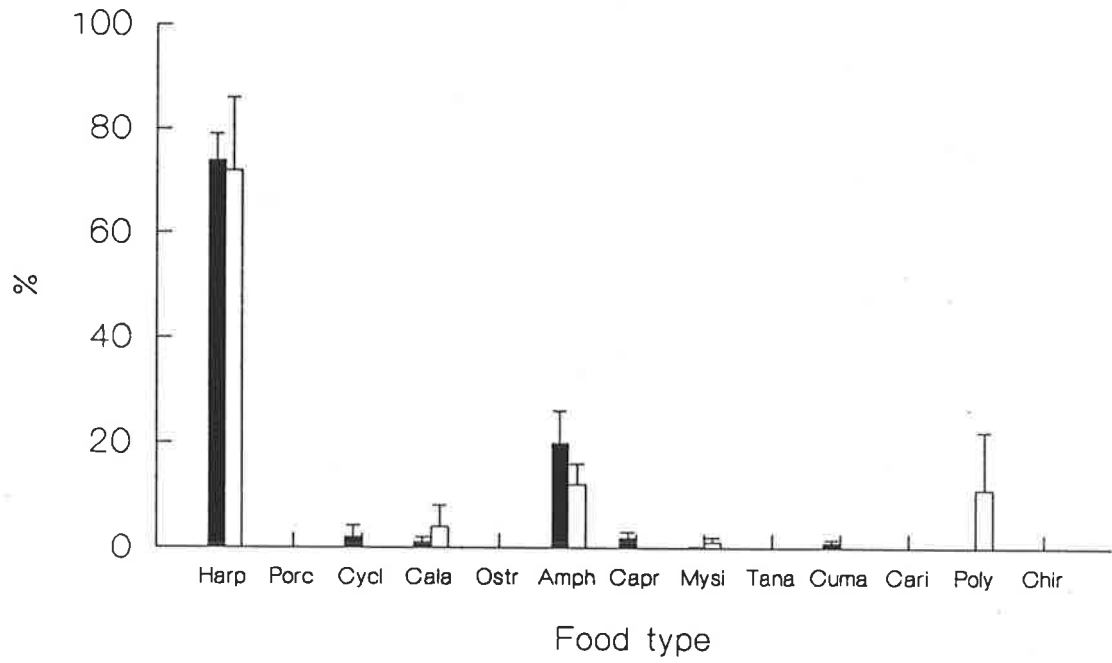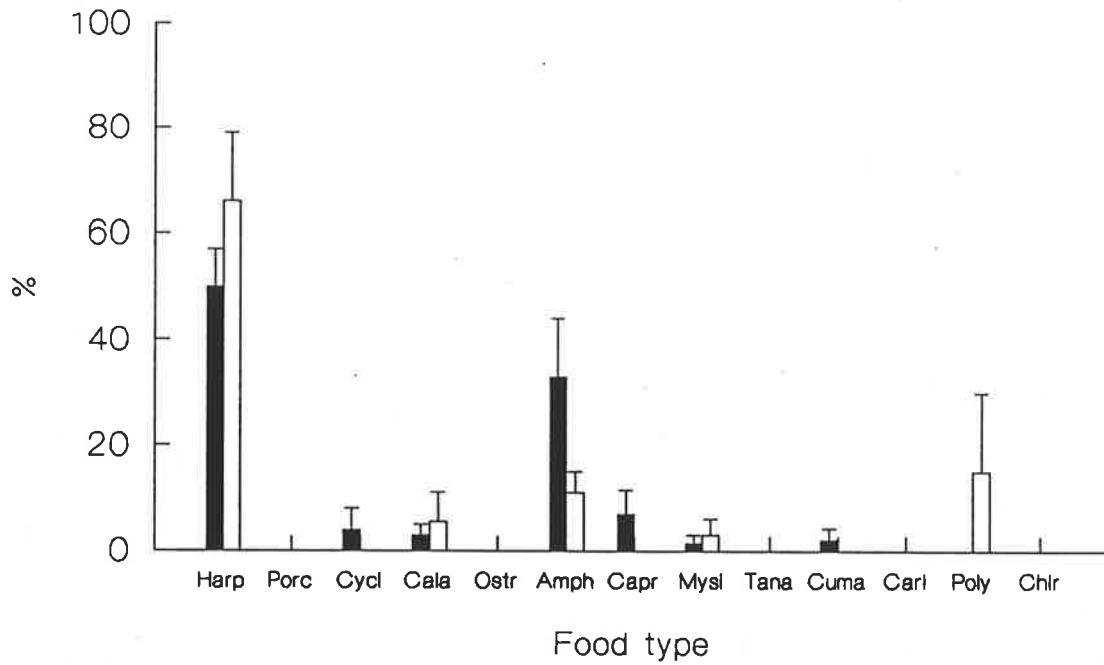
☐ Unvegetated
■ Eelgrass

Fig. 4.13 Stomach contents of *Sillaginodes punctata* at night in October 1991 by habitat, based on a) abundance, and b) weight. Details as for Fig. 4.1. Fish were caught at only one unvegetated site, so no standard error is shown

a

b
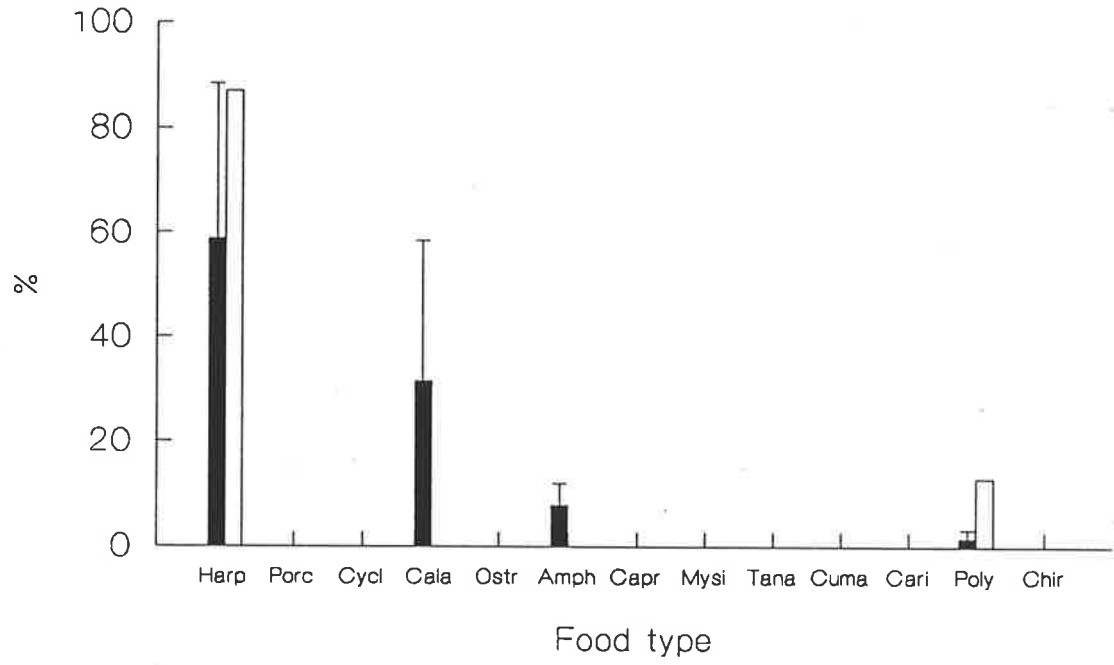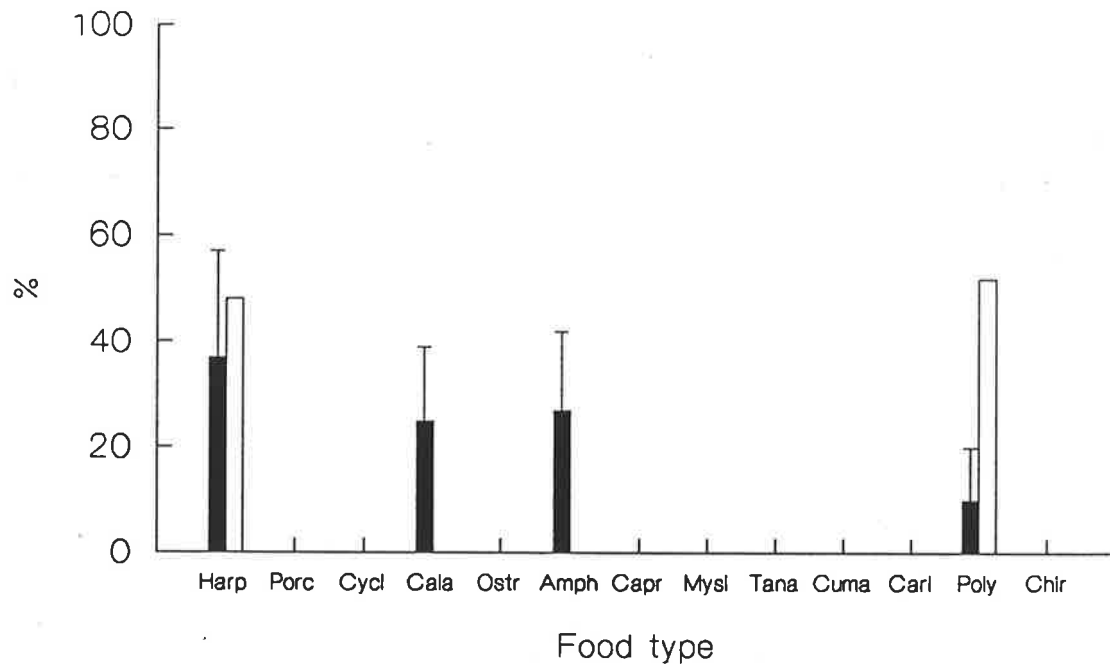
□ Unvegetated
■ Eelgrass

Chapter 5

A comparison of epifauna from eelgrass and unvegetated habitats

5.1 Introduction

Having shown unequivocally in Chapter 3 that fish assemblages associated with seagrass and unvegetated habitats in the Barker Inlet region differ, I now raise the question of whether those differences in fish assemblages could be explained by differences in food availability in the two habitats. Previous studies in seagrass meadows in Australia and elsewhere have shown that the majority of fish of the meadows and adjacent flats predominantly eat epifaunal invertebrates, which are associated with the surface of either the sea-bed or vegetation, rather than infaunal invertebrates, which are buried in the sediment (Klumpp *et al.* 1989). The epifaunal taxa of most importance in the diet of fish include crustaceans, polychaetes and molluscs (Klumpp *et al.* 1989). For *Sillaginodes punctata* in particular, the main prey are epifaunal crustaceans and polychaetes (see Chapter 4).

The abundance of epifaunal invertebrates associated with seagrass is usually greater than that associated with adjacent unvegetated patches (Orth *et al.* 1984). This difference is more obvious for epifauna than for infauna (Howard *et al.* 1989, Edgar *et al.* 1994). Epifaunal assemblages associated with adjacent patches of different species of seagrass are often more similar than those associated with patches of the same species of seagrass separated by large distances (Howard *et al.* 1989). Artificial seagrass placed in unvegetated areas near natural seagrass beds attract a fauna similar to that in the natural beds (Howard *et al.* 1989, Edgar 1990b). Howard *et al.* (1989) suggest that the type of seagrass may be less important than the presence of seagrass. Larger epifaunal invertebrates (macrofauna, defined as animals retained on 0.5 mm mesh) are, however,

capable of selecting amongst different densities of seagrass (Leber 1985).
Bell & Westoby (1986c) manipulated seagrass densities in field experiments and used predator exclusion cages to show that decapods were more common in denser seagrass regardless of the presence or absence of predators. They showed convincingly that low decapod numbers in patches with less dense seagrass cover were not due to increased predation, and concluded that decapods select habitat. Stoner (1980) demonstrated that amphipods can also detect and respond to differences in canopy density. The high mobility of epifaunal invertebrates, even in their adult stages, enhances their ability to exercise behavioural selection for seagrass of differing densities. Although less experimental work has been done on meiofauna (Howard *et al.* 1989), defined as animals passing through 0.5 mm mesh but retained on 0.1 mm mesh, harpacticoid copepods are known to colonise artificial seagrass placed near natural seagrass beds (Bell & Hicks 1991).

The aim of the current work was to determine whether the abundance, biomass or production of epifaunal taxa differed between seagrass and unvegetated habitats, in a region where patches of seagrass are known to support more fish and different fish assemblages than adjacent unvegetated patches.

5.2 Materials and Methods

The survey of epifaunal invertebrates was done in conjunction with the survey of fish in the Barker Inlet - Port River study region from January 1990 to February 1991. As in the case of the fish survey, the benefits of natural interspersion of the two habitats within the estuary and avoidance of the southern part of the estuary affected by warm-water effluent from the Torrens Island Power Station (see Fig. 3.2 in Chapter 3) apply. The sites used for epifaunal collections were the same as those selected for fish collections. A sample of epifauna was taken in water between 30 and 50 cm deep, adjacent to and

simultaneously with the hauling of fish nets. A 95 μm mesh net with a 25 x 25 cm

opening was used, following the method of Sergeev *et al.* (1988) in which the net was

placed rapidly over the canopy onto the sediment. Whilst the net was held in place,

shears were slipped under the net, and seagrass, where present, was cut level with the

sediment surface. In habitats without seagrass, the same action was taken, ensuring that

the sediment surface was ruffled as it was where seagrass was present. The net was then

slipped off its frame and dragged shut along the sediment surface. Animals were later

separated into sieve size classes of 2 mm, 1 mm, 500 μm, 250 μm, 125 μm and 75 μm

before being identified to major taxa and counted. Numbers of very abundant taxa were

counted from random subsamples with the aim of counting between 50 and 200

individuals of each taxon per sieve size in any sample. Twenty-one taxa were used, 13

crustacean and 8 others (Table 5.1). Nematodes were counted but, as they are typically

not a component of fish diets, were treated separately in analyses. Nematode numbers

are presented here but have been excluded from estimates of total epifaunal abundance.

Animals from the largest sieve were ashed and ash-free dry weights (AFDW) were

calculated. Ash-free dry weights for other sieves were calculated by converting

abundances for each taxon for each sieve size using Edgar's (1990a) equation,

$\log B = a + b \, \log S$ (where B = AFDW (mg), S = sieve mesh size (mm) and a and b

vary depending on broad taxonomic category). Since each sieve size retains animals

ranging from that sieve size to the next, S is expressed as a geometric mean calculated

using the equation, $\log S = (\log S_i + \log S_{i+1})/2$, in which S_i = mesh size of the ith sieve

and S_{i+1} = mesh size of the next size up (Edgar 1990a). Mean ash-free dry weights for

each taxonomic group for each sieve size are shown in Table 5.2. The slope of lines

based on Edgar's (1990a) equations is less than would be expected if body weight

increases with the cube of body dimensions. One possible reason for this is that the

volume of larger individuals may include proportionately more inorganic material, such

as shell or exoskeleton (Edgar 1990a). Edgar warns that the relationship between sieve

mesh size and AFDW for polychaetes depends on the form of polychaetes. As in Edgar's

(1990a) study, the majority of polychaetes caught during my surveys were epifaunal, and string-shaped forms such as capitellids were rarely collected; Edgar's equation is therefore appropriate.

Epifaunal production is a more useful measure than abundance, but field measurements of production are extremely difficult. A useful index of community production has been developed by Edgar (1990a) using the relationship between epifaunal biomass and production. Smaller individuals have a relatively higher production rate than larger individuals, and the abundance of different sized animals can be used to estimate production (Edgar 1990a, Edgar & Shaw 1993, Edgar *et al.* 1994). Edgar (1990a) used production rates for small, benthic invertebrates from several published sources to determine the relationship between biomass and daily production. Biomass alone explained much of the variability in production, and when water temperature was also taken into account, the two factors explained very nearly all of the variability in production (Edgar 1990a). As an approximate indication, at least, of epifaunal production in eelgrass and unvegetated habitats, I have used Edgar's (1990a) equation, $P = 0.0049 \ B^{0.80} \ T^{0.89}$, relating production (P, μg/day) to sample AFDW (B, μg) and water temperature (T, $^{\circ}$C). Since temperature did not differ significantly between eelgrass and unvegetated sites at any sampling period, the temperature for all sites was taken as the mean value of all sites at that sampling period (values were intermediate between mean temperatures at eelgrass and unvegetated sites shown in Table 3.5). Production, as calculated using Edgar's equation, can be considered as a general index of community processes such as respiration and consumption as well as of community production (Edgar 1993), although for the purpose of comparing availability of food to fish in different habitats it is used here as an index of production. Production values are useful in that they permit comparisons between habitats using data from a wide range of size-classes without being dominated by a particular size class (Edgar 1994).

Water temperature and salinity measurements were taken as described in Chapter 3.

Data Analysis

Multivariate data were transformed and analysed as in Chapter 3. Comparisons of epifaunal assemblages (described both by abundance and biomass (AFDW)) from seagrass and unvegetated habitats were made using ANOSIM. Assemblages from the two waterways, Barker Inlet and Port River, were compared using a two-way crossed ANOSIM with habitat as the second factor. Relationships amongst assemblages are presented as two-dimensional MDS ordination plots, and the association of the environmental variables water temperature, salinity and distance to open water, with patterns in biotic data, were measured using the weighted Spearman's coefficient recommended by Clarke & Ainsworth (1993).

Comparisons were made for each of the five periods separately, but an MDS ordination was also performed on data from all periods combined, and a two-way crossed ANOSIM was used to test differences amongst periods after taking into account differences between habitats.

The abundance and biomass of epifauna (all taxa combined and key taxa separately) and total epifaunal production from the two habitats were compared using one-way ANOVA.

5.3 Results

Two-dimensional ordination plots of epifaunal assemblages show a very similar pattern for both abundances (Fig. 5.1) and biomasses (Fig. 5.2). At every period, there is strong grouping of seagrass and unvegetated sites. Assemblages were significantly different using both variables, at each sampling period, as tested with ANOSIM (Table 5.3).

After accounting for differences due to habitat, assemblages from the two waterways, Barker Inlet and Port River, differed in some periods but not at others (Table 5.4).

The correlations between epifaunal assemblages and environmental variables differed depending on the sampling period, but were weak at all periods and were secondary to the main difference between eelgrass and unvegetated sites. Salinity was not correlated with assemblages at any period, and no environmental variable was correlated with assemblages in October 1990 and February 1991. In January 1990, distance to open water was the most closely correlated variable (abundance: $\rho_w = 0.194$; biomass: 0.265); although no formal test of this correlation is available, these values are low (Clarke & Ainsworth, 1993), and demonstrate only a weak association between distance and epifauna. Despite thorough interspersion of eelgrass and unvegetated sites (as shown in Fig. 3.1), a test comparing mean distance to open water of eelgrass (mean distance = 4.2 km) and unvegetated (6.6 km) sites detected a significant difference (t-test, after check for homoscedasticity: $t = 2.858$, $p = 0.011$). This suggests that, in January 1990, the difference between eelgrass and unvegetated sites could have been confounded with the association of epifauna with distance to open water. Given the weakness of the association with distance, however, it is not possible that the marked distinction between assemblages of eelgrass and unvegetated habitat is solely the result of the greater distance to open water of unvegetated sites. It is possible, though, that the weak association with distance is a result of the marked differences between assemblages of the two habitats. In April 1990, water temperature was the variable most closely correlated with assemblages (abundance: $\rho_w = 0.264$; biomass: 0.188). Again, these values are low, and demonstrate only a weak association between temperature and epifauna. The mean temperature at eelgrass and unvegetated sites was not significantly different (as stated in Chapter 3). In August 1990, distance to open water was the variable most closely correlated with assemblages (abundance: $\rho_w = 0.390$; biomass: 0.453), and the values, whilst not showing a strong correlation (Clarke & Ainsworth 1993), do imply a moderately close association. A simple overlay of distance to open

water onto MDS plots of epifaunal assemblages for abundance (Fig. 5.3a) and biomass (Fig. 5.3b) shows no obvious pattern for eelgrass sites but for unvegetated sites suggests that distance is associated with three separate groups. Both for abundance and biomass, one site high and far to the left of the plot has a small distance, two sites lower on the far left have large distances, and four sites near the centre of the plot have intermediate distances. Each of the two groups with more than one site includes sites from both waterways, Barker Inlet and Port River, and they are not grouped by temperature or salinity, so differences amongst the groups can best be explained by distance to open water.

When all periods are combined, the distinction between eelgrass and unvegetated sites remains the overwhelming difference based on both abundance (Fig. 5.4) and biomass (pattern very similar to Fig. 5.4, and not shown). After accounting for differences between habitats, sampling periods are, however, also different (Table 5.5). Pairwise comparisons on abundances show that all periods differ except January 1990 and February 1991. Given that sampling actually occurred at the end of January 1990 and the beginning of February 1991, these two period are almost identical seasonally, and the similarity of assemblages at these two sampling periods may reflect a seasonal pattern. Pairwise comparisons on biomass data showed a similar pattern except that assemblages from August 1990 were also found to be not significantly different from February 1991 and October 1990.

Mean abundances and biomasses of all taxa combined (excluding nematodes) were significantly greater in eelgrass than in unvegetated habitat at all sampling periods (Table 5.6). Abundances and biomasses of all taxa important in distinguishing between assemblages of the two habitats (Table 5.3) were also significantly greater in eelgrass than in unvegetated habitat at all periods (Table 5.6). Taxa important in distinguishing between assemblages from the two habitats were similar at all periods, with amphipods and harpacticoid copepods important at all periods and tanaids, isopods and polychaetes

important at all but one period. Nematodes were analysed separately but showed the same consistent pattern of greater abundance and biomass in eelgrass than in unvegetated habitat at all periods. When nematodes were included in multivariate analyses of assemblages, they were important at several periods in distinguishing between the two habitats. The inclusion of nematodes did not alter the order of importance of other taxa and altered ANOSIM results very little.

Cumaceans were found in small numbers from a few sites at each sampling period. Although they provided only a minor contribution to differences between assemblages from the two habitats, there is some evidence that cumaceans occur more frequently in unvegetated than in eelgrass habitat. Mean cumacean abundances and biomasses were lower in eelgrass than in unvegetated habitat at all periods (Table 5.7). Cumaceans were absent from many sites in both habitats, and transforming data using $\log_{10} (x + 1)$ failed to render data normal. A non-parametric Mann-Whitney U-test with a correction for tied ranks was therefore used to test whether median cumacean abundance and biomass differed between the two habitats. In January and April 1990 no cumaceans were collected in eelgrass, and no test was done for these periods. Despite mean abundances and biomasses being greater in unvegetated habitat at all periods, no significant differences between medians at any one period were found (Table 5.7). The sign test was used to test the significance of the mean abundance and biomass being higher in unvegetated habitat at all periods ($n = 5$, $p = 0.031$). The evidence from all periods taken together, therefore, is that cumaceans are more often found over unvegetated habitats.

Total epifaunal production and crustacean production for the two habitats are shown for each sampling period in Table 5.8. Results of statistical comparisons between habitats were the same for total production and crustacean production; at all sampling periods production was significantly higher in eelgrass than in unvegetated habitat (ANOVA results; total epifaunal and total crustacean production: All periods, $p < 0.001$).

As stated in Chapter 3, although water temperature and salinity fluctuated between sampling periods, within any period no differences were detected between eelgrass and unvegetated sites.

5.4 Discussion

As expected from previous published work (Orth *et al*. 1984), epifauna associated with eelgrass differed from that associated with unvegetated habitat. Total abundance, biomass and production were greater in eelgrass. Epifaunal assemblages from eelgrass and unvegetated areas were different at all sampling periods. This habitat difference was clearly evident over and above changes in epifauna with time (possibly with a seasonal component), associations of epifauna with water temperature and distance to open water, and differences at some periods between the two waterways. At the taxonomic level used here, differences between assemblages from eelgrass and unvegetated habitats lay mainly in abundances and biomasses of taxa rather than presence or absence of taxa. Cumaceans were an exception, being absent (or at least not collected) at eelgrass sites in January and April 1990. Taxa having much greater abundance in eelgrass habitat included both macrofaunal groups (such as amphipods and isopods) and meiofaunal groups (such as harpacticoid copepods). Several of the taxa found in greater numbers in eelgrass, most notably harpacticoid copepods, amphipods and polychaetes, form a major part of the diet of most fish species associated with shallow inshore areas (Klumpp *et al*. 1989) and of *Sillaginodes punctata* in particular (Chapter 4).

The much greater production of epifauna in eelgrass areas is consistent with a model explaining the importance of seagrass in which small fish are more abundant in seagrass because of greater food availability there.

Table 5.1 List of taxa into which animals were grouped

Abbreviations shown are those used in Table 5.3.

	Crustacea		Non-crustacea
	Brachyura	Pol	Polychaeta
	Caridea		Gastropoda
	Mysidacea		Bivalvia
Amp	Amphipoda - Gammaroidea	Oph	Ophiuroidea
	Amphipoda - Caprellidea		Actiniaria
Tan	Tanaidacea		Echinodermata, larvae
Iso	Isopoda		Chironomid, larvae
	Cumacea		Nematoda
Har	Copepoda - Harpacticoida		
	Copepoda - Cyclopoida		
	Copepoda - Calanoida		
	Copepoda - nauplii (unidentified)		
	Ostracoda		

Table 5.2 Mean ash-free dry weights (mg) of different forms of epifaunal invertebrates in each sieve size-class

Values were calculated using Edgar's (1990a) equation: $\log B = a + b \log S$ (where B = AFDW (mg), S = sieve mesh size (mm) and a and b vary depending on taxonomic category). Sieve size (S) is a geometric mean, as explained in text. Taxa not fitting within any of the named categories shown were treated as general forms. Crustacea includes all crustaceans except caprellid amphipods. No value is shown where category was not found in that sieve size.

Sieve size (mm)	General	Crustacea	Caprellidea	Polychaeta	Mollusca
1.0	0.2448	0.2302	0.1181	0.2440	0.2484
0.5	0.0392	0.0361	0.0316	0.0433	0.0367
0.25	0.0063	0.0057		0.0077	0.0054
0.125	0.0010	0.0009		0.0014	0.0008
0.075	0.0002	0.0002		0.0003	

Table 5.3 Results of ANOSIM comparisons between epifaunal assemblages from eelgrass and unvegetated habitats

Results are probabilities. Contributing taxa are those making a consistently large contribution to differences between samples from the two habitats, listed in order of decreasing importance.

Sampling period	Variable	ANOSIM result	Main contributing taxa
January 1990	Abundance	< 0.001	Iso, Har, Pol, Tan, Amp
	Biomass	< 0.001	Iso, Amp, Har
April 1990	Abundance	< 0.001	Har, Tan, Amp, Pol
	Biomass	< 0.001	Har, Tan, Amp, Pol
August 1990	Abundance	< 0.001	Iso, Har, Pol, Oph, Amp
	Biomass	< 0.001	Iso, Har, Oph, Amp, Pol
October 1990	Abundance	< 0.001	Iso, Amp, Har, Tan
	Biomass	< 0.001	Iso, Har, Tan, Amp
February 1990	Abundance	< 0.001	Har, Iso, Tan, Pol
	Biomass	< 0.001	Tan, Har, Iso, Amp

Table 5.4 Results of two-way ANOSIM comparisons of epifaunal assemblages between habitats and between the two waterways, Barker Inlet and Port River

Results are probabilities (ns = not significant). Results for the factor habitat were all $p < 0.001$, and are not shown. Probabilities for factor waterway relate to the question of whether assemblages differ between waterways after accounting for differences due to habitat, and vice versa for factor habitat.

Sampling period	Variable	ANOSIM result, factor Habitat	ANOSIM result, factor Waterway
January 1990	Abundance	< 0.001	0.001
	Biomass	< 0.001	0.011
April 1990	Abundance	< 0.001	0.285 ns
	Biomass	< 0.001	0.309 ns
August 1990	Abundance	< 0.001	0.356 ns
	Biomass	< 0.001	0.844 ns
October 1990	Abundance	< 0.001	0.005
	Biomass	< 0.001	0.002
February 1990	Abundance	< 0.001	0.241 ns
	Biomass	< 0.001	0.125 ns

Table 5.5 Results of two-way ANOSIM comparisons of epifaunal assemblages between habitats and amongst sampling periods on data from all periods together

Results are probabilities. Pairwise tests are for differences between pairs of periods: Jan = January 1990, Apr = April 1990, Aug = August 1990, Oct = October 1990, Feb = February 1991. Significance level for each pairwise comparison is 0.005 so that overall significance level for ten comparisons is 0.05 (ns = not significant). Pairwise tests for habitat are unnecessary because there are only two habitats.

Variable	Global ANOSIM result	Pairwise comparison	ANOSIM result
Abundance	Habitat < 0.001	Jan, Apr	< 0.001
	Period < 0.001	Jan, Aug	< 0.001
		Jan, Oct	< 0.001
		Jan, Feb	0.020 ns
		Apr, Aug	< 0.001
		Apr, Oct	< 0.001
		Apr, Feb	< 0.001
		Aug, Oct	0.002
		Aug, Feb	0.001
		Oct, Feb	< 0.001
Biomass	Habitat < 0.001	Jan, Apr	< 0.001
	Period < 0.001	Jan, Aug	< 0.001
		Jan, Oct	< 0.001
		Jan, Feb	0.062 ns
		Apr, Aug	< 0.001
		Apr, Oct	< 0.001
		Apr, Feb	< 0.001
		Aug, Oct	0.020 ns
		Aug, Feb	0.083 ns
		Oct, Feb	< 0.002

Table 5.6 Comparisons of abundances and biomasses of total epifauna and key taxa from eelgrass and unvegetated habitats

Abundances are means (individuals/net). Precision estimates are not shown as all means have been tested for significance using ANOVA. ANOVA results are probabilities.

a) January 1990

Taxon	Abundances			Biomasses		
	Eelgrass	Unveg.	ANOVA result	Eelgrass	Unveg.	ANOVA result
All taxa combined	2583	551	< 0.001	66.3	6.6	< 0.001
Amphipods	70	5	0.008	6.9	0.3	0.001
Tanaids	22	6	0.001	2.0	0.3	0.009
Isopods	47	1	< 0.001	26.9	0.3	< 0.001
Harpacticoids	2076	412	< 0.001	7.4	1.8	< 0.001
Polychaetes	128	40	0.001	7.0	1.3	0.003
Nematodes	832	148	< 0.001	4.9	0.8	< 0.001

b) April 1990

Taxon	Abundances			Biomasses		
	Eelgrass	Unveg.	ANOVA result	Eelgrass	Unveg.	ANOVA result
All taxa combined	1462	302	< 0.001	24.5	8.2	< 0.001
Amphipods	90	15	< 0.001	9.2	2.1	0.002
Tanaids	23	1	< 0.001	3.2	0.1	< 0.001
Harpacticoids	1242	261	< 0.001	4.2	0.9	< 0.001
Polychaetes	15	7	0.060	1.3	0.6	0.020
Nematodes	854	163	< 0.001	3.0	0.5	< 0.001

c) August 1990

Taxon	Abundances			Biomasses		
	Eelgrass	Unveg.	ANOVA result	Eelgrass	Unveg.	ANOVA result
All taxa combined	1174	151	< 0.001	29.3	2.6	< 0.001
Amphipods	69	3	< 0.001	14.4	0.3	< 0.001
Isopods	7	0	< 0.001	5.1	0.0	< 0.001
Harpacticoids	1014	102	< 0.001	3.2	0.3	< 0.001
Polychaetes	33	3	< 0.001	0.9	0.3	0.005
Ophiuroids	3	0	0.001	0.7	0.0	0.006
Nematodes	792	185	0.007	2.8	0.5	0.001

Table 5.6 (cont.)

d) October 1990

Taxon	Abundances			Biomasses		
	Eelgrass	Unveg.	ANOVA result	Eelgrass	Unveg.	ANOVA result
All taxa combined	2660	439	< 0.001	53.1	5.2	< 0.001
Amphipods	139	6	< 0.001	11.7	0.8	< 0.001
Tanaids	73	1	< 0.001	3.6	0.1	0.010
Isopods	6	0	< 0.001	2.8	0.0	< 0.001
Harpacticoids	1789	181	< 0.001	8.4	0.7	< 0.001
Nematodes	1321	110	< 0.001	6.2	0.4	< 0.001

e) February 1991

Taxon	Abundances			Biomasses		
	Eelgrass	Unveg.	ANOVA result	Eelgrass	Unveg.	ANOVA result
All taxa combined	1443	196	< 0.001	28.6	4.3	< 0.001
Amphipods	34	8	0.001	4.2	0.6	0.001
Tanaids	26	0	< 0.001	1.4	0.0	0.001
Isopods	17	1	< 0.001	9.5	0.3	< 0.001
Harpacticoids	1195	152	< 0.001	4.1	0.5	< 0.001
Polychaetes	40	12	0.011	2.8	0.5	0.012
Nematodes	809	203	0.002	3.5	0.7	< 0.001

Table 5.7 Comparisons of abundances and biomasses of cumaceans from eelgrass and unvegetated habitats

Abundances are means (individuals/net) with medians shown in parentheses. Precision estimates are not shown as medians have been tested for significance using Mann-Whitney U-tests. Mann-Whitney U-test results are probabilities; all are not significant at $\alpha = 0.05$. Tests were not done for periods when no cumaceans were collected from eelgrass habitat (January 1990 and April 1990).

Sampling period	Abundances			Biomasses		
	Eelgrass	Unveg.	M-W result	Eelgrass	Unveg.	M-W result
January 1990	0 (0)	0.727 (0)		0 (0)	0.097 (0)	
April 1990	0 (0)	0.100 (0)		0 (0)	0.023 (0)	
August 1990	0.125 (0)	1.000 (1)	0.062	0.005 (0)	0.175 (0.036)	0.053
October 1990	0.750 (0)	3.857 (2)	0.111	0.051 (0)	0.233 (0.072)	0.200
February 1991	0.400 (0)	2.778 (0)	0.208	0.014 (0)	0.402 (0)	0.170

Table 5.8 Total epifaunal production and total crustacean production
(μg/day/0.0625m^2) in eelgrass and unvegetated habitats at each sampling period

Numbers are means. Precision estimates are not shown as all means have been tested for
significance using ANOVA. ANOVA results for total epifaunal production are the same
as those for total biomass in Table 5.5.   ANOVA results for total crustacean production
are $p < 0.001$ at all sampling periods.

Sampling period	Total epifaunal production		Total crustacean production	
	Eelgrass	Unveg.	Eelgrass	Unveg.
January 1990	539	86	407	53
April 1990	249	94	212	46
August 1990	199	27	171	12
October 1990	391	59	241	37
February 1991	339	73	265	39

Fig. 5.1 Two-dimensional MDS ordination plots of epifaunal assemblages based on abundance: (a) January 1990 (stress value (Kruskal's formula 1) = 0.127),
(b) April 1990 (0.141), (c) August 1990 (0.076), (d) October 1990 (0.088),
(e) February 1991 (0.109)
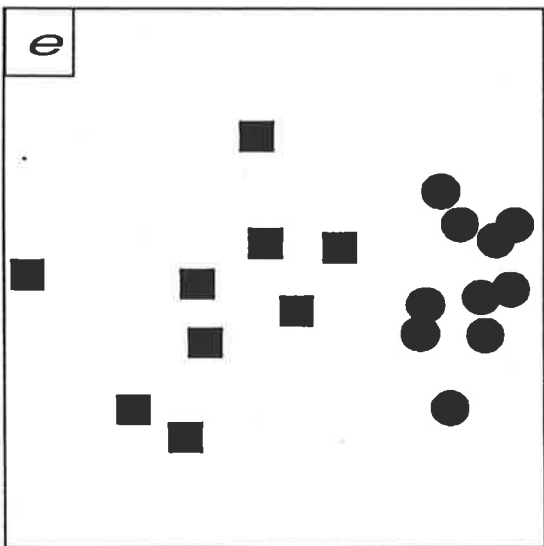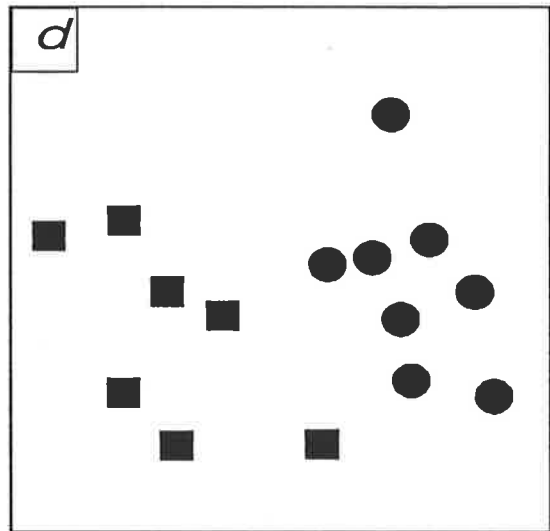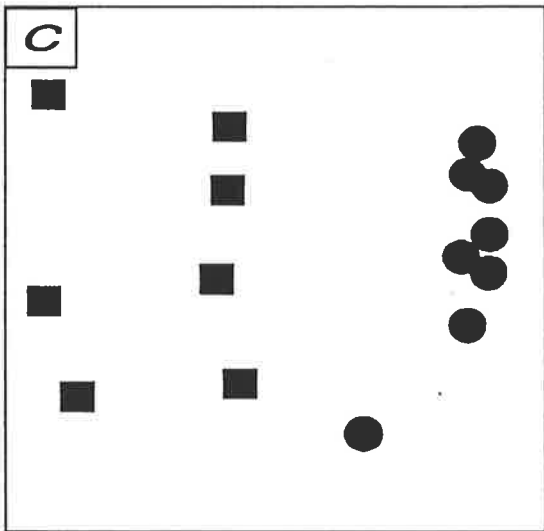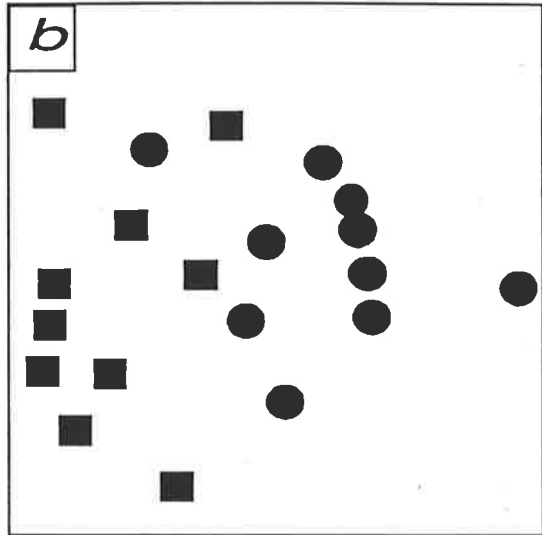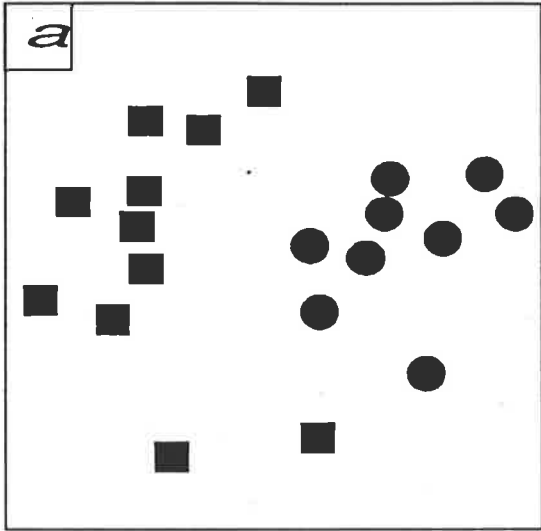
Eelgrass ●

Unvegetated ■

Fig. 5.2 Two-dimensional MDS ordination plots of epifaunal assemblages based on biomass: (a) January 1990 (stress value = 0.135), (b) April 1990 (0.153), (c) August 1990 (0.061), (d) October 1990 (0.099), (e) February 1991 (0.118)
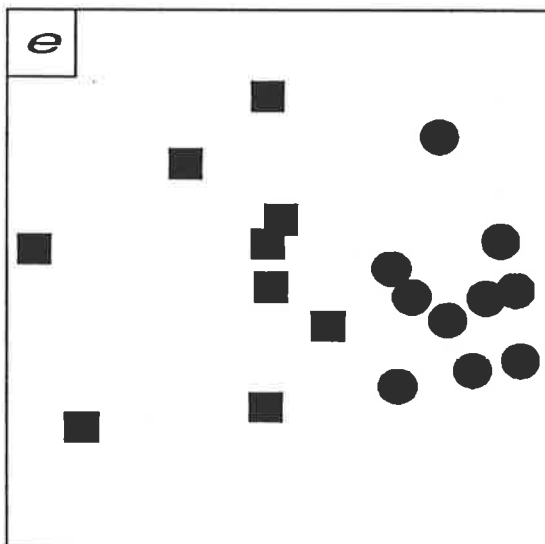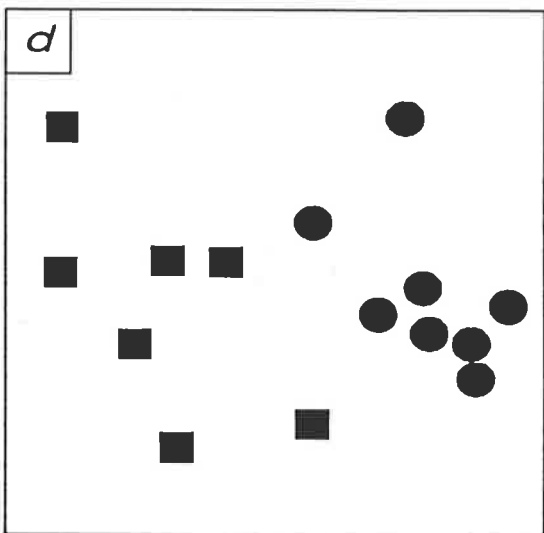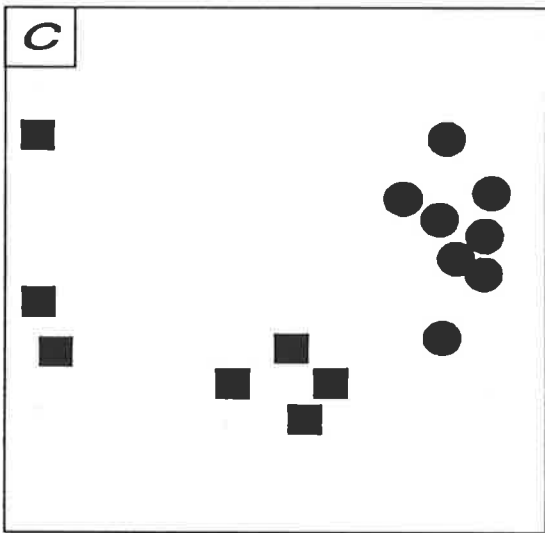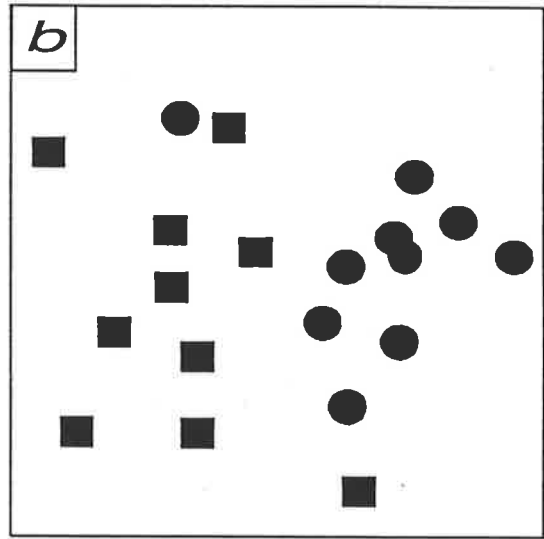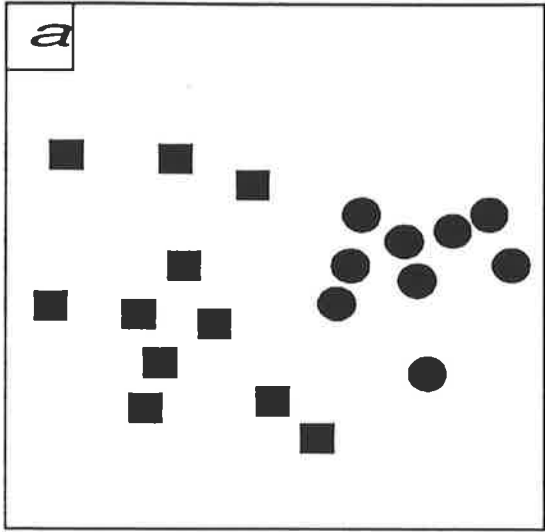
Eelgrass

Unvegetated

Fig. 5.3 Overlay of distance from site to open water onto MDS ordination plots for August 1990 using a) abundance (see Fig. 5.1c) and b) biomass (see Fig. 5.2c). Diameter of circle is proportional to distance. Smallest circle in any plot = 1.0 km, largest = 9.1 km
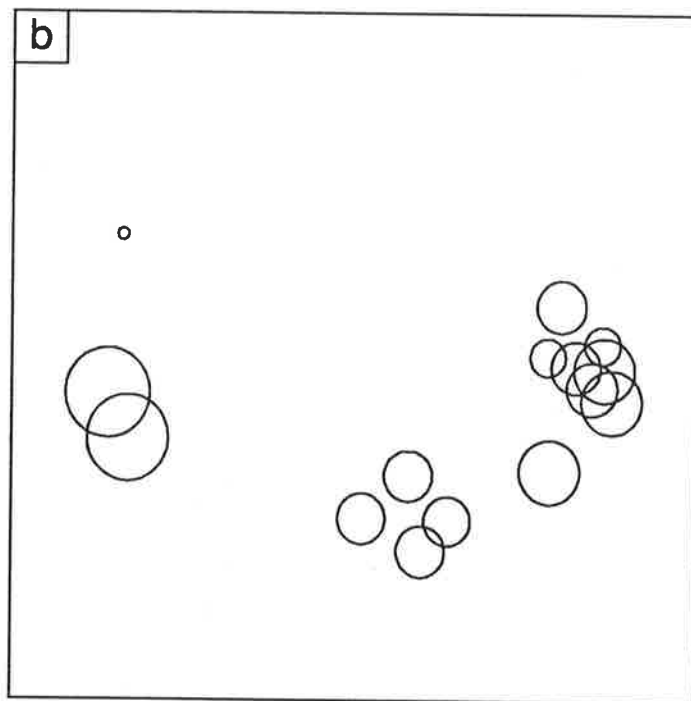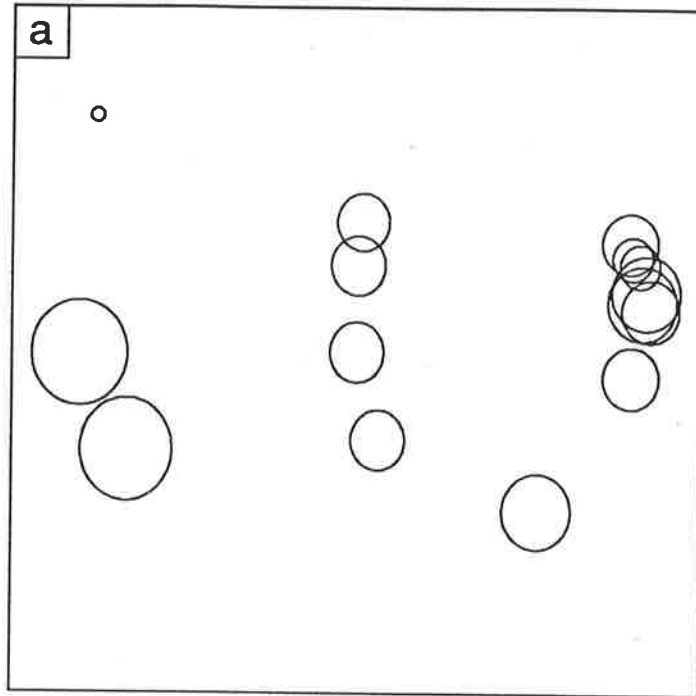
Fig. 5.4 Two-dimensional MDS ordination plot of epifaunal assemblages over all periods combined. Stress = 0.166. Sites of a given habitat (eelgrass or unvegetated) at a given period have been combined and plotted at their centroid. Periods are indicated by numbers: 1 = January 1990, 2 = April 1990, 3 = August 1990, 4 = October 1990, 5 = February 1991
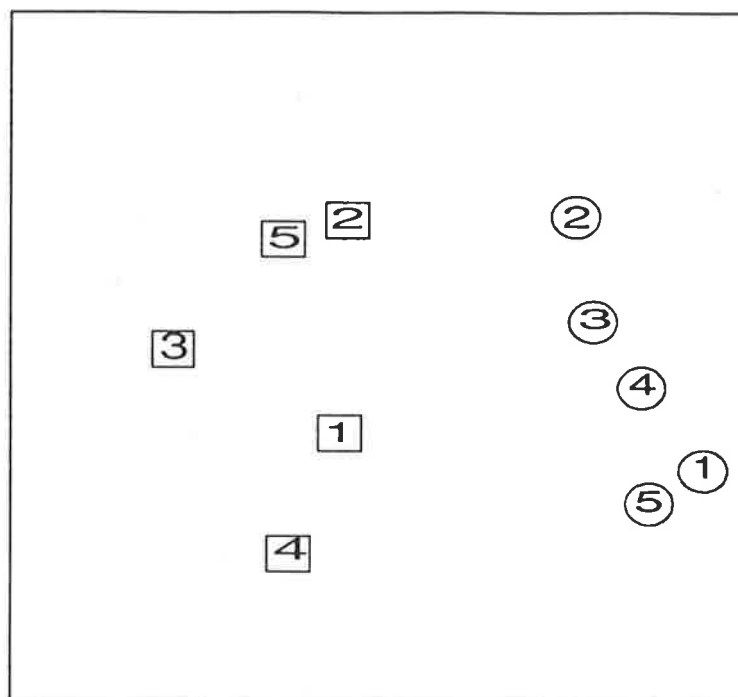
○ Eelgrass

□ Unvegetated

Chapter 6

Removal of seagrass canopy: effects on small fish

6.1 Introduction

Unvegetated areas adjacent to seagrass meadows typically have different fish assemblages, usually with fewer fish and fewer species (Bell & Pollard 1989); this pattern has been demonstrated in Chapter 3 for the Barker Inlet region. According to the model of Bell & Westoby (1986b), the lower abundance of fish in unvegetated areas is the result of fish choosing to settle in seagrass beds in preference to adjacent unvegetated areas. The difference between fish assemblages of seagrass and unvegetated areas is, however, only an association. Fish may simply be attracted to more abundant food in seagrass. Environmental factors (such as measures of water quality) concomitant with, or resulting in, seagrass absence may also be the cause of differing fish assemblages.

Attempts to demonstrate the importance of seagrass have mostly involved the construction of patches of artificial seagrass in unvegetated areas. The question posed is: what is the effect on fish of placing seagrass mimic in positions having all other factors consistent with absence of seagrass? An alternative is to remove seagrass from areas where it is naturally occurring. The question them becomes: what is the effect on fish of removing seagrass from positions having all other factors consistent with seagrass presence? This more closely matches the question: what is the effect of seagrass loss on fish? The disadvantages of seagrass removal are firstly that regrowth necessitates either a short-term experiment or repeated removal, and secondly that seagrass removal is irresponsible except when working with species that recover quickly.

The aim of the present study was to determine the effects on small fish distribution of removing above-ground vegetation (seagrass canopy). If the seagrass canopy is important, for whatever reason, then patches from which the vegetation has been removed should support fewer fish and different fish assemblages than seagrass patches. Moreover, if the seagrass canopy is the important difference between seagrass and unvegetated habitat, then fish assemblages associated with patches from which the seagrass canopy has been removed should match assemblages from patches which were unvegetated prior to the experiment. If small fish are less abundant in unvegetated patches because they do not settle there then, as predicted above, the numbers of fish in patches cleared of seagrass should match the number from areas unvegetated prior to the experiment. If, on the other hand, small fish are attracted to seagrass directly to feed upon more abundant prey, then the number of fish in patches cleared of seagrass should match prey abundance and production associated with the modified habitat and will not necessarily be the same as fish numbers from areas unvegetated prior to the experiment. Prey abundance was measured as part of this experiment and is reported in Chapter 7.

The contents of this chapter are substantially the same as those in Connolly (in press) entitled "Removal of seagrass canopy: effects on small fish and their prey", which is included as Appendix A.3. A summary of results of prey availability is included in the paper but these have been presented more fully in Chapter 7 of this thesis.

6.2 Materials and Methods

The Barker Inlet - Port River region is strongly tidal, typically with two tides per day, with a maximum tidal amplitude of 2 m, and fish occupying the lower intertidal zone must choose anew the habitat over which they swim on every incoming tide. The experiment was situated in an area dominated by *Zostera muelleri*, a fast growing, colonising species. The experiment was done in September 1991, and was timed to coincide with the seasonal recruitment into the estuary of juvenile *Sillaginodes punctata*.

Fish were collected from the following four habitats (treatments) marked as 5.5 x 5.5 m squares:

1) eelgrass in natural state (control = C),

2) eelgrass removed by cutting with shears at the sediment surface whilst emergent on low tides (removed = R),

3) eelgrass uncut, but with equivalent time and effort spent at site mimicking cutting (procedural control = P), and

4) unvegetated mudflat (unvegetated = U).

Six eelgrass sites were assigned to each of the first three treatments in a randomised block design. That is, one replicate of each of the first three treatments was assigned at random to six randomly selected areas (blocks) along a 1 km stretch of shore. The unvegetated treatment could not be randomly assigned. Instead, the nearest unvegetated site to the block occurring at the same height in the intertidal was selected as the unvegetated patch. The blocked design guaranteed interspersion, which is important because of the potential patchiness of fish abundances.

Patches were prepared over several days, and fish were collected 14 days later. This was a short enough interval to avoid eelgrass regrowth. The order in which patches were prepared and therefore netted was chosen so that on any day only one patch within a

block was netted, so as to avoid disturbance of nearby patches. During the experiment the netting schedule was disrupted by inclement weather and attempts to collect fish from one block were abandoned in a bid to return to schedule. Fish were collected only from a 5 x 5 m square in the centre of each patch, avoiding the edges of habitats. Fish were netted using a buoyant pop net released in water depths from 40 - 100 cm on an incoming daytime tide. The pop net was designed to collect fish neatly from experimental plots, a situation for which more conventional seine netting is too unwieldy (Chapter 2). All fish were identified and counted. Species considered to be pelagic (*Atherinosoma microstoma* - Atherinidae, *Arripis georgianus* Valenciennes - Arripidae and *Spratelloides robustus* - Clupeidae) were excluded from some analyses.

The amount of food available to fish within each patch was estimated by sampling the epifauna. The results of these collections are reported in Chapter 7.

The surface area of eelgrass leaves within all patches that supported eelgrass prior to the experiment was estimated before setting up the experiment and again on the day after fish collection. Leaf area was calculated for each patch from measurements of the number of leaves per 400 cm^2 quadrat, and the length and width of ten leaves, at five randomly selected sites. Prior to the experiment, leaf area did not differ between patches selected for the three treatments involving eelgrass (C: 1.54 m^2 leaf area/ m^2 sediment surface; P: 1.31; R: 1.39; ANOVA: p = 0.651). After removal, the leaf area within patches of treatment R was reduced almost to zero, whilst patches of P remained similar to patches of C (C: 1.55; P: 1.46; R: 0.02; ANOVA: p < 0.001; Tukey HSD pairwise comparisons: C P R).

Data Analysis

The number of fish (all species combined and key species separately) from the four habitats were compared using a randomised block analysis of variance (ANOVA); this is equivalent to a mixed model, two-way ANOVA without replication, in which "habitat" is the fixed factor and "block" the random factor. Results of the significance test for effects of block have been reported, but should be treated cautiously since they depend on the untested assumption that the interaction effect is small (Zar 1984). Furthermore, the intention of allocating treatments to blocks was to guarantee interspersion of treatments rather than to search for differences in fish abundances along the coast. However, by removing the variance due to block, a more sensitive test for differences amongst habitats is made than would be the case with a simple one-way ANOVA. Significant ANOVA results were followed by Tukey HSD pairwise comparisons between habitat means. The order in which means are displayed in Tukey results are based on means of transformed data and therefore sometimes varies slightly from the order of mean raw abundance shown in Table 6.1. *Atherinosoma microstoma* is a species that schools strongly, unlike the other species analysed. This behaviour results in large fluctuations in number per net since catch rates are either zero or, if a school happens to be caught, in the order of 100 individuals. $\text{Log}_{10} (x + 1)$ transformation failed to render data normal, and *A. microstoma* numbers were therefore analysed using Friedman's non-parametric equivalent to the ANOVA described above (Zar 1984). Sample variances generally increased with increasing means, and analyses were performed on $\log_{10}$ transformed data after checking that the transformation increased homoscedasticity. Significance levels are 0.05 throughout.

Fish assemblages from the four habitats were compared using an analysis of similarities (see Chapter 3 for discussion of the advantages of this method). ANOSIM compares ranked similarities between and within groups selected *a priori* (here the four habitats). Since habitat differences could have been obscured by any block effect, a two-way

ANOSIM without replication (randomised block ANOSIM), equivalent to the univariate ANOVA described above, was also used to test simultaneously for differences amongst habitats and blocks (Clarke & Warwick 1994). Although this test is more sensitive to differences amongst habitats than a one-way ANOSIM, it cannot be used to find differences between pairs of habitats once a significant global habitat effect has been shown (Clarke & Warwick 1994). Pairwise ANOSIM tests were therefore done following the one-way ANOSIM. All ANOSIM tests involved 5000 simulations using the PRIMER package.

The relationships amongst assemblages from each patch are graphically represented using non-metric multidimensional scaling (MDS), which is described in Chapter 3. For comparisons of fish assemblages among the four habitats, raw counts were transformed using $x^{0.25}$ for the reasons described in Chapter 3. The Bray-Curtis similarity coefficient was used.

6.2 Results

A total of 2170 fish of 11 species were caught during the study, including 504 individuals of the three species categorised as pelagic. The mean number of individuals of each species and for all species together from each habitat is shown in Table 6.1.

Total fish abundance in habitat P was greater than in habitat U. Fish abundances in habitats C and R were not different from each other and were intermediate between the other two habitats (ANOVA: Habitat - $p = 0.034$, Block - $p = 0.654$; Tukey: P C R U).

Excluding pelagic species, more fish were caught in habitats C, P, and R than in U. Differences between catches in the first three habitats were not significant (ANOVA: Habitat - $p = 0.003$, Block - $p = 0.232$; Tukey: <u>R P C</u> U).

Abundances of *Sillaginodes punctata* were higher in habitat R than in U. Abundances in habitats P and C were not different from each other and were intermediate between the other two habitats (ANOVA: Habitat - $p = 0.022$, Block - $p = 0.051$; Tukey: <u>R P C</u> U).

Comparisons for *Favonigobius lateralis* and *Atherinosoma microstoma* detected no significant differences in abundance amongst habitats (*F. lateralis* - ANOVA: Habitat - $p = 0.498$, Block - 0.182; *A. microstoma* - Friedman's: Habitat - $p = 0.316$, no test for block). These non-significant results are more meaningful if the statistical power of the tests is examined. In the case of the fixed factor in a randomised block ANOVA, effect size can be specified as the difference between the two most extreme means (Zar 1984). For the test amongst means of *F. lateralis* abundance, I consider it important to detect a departure from the null in which one treatment has a mean 50% lower than other treatments. An example of this effect size for *F. lateralis* would be the following means (units are fish/net): $C = P = R = 50$, $U = 25$. On $\log_{10}$ data this translates to: $C = P = R = 1.7$, $U = 1.4$. The chance of detecting a difference amongst habitats in mean abundance of *F. lateralis* with the effect size specified above was 0.22 ($\beta = 0.78$) (Equ. 13.33; Zar, 1984). No formal power calculations are possible on the Friedman's non-parametric test of *Atherinosoma microstoma* abundances, but it is possible to apply the known power efficiency of Friedman's test compared with the equivalent ANOVA (0.76 for 4 treatment means; Zar, 1984) to an estimate of what power would have been if an ANOVA had been applied to the data. The effect size for *A. microstoma* would be similar to that for *F. lateralis* and degrees of freedom are identical, but variance is larger and therefore power would be something less than the figure of 0.22 for *F. lateralis*. This figure would be reduced further upon application of the power efficiency factor

(multiply estimated power of ANOVA by 0.76), and the best estimate of power to detect a difference amongst median abundances of *A. microstoma* is therefore considerably less than 0.2. The low power in tests of *F. lateralis* and *A. microstoma* abundances suggests that, although no differences were detected, it should not be concluded that there are no biologically important differences amongst abundances of these species. Rather, the test results demonstrate a need for increased numbers of patches.

No clear differences between habitats are discernible in the ordination plots showing relationships amongst fish assemblages from each patch for all fish species (Fig. 6.1a) and non-pelagic species only (Fig. 6.2a). Ignoring any block effects, statistical comparisons of fish assemblages found no significant differences amongst habitats whether or not pelagic species were included (One-way ANOSIM: All fish - $p = 0.526$; Pelagic species excluded - $p = 0.663$). Ordination plots including (Fig. 6.1b) and excluding (Fig. 6.2b) pelagic species show some signs of grouping according to block. Block effects are not significant, however, and nor are differences amongst habitats after removing effects of block (Two-way ANOSIM: All fish, Factor Habitat - $p = 0.098$, Factor Block - $p = 0.592$; Pelagic species excluded, Habitat - $p = 0.328$, Block - $p = 0.115$). No formal power calculations are currently possible with the ANOSIM method, but the small number of replicate patches serves as a reminder that a Type II error is possible.

6.4 Discussion

The differences in fish catches from patches of the four habitats were in overall fish abundance and abundance of *Sillaginodes punctata* rather than in assemblage composition. Surveys of eelgrass and unvegetated patches in the region (Chapter 3) showed unequivocal differences between assemblages of the two habitats at all times of year. The patch sizes of unvegetated habitat in the present experiment were smaller than the smallest patches netted during survey work, and this difference in scale may explain why assemblage differences between undisturbed eelgrass and unvegetated patches were not apparent in the current experiment. Ferrell & Bell (1991) have shown that the distance of unvegetated sites from eelgrass affects how different the fish assemblages are from those of adjacent *Zostera* beds. The total area netted in this experiment was only about one third of the area netted with seine nets during each survey period. Less common species were therefore less likely to be caught during this experiment, and so species typical of a habitat without being abundant there, such as the syngnathid *Stigmatopora nigra* and the odacid *Haletta semifasciata* (Valenciennes) from eelgrass habitat, were not caught. The number of *Favonigobius lateralis* was similar at all habitats. This species was also found in similar numbers over eelgrass and unvegetated habitat during survey work. *F. lateralis* individuals are intimately associated with the seabed and even in eelgrass areas tend to occur in bare patches between clumps of eelgrass. The fish are well camouflaged when they are over sediment.

Total fish numbers tended to be lower over habitat U than over the other habitats, and these differences were clearer when pelagic species were excluded. A similar pattern was found for numbers of *Sillaginodes punctata*. Surveys in Chapter 3 also showed lower total fish abundances and fewer *S. punctata* over unvegetated habitat. The disturbance associated with eelgrass removal (habitat P) on its own had no marked effect on fish numbers.

If the eelgrass canopy itself is the characteristic of eelgrass habitat important in attracting an increased abundance of small fish compared with adjacent unvegetated areas, then fish numbers should have declined in the treatment from which eelgrass was removed. In this experiment fish numbers over habitat R were a little lower than in habitat P, but did not match the much lower numbers found in habitat U. Moreover, when considering only non-pelagic species, for which benthic habitat was expected to be especially important, numbers over habitat R were not lower than in habitats C and P. It must be concluded that over the length of this experiment, removal of eelgrass canopy did not cause fish to distribute themselves in a way consistent with the predictions of a model in which the eelgrass canopy alone is of major importance to small fish.

Two other possible explanations for the failure of fish numbers to fulfil expectations need examining. Firstly, the duration of the experiment was short relative to the seasonal settlement patterns of fish, and longer term manipulations of habitat, provided that they deal adequately with seagrass regrowth, may allow time for changes in physical factors such as sediment grain size affected by the presence of seagrass. As a test of the importance of seagrass canopy *per se*, however, the duration of this experiment was satisfactory, because fish were forced away from the area on every low tide, and could be expected to redistribute themselves semi-diurnally. Secondly, since patches of habitat U did not receive the disturbance inflicted on patches of R during eelgrass removal, the greater abundance of fish in habitat R compared with that in habitat U could be the result of the difference in degree of disturbance. Another treatment in which unvegetated patches received the disturbance of simulated eelgrass removal could have been used. The same disturbance in eelgrass patches did not alter fish numbers, generating some confidence that disturbance was not important when comparing habitat R with habitat U; that possibility has not, however, been altogether removed.

Table 6.1 Fish abundance by habitat

Numbers for each habitat are means, with standard errors in parentheses (n = 5).
Total abundance is number of individuals.

	Control	Procedural control	Removed	Unvegetated	Total abundance	% of all fish
Favonigobius lateralis	37.2 (5.9)	56.8 (17.3)	49.6 (12.4)	26.4 (5.3)	850	39
Sillaginodes punctata	45.0 (17.7)	40.6 (9.8)	51.8 (14.9)	14.2 (3.3)	758	35
Atherinosoma microstoma	62.2 (57.1)	29.8 (18.1)	0.4 (0.4)	5.0 (4.5)	487	22
Kaupus costatus	1.2 (0.4)	1.6 (0.9)	0.8 (0.4)	0.2 (0.2)	19	1
Tetractenos glaber	1.2 (0.7)	0.8 (0.4)	0.6 (0.4)	0.8 (0.6)	17	1
Spratelloides robustus	0.4 (0.4)	2.8 (2.8)	0	0	16	1
Gymnapistes marmoratus	0.6 (0.4)	1.4 (1.2)	0.2 (0.2)	0.4 (0.4)	13	1
Heteroclinus perspicillatus	0.8 (0.5)	0.2 (0.2)	0	0	5	< 1
Rhombosolea tapirina	0	0	0.2 (0.2)	0.4 (0.4)	3	< 1
Arripis georgianus	0	0	0	0.2 (0.2)	1	< 1
Hyporhamphus melanochir	0.2 (0.2)	0	0	0	1	< 1
All species combined	148.8 (55.7)	134.0 (28.4)	103.6 (20.5)	47.6 (9.7)	2170	

Fig. 6.1 Two-dimensional MDS ordination plot of fish assemblages, all species included: a) by habitat, C = Control, P = Procedural control, R = Removed, U = Unvegetated, and b) by block. Stress value (Kruskal's formula 1) = 0.162
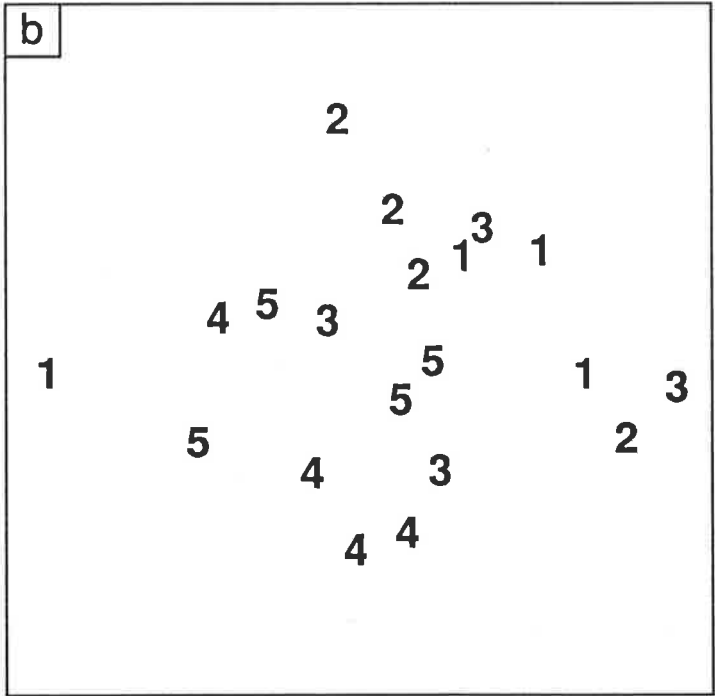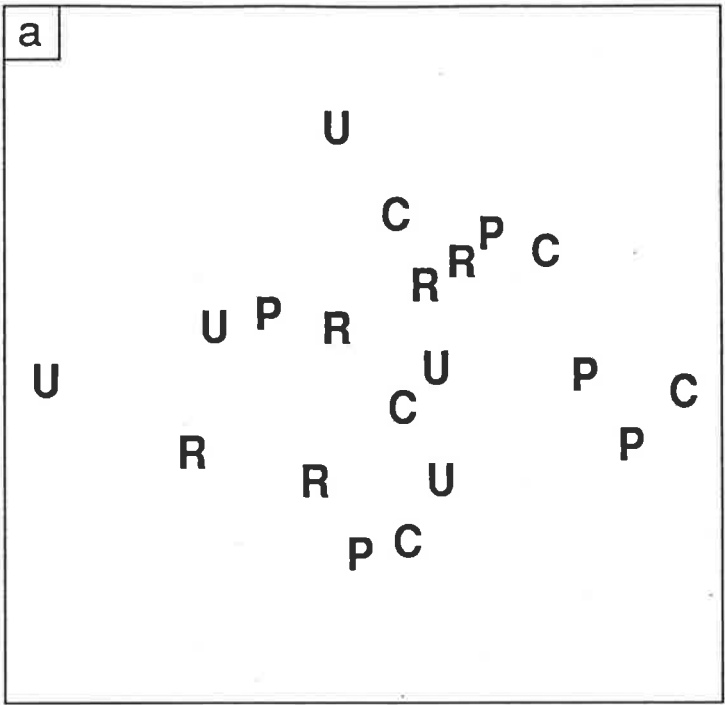
Fig. 6.2 Two-dimensional MDS ordination plot of fish assemblages, pelagic species excluded: a) by habitat, lettering as for Fig. 6.1, and b) by block. Stress value (Kruskal's formula 1) = 0.136

a

b
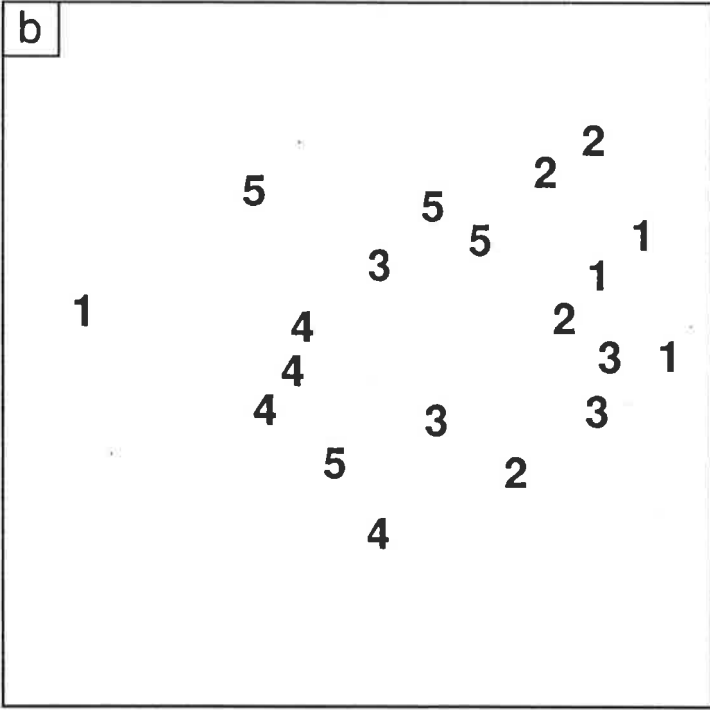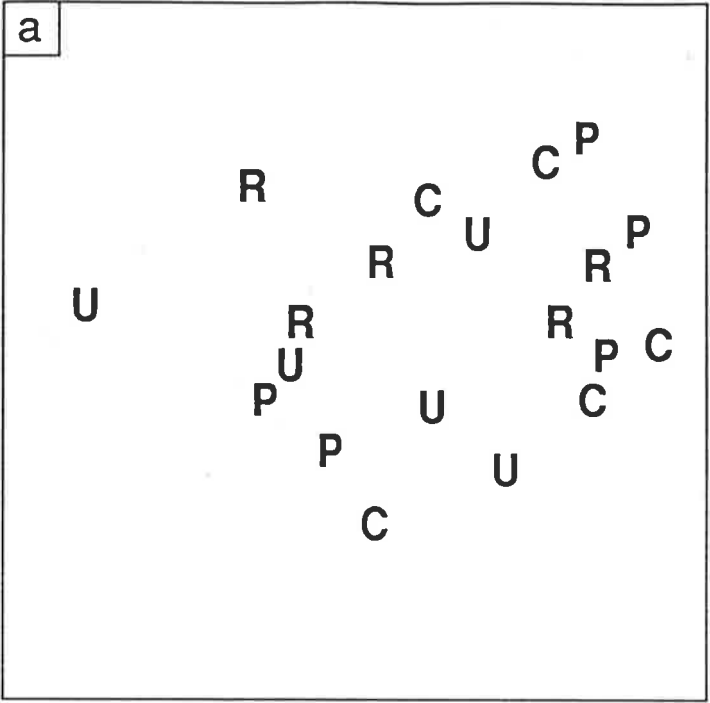
Chapter 7

Removal of seagrass canopy: effects on epifauna

7.1 Introduction

When patches of eelgrass canopy were removed in the experiment reported in Chapter 6, abundances of small fish did not decline to levels found over patches unvegetated prior to the experiment. This result is not consistent with predictions from a model in which the greater abundance of small fish in seagrass compared with unvegetated habitat is explained by habitat selection. If, however, small fish are attracted to seagrass directly to feed upon more abundant prey, then the number of fish in patches cleared of seagrass should match prey abundance and production.

Epifaunal invertebrates, especially crustaceans, are the predominant prey of most small fish associated with soft-substratum habitats (Klumpp *et al.* 1989, and see Chapter 4). The abundance of epifaunal invertebrates associated with seagrass is usually greater than that associated with adjacent unvegetated patches (Orth *et al.* 1984), and has been shown to be so in the Barker Inlet region (Chapter 5).

In this chapter, the epifauna associated with the four habitats from which fish were sampled in Chapter 6 is described and compared, with the aim of determining whether levels of prey availability match abundances of small fish more closely than do levels of eelgrass canopy cover.

The contents of this chapter are substantially the same as those in the manuscript submitted for publication entitled "The effects of removal of seagrass canopy on assemblages of small, motile invertebrates", which is included as Appendix A.4. This

chapter, however, includes a synthesis of fish and invertebrate data which is part of Connolly (in press) included as Appendix A.3.

7.2 Materials and Methods

Epifauna were collected from the 24 plots in the experiment described in Chapter 6. The experimental treatments and selection of sites are as described in Chapter 6. Epifauna were sampled on the day prior to collection of fish (i.e. 13 days after preparation of treatments). Epifauna were sampled from patches in the block not sampled for fish (Chapter 6), and data from patches in this block are included in comparisons of epifauna from the four habitats but are excluded from the test of association between fish abundances and epifaunal production.

Epifauna were collected from three randomly placed sites within each patch, subject to the restriction that a 0.5 m wide strip around the perimeter of the patch was avoided. Collections were made on the daytime rising tide in water depths between 30 and 50 cm. The methods of collection, sorting and counting were as described in Chapter 5. Taxa were similar to those listed in Table 5.1 and are listed in Table 7.1. As in Chapter 5, nematodes were treated separately in analyses because they are typically not an important component of the diets of small fish. Nematode numbers are excluded from estimates of total epifaunal abundance.

Ash-free dry weights (AFDW) were calculated as in Chapter 5, except that animals retained in the largest sieve-size (2 mm) were not weighed. Rather, the mean weight of these animals was estimated by arbitrarily setting the upper mesh-size at 4 mm. The AFDW of animals retained on the 2 mm mesh was therefore calculated as if all animals had first passed through a 4 mm sieve. The mean AFDW of animals in different categories retained on a 2 mm sieve (which can be added to the values for finer sieves

shown in Table 5.2) were as follows (in mg): General, 1.5291; Crustacea, 1.4673; Caprellidea, 0.4409; Polychaeta, 1.3745; Mollusca, 1.6802. Although Edgar (1990a) recommends that animals retained on the largest sieve-size be weighed directly (the procedure used in Chapter 5), this is labour intensive. The setting of an upper mesh-size of 4 mm underestimates the AFDW of the few exceptionally large individuals caught. These animals, such as smooth pebble crabs (*Philyra laevis* Bell), juvenile blue swimmer crabs (*Portunus pelagicus* L.), and large specimens of shrimp, are too large to be eaten by the small fish targeted during fish collections. According to G. Edgar & C. Shaw (unpublished data, cited in Edgar *et al.* (1994)), for example, fish settling in shallow, soft-substratum habitats mainly eat prey of a size retained on mesh in the range 0.25 to 2 mm. The arbitrary setting of an upper mesh-size of 4 mm, whilst being less accurate than the direct weighing of large individuals, is much faster, and tends to down-play the importance of large animals which are of marginal relevance as fish prey.

Epifaunal production was estimated using the method described in Chapter 5, using the mean water temperature measured on collection days of $16.0^{\circ}C$ in Edgar's (1990a) equation relating daily production to biomass and temperature.

The surface area of eelgrass leaves before setting up the experiment and at the time of sampling epifauna is reported in Chapter 6.

Data Analysis

Epifaunal assemblages (described both by abundance and biomass (AFDW)) from the four habitats were compared using ANOSIM. A two-way ANOSIM without replication (randomised block ANOSIM, as used on fish assemblages in Chapter 6) was used to test for habitat and block effects, using averages of the three samples from each patch. This analysis makes a sensitive test for habitat effects, but cannot be used to find differences

between pairs of habitats once a significant global habitat effect has been shown (Clarke & Warwick 1994). A nested ANOSIM was also done, ignoring blocks, and treating patches as a nested factor (patch) within the main factor (habitat). This nested ANOSIM tested whether assemblages differed amongst the four habitats by treating the three samples from each patch as a single collective estimate of the fauna from the patch. After a significant difference was detected using this global ANOSIM test, ANOSIM was employed to test pairwise differences between habitats. All ANOSIM tests involved 5000 simulations using the PRIMER package.

The relationships amongst assemblages from each patch are graphically represented using non-metric multidimensional scaling (MDS, described in Chapter 3). The ordinations presented were done on data averaged over the three samples within each patch to simplify presentation and make habitat groupings clearer. Ordinations were also done using all 72 individual samples, and the resultant habitat groupings were very similar to groupings using averaged data.

Data were transformed as in Chapter 6, and the Bray-Curtis similarity coefficient was again used. The similarity matrix used in MDS and ANOSIM has also been used to highlight taxa making a large contribution to between-group differences (see Chapter 3). The association between epifaunal assemblages and the position of a patch along the shore was measured using the weighted Spearman coefficient (ρ_w) as described in Chapter 3. The position of a patch was described as the distance from the first patch at one end of the experiment. For this analysis the data from the three samples within a patch were averaged to give just one assemblage per patch.

The abundance and biomass of epifauna (all taxa combined and key taxa separately) and total epifaunal and crustacean production from the four habitats were compared using analysis of variance (ANOVA). With three samples from each patch, the design could be considered as a two-way (randomised block) ANOVA with no replication, with multiple

values within a patch nested within the (figurative) interaction term. Adding a nested factor to a randomised block ANOVA, which in itself requires some risky assumptions, would make a very tenuous test given the small number of samples at all levels. I avoided this by testing data in two ways, matching the multivariate analyses described above. First, the three measures within a patch were averaged and data were tested using the same randomised block ANOVA used to test fish abundances. Since the primary aim of allocating treatments to blocks was to guarantee interspersion rather than to search for differences along the coast, I then tested by ignoring blocks and treating patches as a nested factor (patch) within the main factor (habitat). Where habitat differences were significant, Tukey HSD pairwise comparisons of habitat means used variance estimates from replicate patches, not from samples nested within patches. Sample variances increased with increasing means, and all univariate analyses were performed on $\log_{10} x$ transformed data (or $\log_{10} (x + 1)$ where zeros occurred) after checking that the transformation increased homoscedasticity. Significance levels are 0.05 throughout.

Pearson's r-test was used to detect association between fish abundances reported in Chapter 6 and total epifaunal production by patch.

7.3 Results

Two-dimensional ordination plots showed a very similar pattern on both abundances (Fig. 7.1a) and biomasses (Fig. 7.1b). Assemblages from patches of habitat U were grouped separately from those of other habitats. Differences amongst the other three habitats were less obvious. Assemblages from habitats C and P overlapped considerably, and those from habitat R, whilst overlapping with C and P, tended to group more distinctly and be positioned closer to habitat U. The two-way ANOSIM without replication showed a strong effect of habitat and not of block on both abundance and

biomass (Abundance: Habitat, $p = 0.008$, Block, $p = 0.55$; Biomass: Habitat, $p < 0.001$, Block, $p = 0.95$). The nested ANOSIM test for differences amongst habitats detected significant differences using both abundance and biomass data (Table 7.2). On abundance data, assemblages from habitat U were different from those of all other habitats, and no differences were detected amongst habitats C, P and R. On biomass data, assemblages from habitat U were different from all other habitats, habitats C and P were not separate, and habitat R was different from C but not from P (Table 7.2). The ANOSIM results confirm the patterns evident in ordination plots, except that habitat R was found to be intermediate between habitat U and habitats C and P on biomass data only. Assemblages differed amongst patches within habitats using both abundance and biomass data.

The correlation between similarities in epifaunal assemblages and in positions of patches along the shore was close to zero for both abundance ($\rho_w = 0.03$) and biomass ($\rho_w = 0.04$). Although no formal test of this correlation is currently available, these values are extremely low (Clarke & Ainsworth 1993) and provide evidence that there was no gradient in epifauna along the shore. A simple overlay of the position along shore of each patch onto the ordination plot of epifaunal assemblages (Figs 7.2 a,b) shows no obvious pattern and supports the view that epifauna did not change systematically along the shore.

When nematodes were included in the analysis, habitat groups on ordination plots were not noticeably altered. The results of ANOSIM pairwise comparisons using biomass data showed the same differences described above for biomass, and using abundance data the pattern of differences upon inclusion of nematodes became the same as that for biomass. On both abundance and biomass, nematodes were the major contributor to differences between several pairs of habitats. The order of importance of other taxa important in differentiating habitats was unchanged upon the inclusion of nematodes in the analysis. Cumaceans remained the most important taxon distinguishing habitat U from the other habitats.

Mean abundances in each habitat of total epifauna and of key taxa contributing to differences amongst assemblages are shown in Table 7.3, along with ANOVA and Tukey results. Using a randomised block ANOVA, no significant block effect was detected for any taxon except cumaceans, and even for this taxon, habitat differences were significant after accounting for effects of block. In all cases, where habitat differences were shown to be significant using the randomised block ANOVA, the nested ANOVA also showed a significant habitat effect. Total abundance was not significantly different between habitats C and P, and was lower in habitat R and lowest in U. Of individual taxa, numbers of harpacticoids and gastropods were not significantly different in habitats C, P and R, but were lower in U. Amphipod numbers were lowest in U, not significantly different in C and P, and in R were intermediate between U and C but not significantly different from P. Polychaete numbers showed the same pattern except that they were not significantly lower in U than in R. The only significant difference in tanaid numbers was between C and U. Numbers of calanoids and ostracods did not differ significantly amongst habitats. Cumaceans were rare in habitats C, P and R, and common in U. Abundances of all taxa combined and several individual taxa differed between patches within habitats; the habitat differences described above, however, were evident over and above differences amongst patches. The overwhelming trend (except for cumaceans) is of abundances being similar in habitats C and P but lower in R and lowest in U.

Nematodes were more numerous than all other animals combined, and abundances showed the same trend evident in total epifaunal abundances. Nematode numbers were highest in habitats C and P, intermediate in R and lowest in U.

Mean biomasses in each habitat of total epifauna and of key taxa contributing to differences amongst assemblages are shown in Table 7.4. No significant block effect was detected for any taxon except ostracods. As for abundances, in all cases, where habitat differences were shown to be significant using the randomised block ANOVA, the nested

ANOVA also showed a significant habitat effect. Total biomass tended to decrease from habitat C to P to R to U. Biomass in habitat C was higher than in R and U but was not significantly different from P. P was higher than U and intermediate between, but not significantly different from, C and R. R was intermediate between, but not significantly different from, P and U. The same differences were found for biomasses of polychaetes and nematodes. Amphipod biomasses were different only between C and U, with P and R being similar to each other and intermediate between C and U. Harpacticoids showed a similar pattern but the biomass in R was not significantly different either from C and P or from U. For tanaids, calanoids and gastropods no differences were detected amongst biomasses from the four habitats; for ostracods, this was true with (randomised block) or without (nested) accounting for the effects of block. Cumacean biomass was low in habitats C, P and R, and was high in U. Total epifaunal biomass was not found to differ amongst patches within habitats, but differences were detected for several individual taxa. The general pattern evident in abundance data of similarity between habitats C and P, with R intermediate and U lowest is also present in biomass data but the trend is weaker. The main difference between abundance and biomass data is that the gap between habitat U and the other habitats is less obvious in biomass data. The narrowing of this gap suggests that the mean biomass of individuals was greater in habitat U than in other habitats. The mean biomass of individuals in each sample was calculated by dividing the total biomass of a sample by the total number of individuals in the sample. The mean biomass of individuals was highest in habitat U (C: mean = 18.1 μg, (s.e. = 3.6); P: 14.2 (2.2); R: 15.3 (4.8); U: 24.2 (4.8)), although differences amongst habitats were not significant when tested using a nested ANOVA (Habitat: $p = 0.4$ ns). Nor did individual biomasses differ significantly amongst patches within habitats (Patch: $p = 0.17$ ns).

Total epifaunal production and crustacean production in each habitat and results of ANOVA tests are shown in Table 7.5. Randomised block tests showed that total production and crustacean production varied significantly with habitat but not with

block. For both total and crustacean production, production in habitat C was higher than in R and U but was not significantly different from P. P was higher than U and intermediate between, but not significantly different from, C and R. R was intermediate between, but not significantly different from, P and U.

Neither total fish abundance nor total non-pelagic fish abundance (from Chapter 6) were correlated with epifaunal production of patches (All species: Pearson's $r = 0.249$, $p = 0.291$; Pelagic species excluded: $r = 0.279$, $p = 0.233$).

The relationship between mean fish abundance and mean epifaunal production by habitat is perhaps of greater importance than the search for a correlation between fish abundance and epifaunal production by patch. The relationship between mean fish abundance and mean epifaunal production by habitat is contrasted in Fig. 7.3 with the relationship between mean fish abundance and mean seagrass cover (leaf area) in the four habitats. Total abundance of all fish species matches epifaunal production rather than seagrass cover, and this is true also when pelagic species are excluded, although fish abundances then match epifaunal production less closely. The same pattern is evident if crustacean production is substituted for total epifaunal production (this is not shown in Fig. 7.3, to retain clarity of presentation).

7.4 Discussion

Expectations based on published surveys showing higher epifaunal abundance, biomass and production associated with seagrass patches compared to adjacent unvegetated patches (Orth *et al.* 1984) were fulfilled in the present study by the marked differences found between undisturbed (control) eelgrass plots and plots unvegetated prior to the experiment (unvegetated). The abundance and biomass of individual taxa were mostly

lower in unvegetated patches, the striking exception being cumaceans, which were more abundant and had higher biomasses in unvegetated patches.

Effects of the disturbance associated with eelgrass removal were weak relative to effects of removing eelgrass. No effects of disturbance alone were detected on assemblages, using either abundance or biomass data. Important taxa analysed separately were mostly found to have lower abundances and biomasses in disturbed (procedural control) plots than in undisturbed (control) plots. These differences, however, were typically smaller than those between disturbed eelgrass (procedural control) plots and plots from which eelgrass had been removed. That is, the removal of eelgrass had a detectable effect on epifauna over and above any effects of disturbance associated with eelgrass removal.

Removal of eelgrass canopy altered assemblages, rendering them more similar than those from intact eelgrass to assemblages from unvegetated habitat. Removal of eelgrass canopy also decreased the abundance and biomass of several key taxa. The overall effect of removing the canopy was to alter the fauna in the direction of that from unvegetated patches. Removal of canopy did not, however, cause the fauna to match that from previously unvegetated habitat. Assemblages from unvegetated habitat were clearly different from all other habitats, and abundances and biomasses of most of the key species were obviously lower than in other habitats. Cumaceans, which were abundant in habitat unvegetated prior to the experiment, were rare or absent in other habitats.

The results show that the eelgrass canopy does have some importance to epifauna, but that the eelgrass canopy itself is not the only difference, and is not the overriding difference, between patches with and without eelgrass. The evidence therefore supports models in which differences between the epifauna of vegetated and unvegetated habitats are not directly linked to the presence of seagrass canopy.

The same types of explanations for higher fish abundances in seagrass have been used to explain higher epifaunal abundances in seagrass compared with unvegetated areas. Habitat selection by invertebrates may explain the greater abundance of epifauna in vegetated compared to unvegetated habitats. Epifauna may preferentially select vegetated habitat. It has been shown that macrofauna (Virnstein & Curran 1986, Bell &Westoby 1986a, Sogard 1989, and review in Howard *et al*. 1989) and meiofauna (Bell & Hicks 1991) move around over the temporal (two weeks) and spatial (tens of metres) scales used in the present study. The tidal water flow in the Barker Inlet - Port River region increases the chance that invertebrates moved about during the experiment, and that their abundances reflected preferences. The results of the present experiment therefore do not support a model of invertebrates selecting vegetated over unvegetated habitats based on the presence of canopy. Alternative explanations for the greater abundance of epifauna in seagrass include the possibility that invertebrates themselves are attracted to higher abundances of food. The possibility that epifauna are more abundant in vegetated than in unvegetated habitats because they are attracted to higher food abundance has not been tested in the present study. Although removal of eelgrass may have lessened the amount of food available to epifauna (food includes any or all of the following: detritus, bacteria, microscopic algae, and perhaps some of the smaller invertebrates themselves), food availability was not measured.

Other explanations for the greater abundance of epifauna in vegetated habitats are (as listed by Lewis (1984)): 1) the presence of physical structure usable as living space, 2) dampened hydrodynamic forces, 3) increased number of microhabitats, and 4) greater stabilisation and deposition of sediment. Results of the present experiment exclude 1) and 2) as plausible possibilities as these explanations are reliant on the immediate presence of above-ground vegetation. The number of microhabitats available to epifauna would have been greatly reduced by the removal of seagrass canopy. All the different heights in the canopy, and positions among shoots, are removed along with the canopy. There may be some difference in the number of microhabitats, however, between patches

from which seagrass was removed and unvegetated patches, because of the presence of the root/rhizome mat in the former habitat. For this reason, the failure of the fauna in these two habitats to match does not necessarily exclude explanation 3) above. Removal of seagrass canopy should render sediment deposition similar to that experienced in unvegetated patches. However, the stability of sediments is very likely to be affected by the retention of the seagrass root/rhizome mat in patches from which canopy was removed, and in any case it may take time after the removal of canopy before sediment becomes similar to that of unvegetated areas. Results therefore do not exclude the possibility that differences in the epifauna from vegetated and unvegetated habitats are caused by differences in sediment characteristics. Another explanation, not previously considered in the literature, is that epifauna are more abundant in vegetated habitats simply because the canopy causes them to swim more slowly there. Results of the present experiment discount this explanation.

As explained in the discussion of results of fish collections (Chapter 6), since patches unvegetated prior to the experiment did not receive the disturbance inflicted on patches from which eelgrass was removed, the difference in degree of disturbance may have caused the failure of the fauna of the two habitats to match. As for fish, disturbance of eelgrass alone had little effect on epifauna, giving some confidence that disturbance was not important when comparing the two habitats without eelgrass canopy.

The differences amongst the epifauna from the four habitats lay in the abundance or biomass of taxa, not in the presence or absence of taxa (except for cumaceans). This result may reflect the gross clumping of species and possibly functional groups into single, higher taxa, so that changes in the fauna at these levels would not have been detected. Nevertheless, Warwick (1988) showed that multivariate analyses at family level reproduced very closely the results obtained at species level, and even analyses at the level of phylum generally agreed surprisingly well with those at lower taxonomic levels. Warwick suggests that for some purposes analyses based on higher taxa may

more closely reflect the information of interest than those based on species. In any case, the significant differences detected amongst epifauna from different habitats demonstrates that the taxa used in this study were adequate for examination of the general question posed about the effects of canopy removal on epifauna.

Results from the present experiment apply only to daytime distributions of epifauna. In another experiment examining the effects on epifauna of manipulating seagrass canopy height (Chapter 8), epifauna were collected during both the night and day. Abundances and biomasses of key taxa and of all taxa combined were higher at night than during the day, and this is typical of seagrass systems (e.g. Howard 1987). The effects of manipulating canopy height, however, were similar at both night and day.

Epifaunal assemblages, and abundances and biomasses of some key species, differed from patch to patch even within habitats. These differences were not correlated with position along the shore, and at this stage must be considered as unexplained variability.

If fish are directly attracted to seagrass areas by the higher levels of epifaunal production, rather than selecting seagrass habitat *per se* and as a consequence gaining access to the greater abundance of prey, then fish abundance in the treatments of this experiment should match epifaunal production. Although no correlation between fish abundance and epifaunal production was demonstrated by patch, mean fish abundances by habitat did match epifaunal production when pelagic fish species were included. When pelagic species were excluded, fish abundances by habitat matched epifaunal production less closely, but still more closely than the match with seagrass cover.

The evidence from this experiment does not support a model in which small fish select seagrass habitat because of the presence of seagrass canopy. The evidence better supports, but does not alone demonstrate, the importance of food in the role of eelgrass as habitat for fish.

Table 7.1 List of taxa into which animals were grouped

Abbreviations shown are those used in Table 7.2.

	Crustacea		Non-crustacea
	Caridea	Pol	Polychaeta
	Mysidacea	Gas	Gastropoda
Amp	Amphipoda - Gammaroidea		Bivalvia
	Amphipoda - Caprellidea		Ophiuroidea
Tan	Tanaidacea		Actiniaria (Anemones)
	Isopoda		Ascidiacea larvae
Cum	Cumacea		Nematoda
Har	Copepoda - Harpacticoida		
	Copepoda - Cyclopoida		
Cal	Copepoda - Calanoida		
	Copepoda - nauplii (unidentified)		
Ost	Ostracoda		

Table 7.2 Results of ANOSIM comparisons amongst epifaunal assemblages

Results are probabilities. Pairwise tests are for differences between pairs of habitats: C = control, P = procedural control, R = removed, U = unvegetated. Significance level for each pairwise comparison is 0.0083 so that overall significance level for six comparisons is 0.05 (ns = not significant). Contributing taxa are those making a consistently large contribution to differences between samples from the two habitats, listed in order of decreasing importance.

Variable	Global ANOSIM result	Pairwise ANOSIM results	Main contributing taxa
Abundance	Habitat < 0.001	C,P 0.593 ns	Amp, Cal, Pol
	Patch < 0.001	C,R 0.017 ns	Tan, Gas, Har
		C,U 0.002	Cum, Pol
		P,R 0.128 ns	Ost, Gas, Har
		P,U 0.002	Cum, Har, Pol
		R,U 0.002	Cum, Har, Cal
Biomass	Habitat < 0.001	C,P 0.517 ns	Pol, Gas, Amp, Tan
	Patch < 0.001	C,R 0.006	Gas, Har, Tan
		C,U 0.002	Har, Pol, Cum
		P,R 0.056 ns	Gas, Cal, Har
		P,U 0.002	Cum, Har
		R,U 0.002	Cum, Har

Table 7.3 Abundances of total epifauna and key taxa in each habitat

Numbers are means (individuals/net) with standard errors in parentheses. ANOVA results are probabilities; ns = not significant.
Tukey results are for comparisons between pairs of habitats (following nested ANOVA) and show significant differences as
letters not grouped by underlining: C = control, P = procedural control, R = removed, U = unvegetated.

| Taxon | Habitat type | | | | Randomised block ANOVA | | Nested ANOVA | | |
	C	P	R	U	Habitat	Block	Habitat	Patch	Tukey results
All taxa combined	903 (152)	794 (132)	381 (56)	148 (21)	< 0.001	0.759 ns	< 0.001	0.016	C P R U
Amphipods	16 (2)	10 (2)	6 (1)	2 (0)	< 0.001	0.507 ns	< 0.001	0.019	C P R U
Tanaids	3 (1)	3 (1)	2 (1)	0 (0)	0.011	0.192 ns	0.017	0.082 ns	C P R U
Cumaceans	1 (1)	1 (0)	0 (0)	12 (2)	< 0.001	0.050	< 0.001	0.198 ns	U C P R
Harpacticoids	590 (101)	613 (122)	282 (35)	105 (18)	< 0.001	0.600 ns	< 0.001	0.001	P C R U
Calanoids	5 (2)	11 (3)	6 (2)	2 (1)	0.298 ns	0.602 ns	0.3 ns	< 0.001	
Ostracods	142 (106)	32 (17)	29 (20)	3 (1)	0.173 ns	0.085 ns	0.17 ns	0.001	
Polychaetes	118 (19)	102 (28)	43 (8)	22 (8)	0.001	0.101 ns	< 0.001	0.003	C P R U
Gastropods	15 (5)	9 (5)	8 (3)	1 (0)	0.009	0.106 ns	0.03	0.001	C P R U
Nematodes	1446 (187)	1487 (326)	350 (60)	97 (20)	< 0.001	0.683 ns	< 0.001	0.063 ns	C P R U

Table 7.4 Biomasses of total epifauna and key taxa in each habitat

Numbers are means (mg) with standard errors in parentheses. ANOVA results are probabilities; ns = not significant.
Tukey results are for comparisons between pairs of habitats (following nested ANOVA) and show significant differences as
letters not grouped by underlining: C = control, P = procedural control, R = removed, U = unvegetated.

| Taxon | Habitat type | | | | Randomised block ANOVA | | Nested ANOVA | | |
	C	P	R	U	Habitat	Block	Habitat	Patch	Tukey results
All taxa combined	17.8 (7.9)	10.8 (2.0)	8.9 (5.4)	3.8 (1.0)	0.039	0.669 ns	< 0.001	0.112 ns	C P R U
Amphipods	2.4 (0.8)	1.1 (0.2)	0.5 (0.2)	0.3 (0.1)	0.030	0.111 ns	< 0.018	0.012	C P R U
Tanaids	0.4 (0.1)	0.3 (0.3)	0.4 (0.2)	0.0 (0.0)	0.229 ns	0.497 ns	0.04 ns	< 0.001	
Cumaceans	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.7 (0.4)	< 0.001	0.081 ns	< 0.001	0.123 ns	U C P R
Harpacticoids	2.6 (0.5)	2.8 (0.7)	1.1 (0.2)	0.5 (0.3)	0.001	0.946 ns	< 0.001	0.001	P C R U
Calanoids	0.1 (0.0)	0.1 (0.0)	0.0 (0.0)	0.0 (0.0)	0.152 ns	0.290 ns	0.18 ns	0.001	
Ostracods	0.5 (0.4)	1.1 (1.1)	2.9 (2.9)	0.0 (0.0)	0.380 ns	0.023	0.7 ns	0.5 ns	
Polychaetes	4.1 (0.8)	2.0 (0.5)	1.2 (0.3)	0.3 (0.1)	< 0.001	0.899 ns	< 0.001	0.039	C P R U
Gastropods	5.3 (4.4)	1.0 (0.4)	2.4 (1.7)	0.5 (0.4)	0.340 ns	0.802 ns	0.26 ns	0.363 ns	
Nematodes	9.7 (2.5)	8.6 (2.7)	6.9 (5.4)	1.7 (1.5)	0.003	0.420 ns	< 0.001	0.108 ns	C P R U

Table 7.5 Total epifaunal and crustacean production in each habitat

Numbers are means (μg/day/0.0625m^2) with standard errors in parentheses. ANOVA results are probabilities; ns = not significant. Tukey results are for comparisons between pairs of habitats (following nested ANOVA) and show significant differences as letters not grouped by underlining: C = control, P = procedural control, R = removed, U = unvegetated.

| Taxon | Habitat type | | | | Randomised block ANOVA | | Nested ANOVA | | |
	C	P	R	U	Habitat	Block	Habitat	Patch	Tukey results
All taxa combined	170 (51)	126 (14)	86 (39)	50 (11)	0.014	0.646 ns	<0.001	0.101	C P R U
Crustacea	84 (17)	77 (11)	51 (27)	37 (9)	0.032	0.286 ns	0.007	0.024	C P R U

Fig. 7.1 Two-dimensional MDS ordination plot of epifaunal assemblages, averaged for the three samples within each patch, based on: a) abundances (stress = 0.143), b) biomasses (stress = 0.150). C = control, P = procedural control, R = removed, U = unvegetated
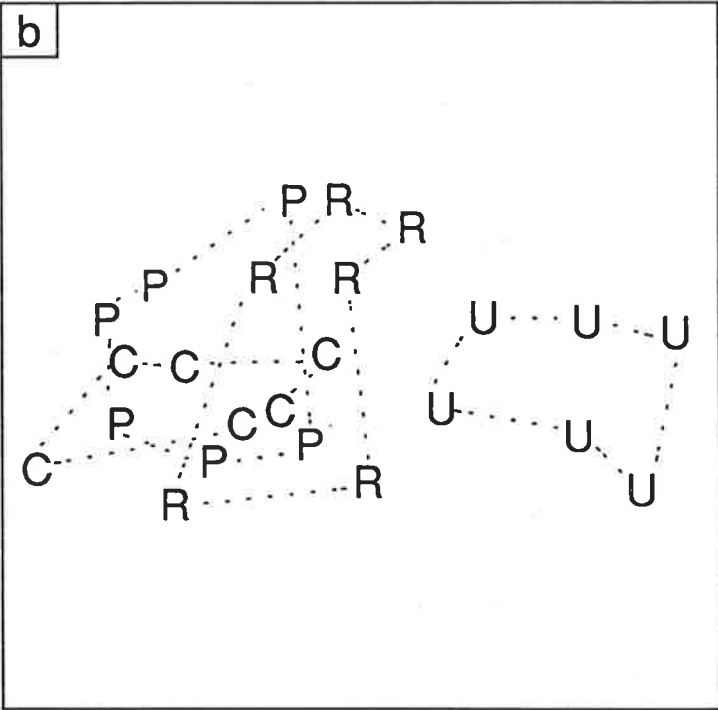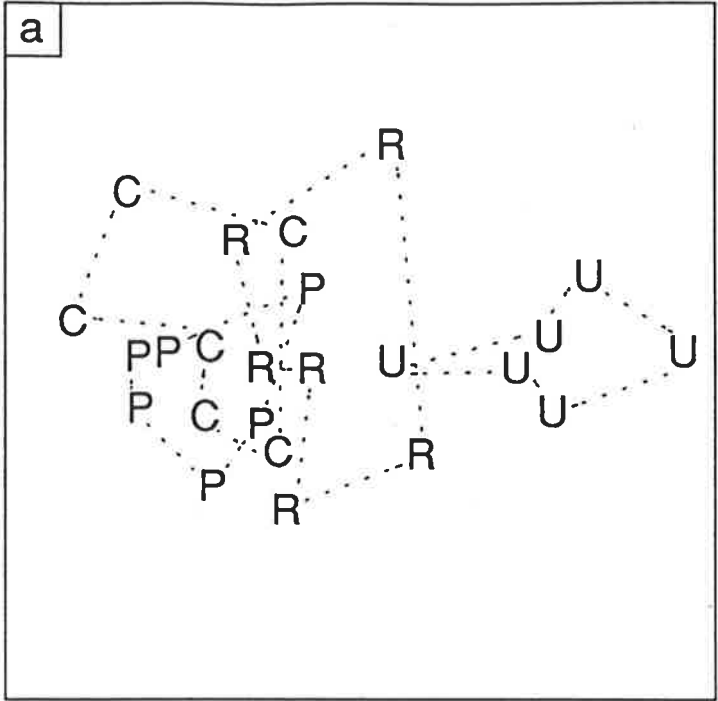
Fig. 7.2 Overlay of position of patch along shore onto MDS ordination plots in Fig. 7.1: a) abundance data, b) biomass data. Diameter of circle is proportional to distance along shore. Smallest circle = 0 m, largest = 900 m
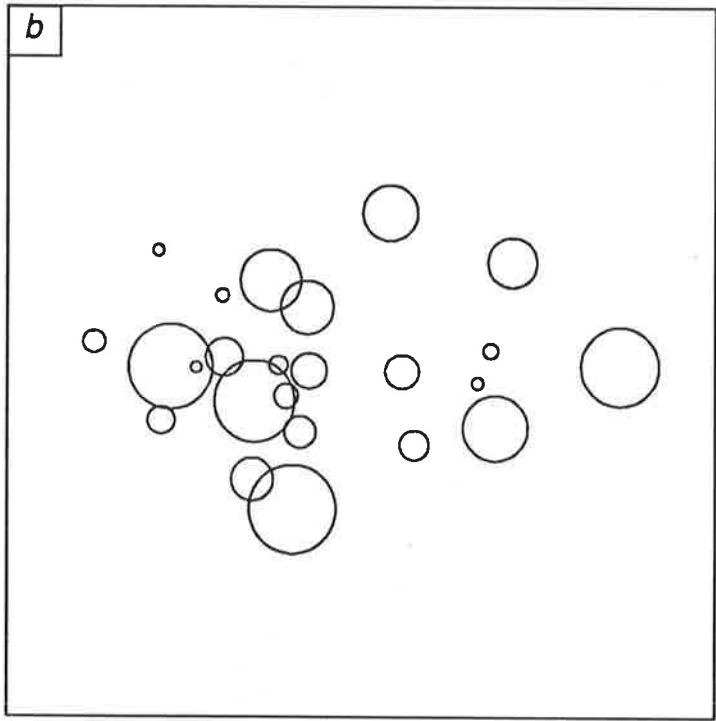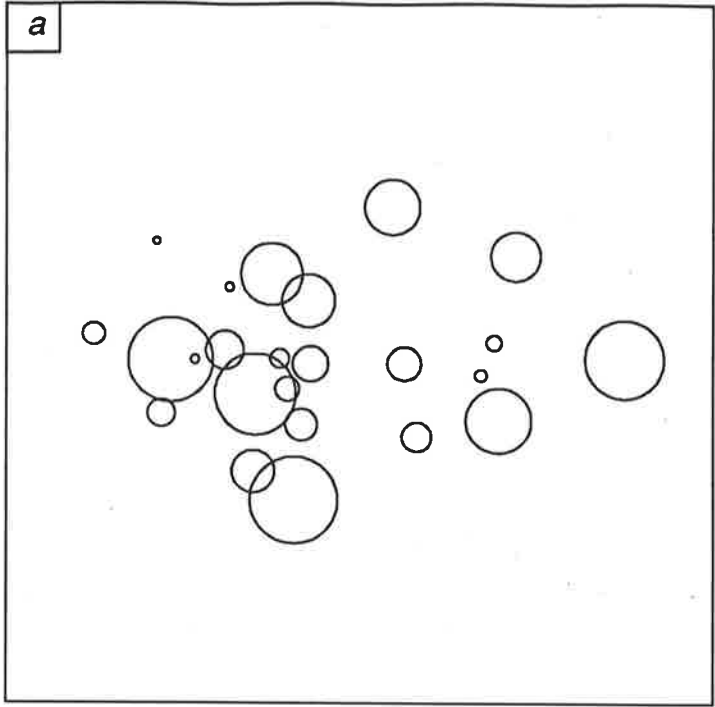
Fig. 7.3 Relationship of total number of fish and total number of non-pelagic fish (fish/net) to epifaunal production (μg/day/0.0625m^2) and seagrass cover (m^2 leaf area/m^2 sediment area) by habitat. All symbols represent means, with common scale. Lines are included to make patterns clear and do not imply that measurements are possible between habitats
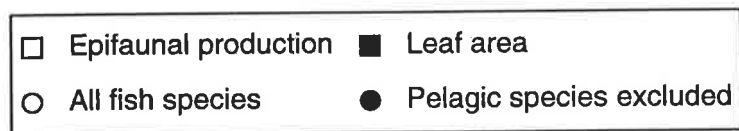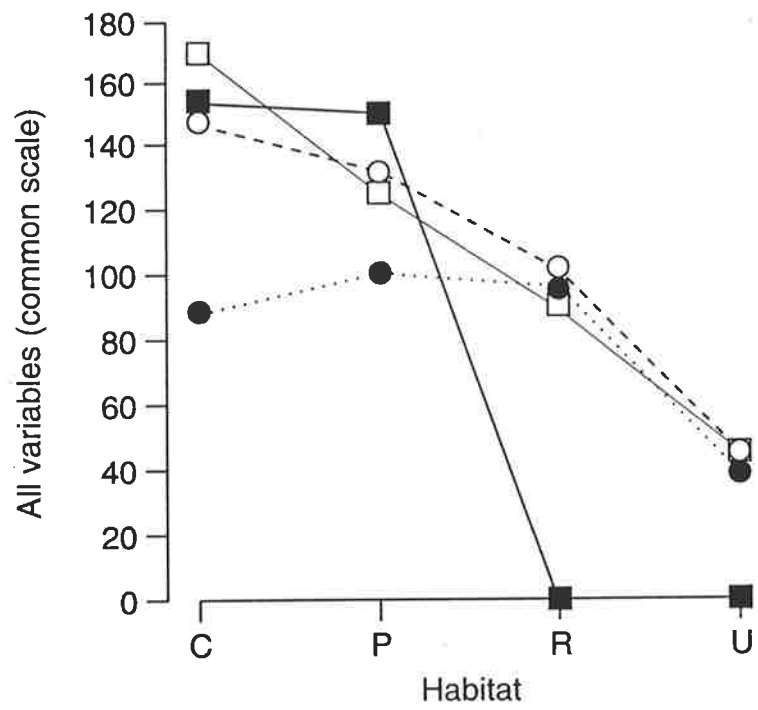
Chapter 8

Altering seagrass canopy height: effects on epifauna of shallow Mediterranean embayments

8.1 Introduction

Previous chapters have examined effects on fauna of total removal of seagrass canopy. The work of Bell & Westoby (1986a,b,c) that encouraged the explanation of small fish distributions in terms of habitat selection involved comparisons of differing levels of seagrass cover. Thinning eelgrass and reducing the height of eelgrass did not have exactly the same effect, but both resulted in reduced abundances of several species of fish and total fish abundances (Bell & Westoby 1986a). These results would be consistent with the simple feeding model if prey availability were also reduced by a reduction in eelgrass cover.

In 1990 I visited the University of Aix-Marseille III and took the opportunity to work in the shallow *Cymodocea* meadows of the Mediterranean Sea, which closely resemble the eelgrass meadows of South Australia. The fish fauna of Mediterranean seagrass meadows has a strong similarity at the familial level with that from seagrass meadows in southern Australia, although the similarity has been more thoroughly examined for the fish fauna of *Posidonia* than of *Zostera* meadows (Pollard 1984). Dietary studies on fish from seagrasses in the Mediterranean Sea (Casabianca & Kiener 1969, Bell & Harmelin-Vivien 1983, Harmelin-Vivien *et al.* 1989, Khoury 1984) and the Black Sea (Duka 1978) support the general dietary patterns of small fish associated with soft-substratum habitats (Klumpp *et al.* 1989).

The aim of this study was to manipulate the seagrass canopy in experimental plots to determine whether alterations to canopy height and surface area would be matched by changes in epifauna. This was done with a view to confirming the possibility that fish abundances can be explained by food availability.

The contents of this chapter are substantially the same as those in the manuscript entitled "The effects of altering seagrass canopy height on small, motile invertebrates of shallow Mediterranean embayments", which is included as Appendix A.5.

8.2 Materials and Methods

Experiments were done in June 1990 in shallow, sheltered embayments in la Lagune du Brusc near the shore of l'Ile des Embiez and l'Etang de Diana on Corsica (Fig. 8.1), where the dominant vegetation is the fine-leaved seagrass *Cymodocea nodosa* (Ucria). These sites are referred to below as Embiez and Diana respectively. Epifauna were collected from the following three habitats (treatments) marked as 1 m x 1 m plots:

1) seagrass uncut (control = C),

2) seagrass canopy cut to one third of original height (partly cut = P), and

3) seagrass canopy removed entirely (removed = R).

Seagrass was cut using hand shears and was shaken vigorously in the water before being removed to minimise the amount of epifauna carried away from the plot. The disturbance associated with cutting was simulated in control plots by spending an equivalent time mimicking cutting.

At each location, six sites were assigned to each of the treatments in a randomised block design. That is, one replicate of each treatment was assigned at random to six areas

(blocks) strung along a 300 m stretch of coast at Diana and placed in a 0.25 ha area adjacent to the coast at Embiez. At Diana an additional four replicates of each treatment were set up for collection of epifauna during the day. All other sampling was done immediately after dusk. The blocked design was intended to guarantee interspersion of treatments. All plots were in water between 30 and 70 cm deep. During the experiments the water height fluctuated 12 cm at Embiez and 2 cm at Diana, but the locations are not truly tidal and plots were never emergent.

Epifauna were collected using a 150 μm mesh net in the manner described in Chapter 5. Samples were taken two days after the setting up of treatments, and the order in which plots were sampled was randomised. One sample was taken approximately in the centre of each plot. Animals were identified and counted as in Chapter 5. Twenty-two taxa were used, 13 crustacean and 9 others (Table 8.1). Nematodes were not counted, because they are typically not an important component in the diet of small fish inhabiting seagrass meadows (Klumpp *et al.* 1989). Ash-free dry weights (AFDW) were calculated as in Chapter 5. Total epifaunal production and crustacean production were also estimated using the method described in Chapter 5, using mean water temperatures during experiments of 24.7°C at Embiez and 25.1°C at Diana.

The length and surface area of seagrass leaves in each plot were estimated prior to cutting and after epifauna collection. Leaf area was calculated for each plot from measurements of the number of leaves per 100 cm^2 quadrat, and the length and width of ten leaves, at three randomly selected places. All vegetation was *Cymodocea nodosa* except for occasional plants of *Zostera noltii* Hornem. at Diana.

Data analysis

Epifaunal assemblages (described both by abundance and biomass (AFDW)) from the three habitats were compared using a one-way ANOSIM. As in Chapter 6, a two-way ANOSIM without replication (randomised block ANOSIM) was also used, to test for habitat differences after allowing for effects of blocks. This analysis cannot be used to test for pairwise differences between habitats, so pairwise tests were done following the one way ANOSIM. Assemblages from the three "locations", Embiez, Diana-Night and Diana-Day, were also compared using a two-way crossed (replicated) ANOSIM with habitat as the second factor. This analysis determines whether assemblages differed amongst the locations after accounting for habitat differences. All ANOSIM tests involved 5000 simulations using the PRIMER package.

The relationships amongst assemblages from each patch are graphically represented using MDS, as in Chapter 3. Data transformation, use of the Bray-Curtis similarity coefficient, and the selection of taxa contributing to between group differences using the similarity matrix, were all as described in Chapter 6.

The abundance and biomass of epifauna (all taxa combined and key taxa separately) and total epifaunal production and crustacean production from the three habitats were compared using the randomised block ANOVA described in Chapter 6 (with the same rationale). Significant habitat effects were followed with Tukey HSD pairwise comparisons. Differences between the three "locations" in the above variables were compared using a two-factor ANOVA with habitat as the second factor. Sample variances increased with increasing means, and all univariate analyses were performed on $\log_{10} x$ transformed data (or $\log_{10} (x + 1)$ where zeros occurred) after checking that the transformation increased homoscedasticity. Significance levels are 0.05 throughout.

8.3 Results

Ile des Embiez

Leaf lengths in the three habitats prior to cutting were: C, mean = 161 mm (s.e. = 8.7); P, 152 (9.9); R, 167 (8.6), and at the time of epifauna collection were: C, 171 (8.8); P, 56 (2.3); R, 18 (2.0). Surface areas prior to cutting were: C, mean = 7.26 m^2 leaf area/m^2 sediment surface area (s.e. = 0.63); P, 6.24 (0.95); R, 7.04 (0.16), and after epifauna collection were: C, 7.58 (0.76); P, 2.18 (0.19); R, 0.13 (0.03).

Two-dimensional ordination plots show strong grouping of plots from the three habitats both on abundance (Fig. 8.2a) and biomass (Fig. 8.3a). Tested with a one-way ANOSIM, assemblages were significantly different using both variables (Table 8.2). Pairwise ANOSIM comparisons using abundance showed that all habitats were different, and using biomass showed that habitat R was different from habitats C and P (Table 8.2). Results of the two-way ANOSIM, however, showed a significant effect of block but not of habitat (Abundance: Habitat, $p = 0.576$, Block, $p = 0.011$; Biomass: Habitat, $p = 0.436$, Block, $p = 0.009$). This suggests that the apparent effect of habitat detected with the one-way ANOSIM is due to differences amongst blocks. Although caution should therefore be used in interpreting the significant habitat differences shown with the one-way ANOSIM, the clear separation of assemblages from the three habitats in Fig. 8.2a and 8.3a, compared with the less obvious separation of blocks in Fig. 8.2b and 8.3b, suggests that meaningful differences existed amongst assemblages from the three habitats. One explanation for the two-way ANOSIM failing to detect habitat differences may be that these were obscured by interaction of habitat and block effects. The test makes the assumption that interaction is negligible (Clarke & Warwick 1994).

Mean abundances for the three habitats of total epifauna and of key taxa contributing to differences amongst assemblages are shown in Table 8.3 along with ANOVA and Tukey

results. The total abundance of epifauna was different for all habitats, being highest in habitat C, intermediate in habitat P and lowest in habitat R. The same pattern was found in abundances of amphipods, the most prominent taxon contributing to differences in assemblages, and harpacticoids. There were fewer tanaids and polychaetes in habitat R than in habitats C and P. Numbers of mysids and gastropods were higher in C than in R but although numbers in P were intermediate, they were not significantly different from either C or R. No differences were detected amongst abundances of calanoids. No taxa differed in abundance amongst blocks. The overwhelming pattern of abundances is of decreasing abundance from C to P to R.

Mean biomasses of total epifauna and of key species are shown in Table 8.4. Differences in total biomass amongst habitats were significant, with biomass in habitat C higher than, but not significantly different from, habitat P, with both of these habitats having significantly greater biomass than R. Harpacticoid, amphipod and polychaete biomasses were highest in C, intermediate in P and lowest in R. Tanaid biomass was higher in habitat C than in R, and intermediate in P but not significantly different from either C or R. Significant differences were not detected in biomasses of other taxa. Only amphipod biomass differed amongst blocks. The general pattern of decreasing abundance from C to P to R was also evident for biomass but was less marked.

Total epifaunal production and total crustacean production both differed amongst habitats (Table 8.5). Total production and crustacean production were lowest in habitat R and not significantly different between C and P (Table 8.5).

Etang de Diana-Night

Leaf lengths in the three habitats prior to cutting were: C, mean = 281 mm (s.e. = 26.3); P, 281 (28.0); R, 268 (25.7), and at the time of epifauna collection were: C, 273 (22.2); P, 82 (6.9); R, 14 (1.9). Surface areas prior to cutting were: C, mean = 9.35 m^2 leaf area/m^2 sediment surface area (s.e. = 1.90); P, 10.19 (2.08); R, 9.15 (1.87), and after epifauna collection were: C, 9.24 (1.78); P, 2.23 (0.34); R, 0.11 (0.02).

Two-dimensional ordination plots show strong grouping of assemblages from the three habitats both on abundance (Fig. 8.4a) and biomass (Fig. 8.5a). Differences amongst habitats were shown to be significant with the one-way ANOSIM (Table 8.6). On both abundance and biomass, all habitats differed according to pairwise ANOSIM tests. Two-way ANOSIM tests also showed significant habitat effects, as well as significant block effects (Abundance: Habitat, $p = 0.002$, Block, $p = 0.028$; Biomass: Habitat, $p = 0.048$, Block, $p = 0.015$).

The total abundance of epifauna was highest in habitat C, intermediate in P and lowest in R (Table 8.7). The same pattern was found for gastropods, the most prominent taxon contributing to multivariate differences, and amphipods, tanaids, harpacticoids and calanoids. Polychaete and chironomid numbers were greater in habitat C and not significantly different between P and R. Isopod numbers were higher in C than in R, and intermediate in P but not significantly different from the other habitats. A Tukey test failed to detect any pairwise differences in anemone numbers despite a significant ANOVA result. This reflects the infrequent occurrence of anemones in habitats P and R. The total numbers of anemones caught were as follows: C, 11; P, 2; R, 2. No taxa were found to differ amongst blocks. As for Embiez data, there was an overwhelming pattern of abundances decreasing from habitat C to P to R.

Total biomass was higher in habitat C than in R, and was intermediate in P but not significantly different from the other habitats (Table 8.8). The biomasses of tanaids, harpacticoids and gastropods were highest in habitat C, intermediate in P and lowest in R. Biomasses of isopods, polychaetes and anemones were higher in habitat C and not significantly different between P and R. Amphipod biomass was lower in habitat R and not significantly different between C and P. No differences in chironomid biomasses were detected between habitats using the Tukey test despite ANOVA results showing a significant habitat effect. Calanoid biomass did not differ amongst habitats. No taxa differed amongst blocks. The general pattern of decreasing biomass from C to P to R is evident but is less marked, judging from total epifaunal biomass, than for abundances.

Total epifaunal production and total crustacean production were both higher in habitat C than in R, and were intermediate in P but not significantly different from the other habitats (Table 8.9).

Etang de Diana-Day

Leaf lengths in the three habitats prior to cutting were: C, mean = 269 mm (s.e. = 25.3); P, 270 (17.7); R, 257 (24.0), and at the time of epifauna collection were: C, 262 (27.2); P, 79 (7.1); R, 13 (2.1). Surface areas prior to cutting were: C, mean = 9.44 m^2 leaf area/m^2 sediment surface area (s.e. = 1.80); P, 9.51 (1.24); R, 9.21 (1.23), and after epifauna collection were: C, 8.94 (1.51); P, 1.99 (0.32); R, 0.09 (0.02).

Two-dimensional ordination plots again show strong grouping of assemblages from the three habitats both on abundance (Fig. 8.6a) and biomass (Fig. 8.7a). Differences were shown to be significant using the one-way ANOSIM (Table 8.10). On abundance data, pairwise ANOSIM comparisons were significant only between habitats C and R. Results for the other comparisons had probabilities not much higher than the 0.05 critical level.

Although no formal statistical power calculations are possible with this method, the low number of plots (four) from each habitat serve as a reminder that the failure to detect differences does not mean that no difference exists. On biomass data, C was different from P and R, with these two habitats not shown to be different, although the above warning about power is again relevant. Two-way ANOSIM tests on abundances showed no habitat or block effects, and on biomasses showed a significant effect of habitat but not of block (Abundance: Habitat, $p = 0.268$, Block, $p = 0.986$; Biomass: Habitat, $p = 0.039$, Block, $p = 0.632$).

The total abundance of epifauna was greatest in habitat C, intermediate in P and lowest in R (Table 8.11). The most conspicuous taxon contributing to differences amongst assemblages was harpacticoids, which showed the same pattern as total epifaunal abundance. Amphipod numbers were lower in habitat R and not significantly different in C and P. Tanaid and polychaete numbers were higher in C than in R, and were intermediate in P but not significantly different from the other habitats. Porcellid, gastropod and bivalve abundances were not found to be different amongst habitats, although porcellid numbers varied by block. Patterns for epifauna collected during the day were similar to those for epifauna collected at night, with total abundances and abundances of several important species decreasing from habitat C to P to R.

Total epifaunal biomass was greatest in habitat C, intermediate in P and lowest in R (Table 8.12). Harpacticoids biomass was higher in C and not significantly different between P and R. Amphipod, tanaid and polychaete biomasses were greater in C than in R, and intermediate in P but not significantly different from the other habitats. Porcellid, gastropod and bivalve biomasses were not significantly different amongst habitats. No taxa differed amongst blocks. As for epifauna collected at night, biomasses of epifauna collected during the day showed the same pattern as abundances but the trend from C to P to R was less obvious.

Estimates of total epifaunal production and crustacean production from daytime collections were highest in habitat C, intermediate in P, and lowest in R (Table 8.13).

Size of animals from different habitats

At all locations the pattern of decreasing abundance from C to P to R was stronger than the pattern for biomass. This implies that the average weight of individual animals increased from C to P to R. The mean biomass of individuals in each sample was calculated by dividing the total biomass of a sample by the total number of individuals in the sample. The mean biomass of individuals at each location was lower in habitat C than in the other two habitats, but differences were not significant (Embiez - C: mean = 15.8 µg, (s.e. = 2.3); P: 18.0 (3.3); R: 18.9 (4.7); ANOVA, $p = 0.945$. Diana-Night - C: 25.9 (3.6); P: 34.1 (4.7); R: 33.2 (4.6); ANOVA, $p = 0.404$. Diana-Day - C: 19.7 (2.5); P: 24.4 (3.4); R: 26.0 (9.2); ANOVA, $p = 0.860$).

Location differences

The two-dimensional ordination plots of assemblages based on abundance and biomass from all locations show strong grouping of habitats within locations, as expected from individual ordinations, but there is also an overriding separation of locations (Fig. 8.8). In plots for both abundance and biomass, habitat groups from Embiez are distinct but close together, and are all entirely separate from those of Diana. Diana-Night and Diana-Day positions overlap, but Diana-Night habitat groups, whilst distinct, are close together, whereas Diana-Day habitat groups are more widely spread. Although the spacing amongst habitat groups is different for Diana-Night and Day, the effect of partly cutting and removing seagrass was the same, with groups C, P and R positioned in that order along a straight line gradient. This gradient is also evident for Embiez habitat

groups. Differences amongst habitats and locations were statistically significant (Table 8.14). ANOSIM comparisons between pairs of habitats were all significant but are not shown in Table 8.14 because they have been reported for each location separately. Pairwise comparisons amongst locations were all significant on both abundance and biomass data; that is, after taking into account habitat differences, assemblages from all three locations were significantly different from each other.

Results of two-factor ANOVA tests on location and habitat for abundance and production data are presented in Table 8.15. Significant interaction was detected on abundance and production data, and probabilities for main effects should be treated with caution. In this case, probabilities for both main effects (location and habitat) are highly significant for both abundance and production, and since interaction tends to diminish main effects, it can be concluded that habitats differed regardless of location and locations differed regardless of habitat. Given the interaction, however, Tukey tests are best used to compare, for example, pairwise differences amongst locations separately for each habitat. Habitat differences are not shown because these have already been reported under separate location sections. Total abundances were higher at Diana-Night than at Embiez for each habitat. Diana-Day abundances were similar to those at Diana-Night in habitat C, but were similar to those at Embiez in habitat P, and even lower than those at Embiez in habitat R. Total production was higher at Diana-Night than at Embiez for each habitat. Production at Diana-Day was similar to that at Diana-Night in habitat C, but was similar to that at Embiez in habitats P and R.

8.4 Discussion

The epifauna of the three experimental habitats was different whether measured as abundance, biomass or production. At each location, total epifaunal production within habitats declined in line with decreasing vegetation cover. Epifaunal assemblages differed amongst habitats, and the types of differences, but not their magnitudes, were consistent at the two sites and at night and day. At each location, the same taxa tended to be dominant numerically and by weight, although the importance of these taxa in distinguishing amongst habitats varied with location. These dominant taxa showed a very strong pattern of decreasing abundance and biomass from habitat C to P to R. As a result, the total abundance and biomass, and therefore estimates of production, declined from C to P to R.

The pattern of decline from C to P to R was less obvious on biomass data than on abundance data. Although differences in mean biomass of individuals were not significant, at each location mean biomass was lowest in habitat C. This can be taken as weak evidence that the relative importance of heavier animals was greater in plots in which seagrass cover was reduced. There are many plausible explanations for any increased importance of heavier animals in habitats from which vegetation had been removed. Predators may have removed animals differentially according to size, or the food resource available in modified habitats may have been more attractive to larger animals. The responses to reduced canopy could be instinctive selection of habitat. An alternative explanation for the increased dominance of larger animals with decreasing canopy cover is that heavier animals, either because of their weight or because they are more powerful swimmers, may have been less likely to be removed along with vegetation at the time of cutting. If smaller animals removed accidentally along with vegetation had not returned by the time epifauna was collected, then the relative importance of heavier animals would increase as the amount of vegetation removed increased.

The differences amongst habitats lay in the abundance or biomass of taxa, not in the presence or absence of taxa. As discussed in Chapter 7, this result may reflect the gross clumping of species and possibly functional groups into single, higher taxa. The taxa used in this study appear adequate, however, when discussing differences in prey availability, at least for fish species that become less abundant in response to a reduction in seagrass canopy. Three species of fish increased in abundance in response to reduction in seagrass canopy in experiments in *Zostera capricorni* beds reported by Bell & Westoby (1986a). To examine the possibility that food availability is the direct cause of increases in abundances shown by some fish species, epifauna may need to be identified to lower levels, or into taxa representing groups of animals actually able to be caught by those fish species.

The epifauna of Embiez and Diana-Night were consistently different. Multivariate analysis showed no overlap of assemblages from the two sites. Epifaunal abundance, biomass and production were always higher at Diana in all habitats. Although the fauna at the two sites differed, the effects of partly cutting and totally removing the seagrass canopy were the same at both sites. Habitat groups in the ordination plots based on abundance and biomass were related in the same way at the two sites. The differences in abundance, biomass and production of habitats were also consistent at both sites.

The effects of partly cutting and removing seagrass were different on the night fauna and day fauna at Diana. Multivariate and univariate analyses on abundance and biomass show that modifying seagrass canopy had the same type of effect on day and night fauna, but that the magnitude of those effects differed with time of day. Habitat groups on the ordination plots for abundance and biomass of day fauna were more spread out than for night fauna. Abundance and production of day fauna matched that of night fauna in control plots, but were as low as or lower than that at Embiez in modified habitats. Plausible explanations for the greater reduction in fauna in modified habitats during the day include the possibility of an increase in predation during the day, or of instinctive

selection of habitat with more canopy cover (behaviour for which the ultimate agent may have been increased risk of predation during the day). Reducing seagrass canopy may increase the number of invertebrates burrowing in sediment during the day, thereby reducing the number in the epifauna. The effect of reducing seagrass canopy on the amount or quality of food available to invertebrates themselves may differ between night and day. Although only 12 hours elapsed between day and night sampling, comparisons between the night and day fauna are confounded by time. The different effects of modifying habitat between the day and night samples may be attributable to changes in environmental factors during that period.

The control and shortened canopy treatments in this study were similar to the control and shortened treatments shown by Bell & Westoby (1986a) to affect fish abundances, and the seagrass of their study (*Zostera capricorni*) is similar in height, width, density, and general form to the seagrass (*Cymodocea nodosa*) in the present study. Results presented here can therefore be sensibly combined with results from the fish studies of Bell & Westoby (1986a) to sharpen explanations of fish distributions.

The aim of this study was to determine whether alterations to the amount of canopy cover affected epifauna. At both sites, and at night and day at Diana, the abundance, biomass and production of epifauna were reduced in line with reduction in canopy height. The results of the present study are consistent with, but do not alone demonstrate the truth of, the model in which fish abundances are explained by food availability.

Table 8.1 List of taxa into which animals were grouped

Abbreviations shown are those used in Tables 8.2, 8.6, 8.10 and 8.14.

	Crustacea		Non-crustacea
	Caridea	Pol	Polychaeta
Mys	Mysidacea	Gas	Gastropoda
Amp	Amphipoda - Gammaroidea	Biv	Bivalvia
	Amphipoda - Caprellidea		Ophiuroidea
Tan	Tanaidacea		Echinodermata, larvae
Iso	Isopoda	Ane	Actiniaria (Anemones)
	Cumacea		Chaetognatha
Har	Copepoda - Harpacticoida	Chi	Chironomidae, larvae
Por	Copepoda - Harpacticoida - Porcellidiidae		Ascidiacea, larvae
Cyc	Copepoda - Cyclopoida		
Cal	Copepoda - Calanoida		
	Copepoda - nauplii (unidentified)		
	Ostracoda		

Table 8.2 Results of ANOSIM comparisons amongst epifaunal assemblages from Embiez

Global test is for any difference amongst habitats using one-way ANOSIM, and pairwise tests are for differences between pairs of habitats. Significance level for each comparison is 0.05 (ns = not significant). Contributing taxa are those making a consistently large contribution to differences between samples from the two habitats, listed in order of decreasing importance.

Variable	Global ANOSIM result	Pairwise ANOSIM results	Main contributing taxa
Abundance	< 0.001	C,P 0.017	Amp, Pol, Gas, Har
		C,R 0.004	Amp, Har, Pol
		P,R 0.009	Amp, Cal, Har, Mys
Biomass	0.001	C,P 0.1 ns	Pol, Har, Tan, Amp, Cal
		C,R 0.002	Har, Amp, Pol
		P,R 0.006	Amp, Har, Cal, Mys, Gas

Table 8.3 Abundances of total epifauna and key taxa in each habitat at Embiez

Numbers are means with standard errors in parentheses. ANOVA results are
probabilities; ns = not significant. Tukey results show significant differences as letters
not grouped by underlining: C = control, P = partly cut, R = removed.

Taxon	Habitat type			ANOVA results		
	C	P	R	Habitat	Block	Tukey results
All species combined	838 (45)	509 (43)	256 (34)	< 0.001	0.505 ns	C P R
Amphipods	64 (8)	28 (2)	10 (2)	< 0.001	0.016	C P R
Tanaids	23 (6)	112 (3)	3 (1)	0.004	0.071 ns	C P R
Harpacticoids	548 (35)	319 (34)	153 (20)	< 0.001	0.327 ns	C P R
Calanoids	15 (4)	19 (3)	22 (9)	0.602 ns	0.136 ns	
Mysids	8 (5)	3 (1)	3 (2)	0.026	< 0.001	C P R
Polychaetes	135 (14)	78 (6)	49 (7)	0.001	0.302 ns	C P R
Gastropods	15 (4)	4 (1)	3 (2)	0.018	0.075 ns	C P R

Table 8.4 Biomasses (AFDW in mg) of total epifauna and key taxa in each habitat at Embiez

Numbers are means with standard errors in parentheses. ANOVA results are probabilities; ns = not significant. Tukey results show significant differences as letters not grouped by underlining: C = control, P = partly cut, R = removed.

Taxon	Habitat type			ANOVA results		
	C	P	R	Habitat	Block	Tukey results
All species combined	13.4 (2.1)	8.9 (1.6)	4.3 (0.9)	0.004	0.232 ns	<u>C P</u> R
Amphipods	4.2 (0.8)	2.2 (0.5)	0.3 (0.1)	< 0.001	0.057 ns	C P R
Tanaids	1.9 (0.6)	0.8 (0.3)	0.3 (0.1)	0.038	0.204 ns	<u>C P R</u>
Harpacticoids	2.1 (0.2)	1.3 (0.3)	0.5 (0.1)	< 0.001	0.205 ns	C P R
Calanoids	0.1 (0.0)	0.1 (0.0)	0.2 (0.1)	0.418 ns	0.373 ns	
Mysids	0.3 (0.1)	0.5 (0.3)	0.1 (0.1)	0.242 ns	0.216 ns	
Polychaetes	2.5 (0.3)	1.0 (0.1)	0.6 (0.1)	< 0.001	0.059 ns	C P R
Gastropods	0.4 (0.1)	1.2 (0.5)	0.1 (0.0)	0.092 ns	0.647 ns	

Table 8.5 Total epifaunal production and total crustacean production (μg/day/0.0625m^2) in each habitat at Embiez

Numbers are means with standard errors in parentheses. ANOVA results are probabilities. Tukey results show significant differences as letters not grouped by underlining: C = control, P = partly cut, R = removed.

| Taxon | Habitat type | | | ANOVA results | | |
	C	P	R	Habitat	Block	Tukey results
All species combined	221 (31)	158 (24)	86 (15)	0.003	0.204 ns	<u>C P</u> R
All crustaceans combined	171 (24)	106 (15)	37 (6)	<0.001	0.029	<u>C P</u> R

Table 8.6 Results of ANOSIM comparisons amongst epifaunal assemblages from Diana-Night

Global test is for any differences amongst habitats using one-way ANOSIM, and pairwise tests are for differences between pairs of habitats. Significance level for each comparison is 0.05 (ns = not significant). Contributing taxa are those making a consistently large contribution to differences between samples from the two habitats, listed in order of decreasing importance.

Variable	Global ANOSIM result	Pairwise ANOSIM results	Main contributing taxa
Abundance	< 0.001	C,P 0.002	Har, Tan, Pol, Gas, Amp
		C,R 0.002	Gas, Iso, Cal, Har
		P,R 0.002	Gas, Amp, Tan, Cal, Har
Biomass	< 0.001	C,P 0.002	Har, Chi, Ane
		C,R 0.002	Gas, Har, Iso, Cal
		P,R 0.002	Gas, Cal, Tan, Amp

Table 8.7 Abundances of total epifauna and key taxa in each habitat at Diana-Night

Numbers are means with standard errors in parentheses. ANOVA results are probabilities; ns = not significant. Tukey results show significant differences as letters not grouped by underlining: C = control, P = partly cut, R = removed.

Taxon	Habitat type			ANOVA results		
	C	P	R	Habitat	Block	Tukey results
All species combined	2554 (314)	912 (57)	521 (59)	< 0.001	0.678 ns	C P R
Amphipods	187 (22)	86 (10)	30 (4)	< 0.001	0.450 ns	C P R
Tanaids	197 (41)	49 (6)	15 (2)	< 0.001	0.647 ns	C P R
Isopods	2 (1)	1 (0)	0 (0)	0.009	0.692 ns	C̲ ̲P̲ ̲R̲
Harpacticoids	1395 (117)	537 (28)	313 (30)	< 0.001	0.657 ns	C P R
Calanoids	16 (4)	5 (2)	0 (0)	< 0.001	0.203 ns	C P R
Polychaetes	569 (135)	140 (13)	78 (9)	< 0.001	0.794 ns	C P̲ ̲R̲
Gastropods	11 (2)	3 (1)	0 (0)	< 0.001	0.379 ns	C P R
Anemones	2 (1)	0 (0)	0 (0)	0.042	0.292 ns	C̲ ̲P̲ ̲R̲
Chironomids	29 (14)	1 (0)	1 (1)	0.001	0.475 ns	C P̲ ̲R̲

Table 8.8 Biomasses (AFDW in mg) of total epifauna and key taxa in each habitat at Diana-Night

Numbers are means with standard errors in parentheses. ANOVA results are probabilities; ns = not significant. Tukey results show significant differences as letters not grouped by underlining: C = control, P = partly cut, R = removed.

Taxon	Habitat type			ANOVA results		
	C	P	R	Habitat	Block	Tukey results
All species combined	67.9 (16.7)	31.3 (5.4)	18.4 (3.9)	0.007	0.627 ns	<u>C P</u> R
Amphipods	16.9 (2.9)	7.8 (1.3)	2.9 (0.5)	0.001	0.821 ns	<u>C P</u> R
Tanaids	11.4 (4.1)	3.3 (0.2)	1.0 (0.5)	< 0.001	0.151 ns	C P R
Isopods	0.8 (0.3)	0.1 (0.1)	0.0 (0.0)	0.004	0.307 ns	C <u>P R</u>
Harpacticoids	3.8 (0.4)	1.5 (0.1)	0.8 (0.1)	< 0.001	0.005	C P R
Calanoids	0.1 (0.0)	0.0 (0.0)	0.0 (0.0)	0.076 ns	0.503 ns	
Polychaetes	12.8 (4.0)	3.7 (0.8)	3.0 (0.6)	0.006	0.924 ns	C <u>P R</u>
Gastropods	4.8 (1.1)	1.5 (0.5)	0.0 (0.0)	< 0.001	0.471 ns	C P R
Anemones	1.5 (0.3)	0.0 (0.0)	0.1 (0.1)	< 0.001	0.319 ns	C <u>P R</u>
Chironomids	0.8 (0.5)	0.0 (0.0)	0.0 (0.0)	0.039	0.466 ns	<u>C P R</u>

Table 8.9 Total epifaunal production and total crustacean production
(μg/day/0.0625m^2) in each habitat at Diana-Night

Numbers are means with standard errors in parentheses. ANOVA results are
probabilities. Tukey results show significant differences as letters not grouped by
underlining: C = control, P = partly cut, R = removed.

Taxon	Habitat type			ANOVA results		
	C	P	R	Habitat	Block	Tukey results
All species combined	812 (151)	434 (55)	277 (51)	0.005	0.602 ns	<u>C P</u> R
All crustaceans combined	568 (101)	347 (42)	226 (42)	0.013	0.565 ns	<u>C P</u> R

Table 8.10 Results of ANOSIM comparisons amongst epifaunal assemblages from Diana-Day

Global test is for any differences amongst habitats using one-way ANOSIM, and pairwise tests are for differences between pairs of habitats. Significance level for each comparison is 0.05 (ns = not significant). Contributing taxa are those making a consistently large contribution to differences between samples from the two habitats, listed in order of decreasing importance.

Variable	Global ANOSIM result	Pairwise ANOSIM results	Main contributing taxa
Abundance	0.001	C,P 0.086 ns	Har, Tan, Pol
		C,R 0.029	Har, Amp, Pol
		P,R 0.057 ns	Biv, Amp, Pol
Biomass	0.001	C,P 0.029	Tan, Har, Biv
		C,R 0.029	Har, Biv, Pol, Gas
		P,R 0.086 ns	Pol, Por, Har

Table 8.11 Abundances of total epifauna and key taxa in each habitat at Diana-Day

Numbers are means with standard errors in parentheses. ANOVA results are
probabilities; ns = not significant. Tukey results show significant differences as letters
not grouped by underlining: C = control, P = partly cut, R = removed.

Taxon	Habitat type			ANOVA results		
	C	P	R	Habitat	Block	Tukey results
All species combined	2308 (625)	451 (87)	143 (20)	< 0.001	0.121 ns	C P R
Amphipods	141 (65)	49 (16)	3 (2)	0.002	0.248 ns	C P R
Tanaids	64 (28)	10 (2)	2 (2)	0.036	0.345 ns	C P R
Harpacticoids	1414 (304)	248 (42)	95 (15)	< 0.001	0.167 ns	C P R
Porcellids	52 (27)	35 (15)	6 (3)	0.133 ns	0.011	
Polychaetes	391 (149)	73 (24)	19 (3)	0.018	0.564 ns	C P R
Gastropods	27 (15)	3 (3)	2 (2)	0.131 ns	0.912 ns	
Bivalves	34 (30)	11 (5)	1 (1)	0.232 ns	0.896 ns	

Table 8.12 Biomass (AFDW in mg) of total epifauna and key taxa in each habitat at Diana-Day

Numbers are means with standard errors in parentheses. ANOVA results are probabilities; ns = not significant. Tukey results show significant differences as letters not grouped by underlining: C = control, P = partly cut, R = removed.

Taxon	Habitat type			ANOVA results		
	C	P	R	Habitat	Block	Tukey results
All species combined	44.9 (11.2)	11.0 (2.4)	3.6 (1.2)	0.001	0.167 ns	C P R
Amphipods	15.4 (7.9)	3.1 (1.2)	0.4 (0.2)	0.015	0.131 ns	C P R
Tanaids	3.2 (1.1)	0.3 (0.1)	0.2 (0.1)	0.036	0.433 ns	C P R
Harpacticoids	5.4 (1.4)	0.8 (0.2)	0.2 (0.1)	< 0.001	0.061 ns	C P R
Porcellids	0.2 (0.1)	0.2 (0.1)	0.0 (0.0)	0.143 ns	0.196 ns	
Polychaetes	6.4 (2.2)	1.0 (0.2)	0.2 (0.1)	0.019	0.590 ns	C P R
Gastropods	2.6 (1.1)	1.7 (1.2)	0.0 (0.0)	0.113 ns	0.316 ns	
Bivalves	0.5 (0.1)	0.3 (0.3)	0.0 (0.0)	0.141 ns	0.415 ns	

Table 8.13 Total epifaunal production and total crustacean production (μg/day/0.0625m^2) in each habitat at Diana-Day

Numbers are means with standard errors in parentheses. ANOVA results are probabilities. Tukey results show significant differences as letters not grouped by underlining: C = control, P = partly cut, R = removed.

| Taxon | Habitat type | | | ANOVA results | | |
	C	P	R	Habitat	Block	Tukey results
All species combined	589 (121)	188 (36)	71 (21)	0.001	0.155 ns	C P R
All crustaceans combined	478 (109)	136 (38)	44 (24)	< 0.001	0.031	C P R

Table 8.14 Results of two-way crossed ANOSIM comparisons amongst epifaunal assemblages of locations and habitats

Global test is for differences amongst habitats or locations, as specified. Pairwise tests for habitats were all significant but are not shown. Pairwise tests amongst locations are shown. Significance level for each comparison is 0.05. Contributing taxa are those making a consistently large contribution to differences between samples from the two habitats, listed in order of decreasing importance.

Variable	Global ANOSIM result	Pairwise ANOSIM results for location	Main contributing taxa
Abundance	Habitat < 0.001 Location < 0.001	EM,DN < 0.001 EM,DD < 0.001 DN,DD < 0.001	Por, Biv, Mys Cal, Cyc, Pol, Har Cyc, Pol, Har
Biomass	Habitat < 0.001 Location < 0.001	EM,DN < 0.001 EM,DD < 0.001 DN,DD < 0.001	Por, Mys, Biv, Gas, Pol Cal, Cyc, Mys, Pol, Har, Gas Cyc, Har, Pol

Table 8.15 Total epifaunal abundance and production comparisons across locations

Results shown as probabilities for two factors and interaction term in two-way ANOVA. Tukey results for Location only, separately for each Habitat, show significant differences as letter not grouped by underlining. EM = Embiez, DN = Diana-Night, DD = Diana-Day.

	Term	ANOVA result	Habitat	Tukey results for Location comparisons
Abundance	Habitat	< 0.001	C	DN DD EM
	Location	< 0.001	P	DN EM DD
	Interaction	< 0.001	R	DN EM DD
Production	Habitat	< 0.001	C	DN DD EM
	Location	< 0.001	P	DN DD EM
	Interaction	0.049	R	DN EM DD

Fig. 8.1 Map showing location of sites

France

Marseilles

Toulon

Spain

Barcelona

Italy

Corsica

Rome

Mediterranean Sea

Sardinia

Le Brusc

Lagune
du
Brusc

Ile des
Embiez

Sampling
Site

Sampling
Site

Corsica

Etang de
Diana
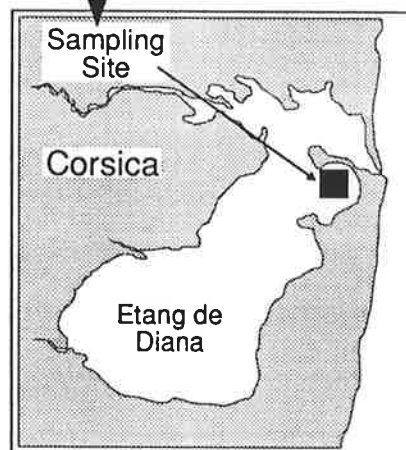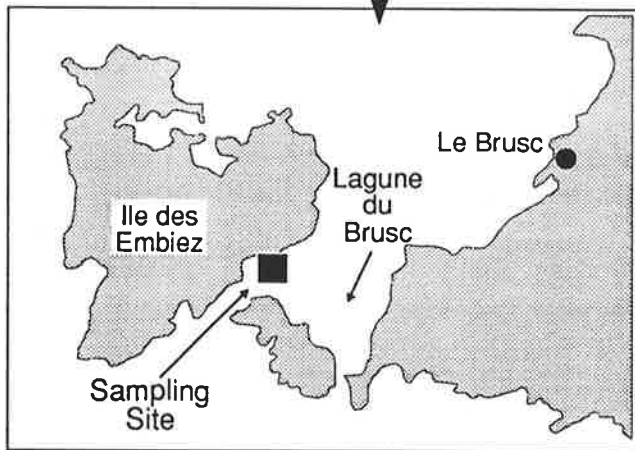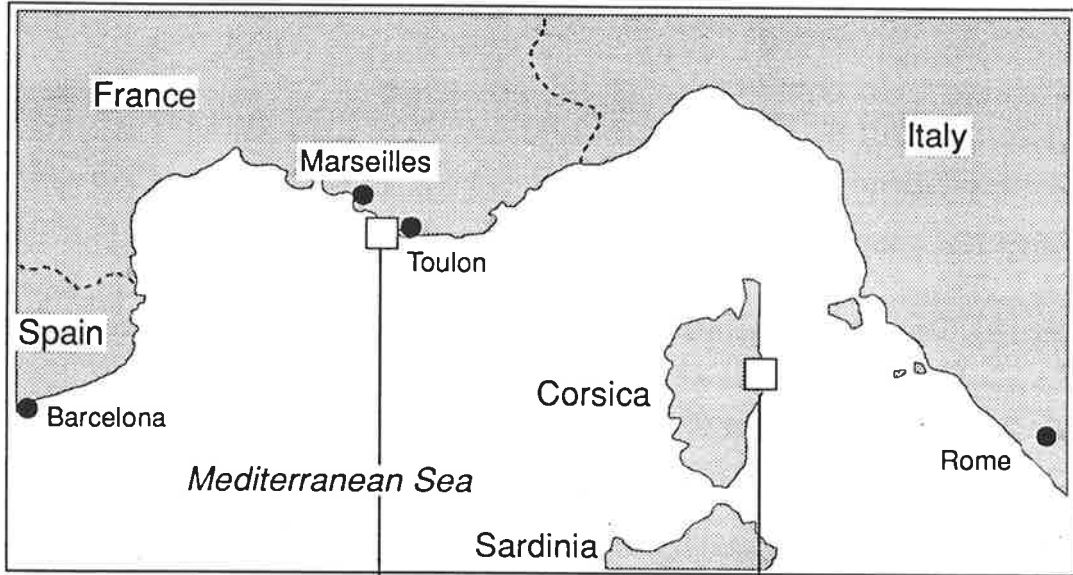
Fig. 8.2 Two-dimensional MDS ordination plots of epifaunal assemblages from Embiez based on abundance (stress = 0.146) labelled by a) habitat, and b) block.
C = control; P = partly cut; R = removed
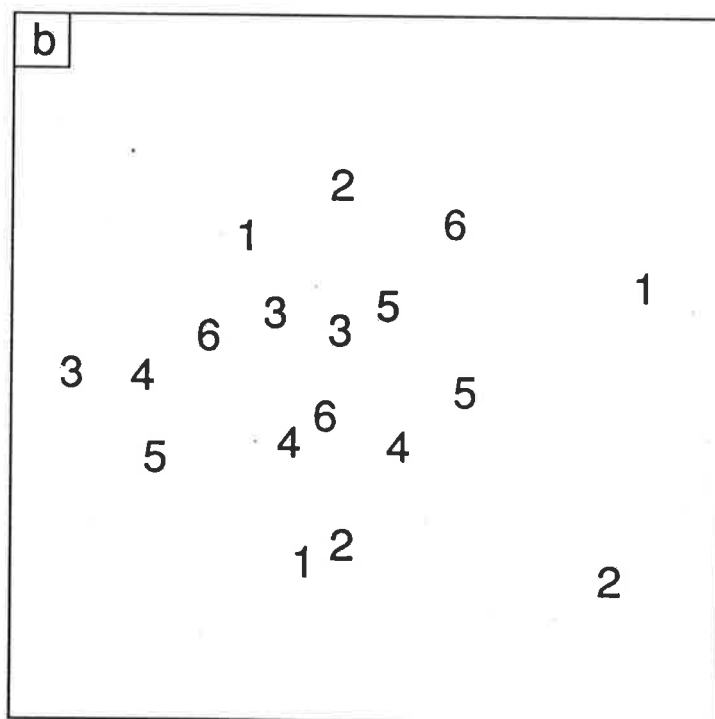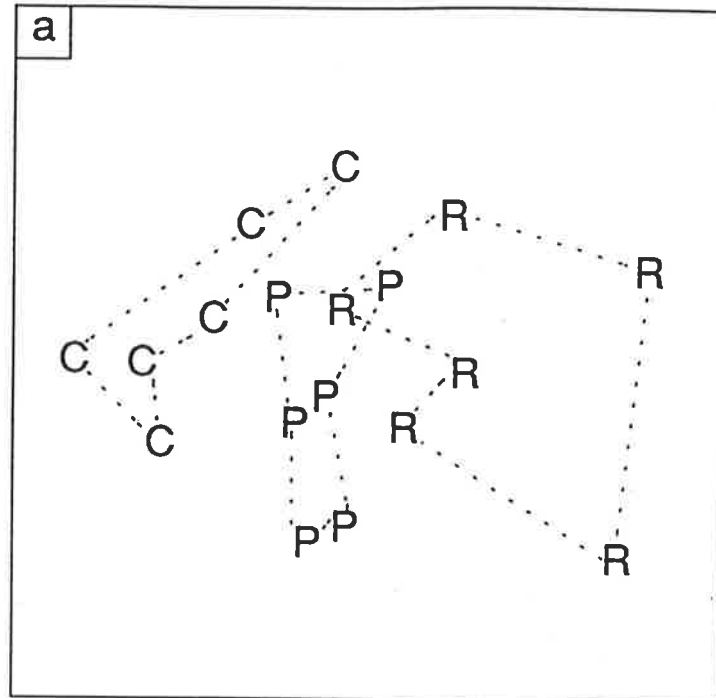
Fig. 8.3 Two-dimensional MDS ordination plots of epifaunal assemblages from Embiez based on biomass (stress = 0.138) labelled by a) habitat, and b) block.
C = control; P = partly cut; R = removed
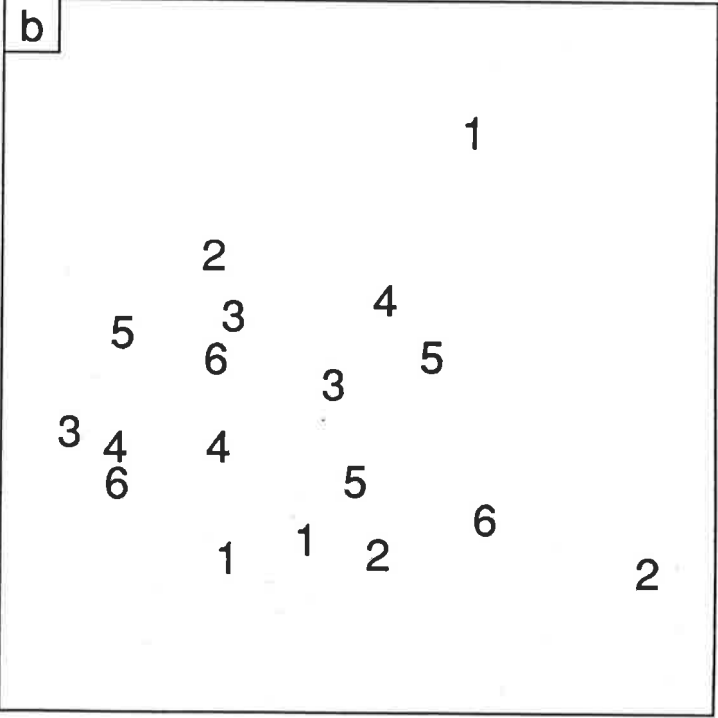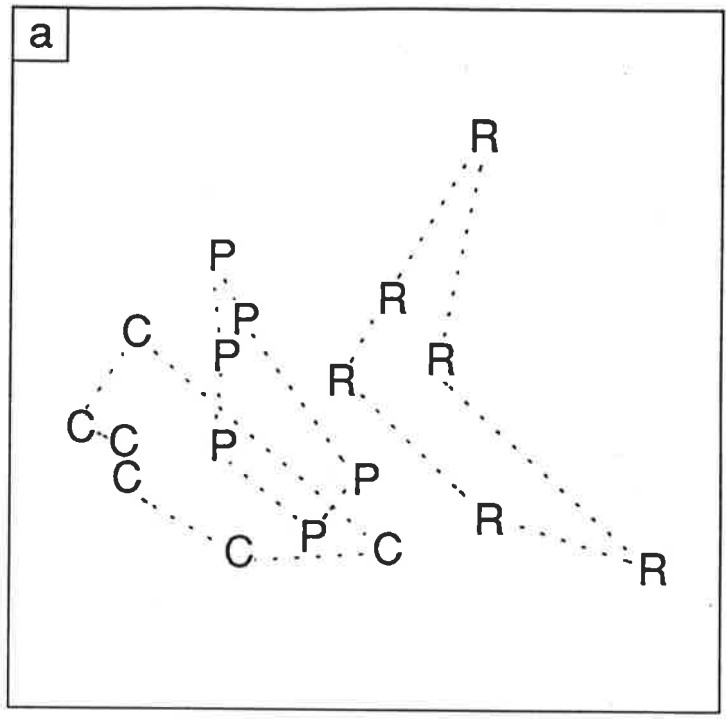
Fig. 8.4 Two-dimensional MDS ordination plots of epifaunal assemblages from Diana - Night based on abundance (stress = 0.068) labelled by a) habitat, and b) block. C = control; P = partly cut; R = removed
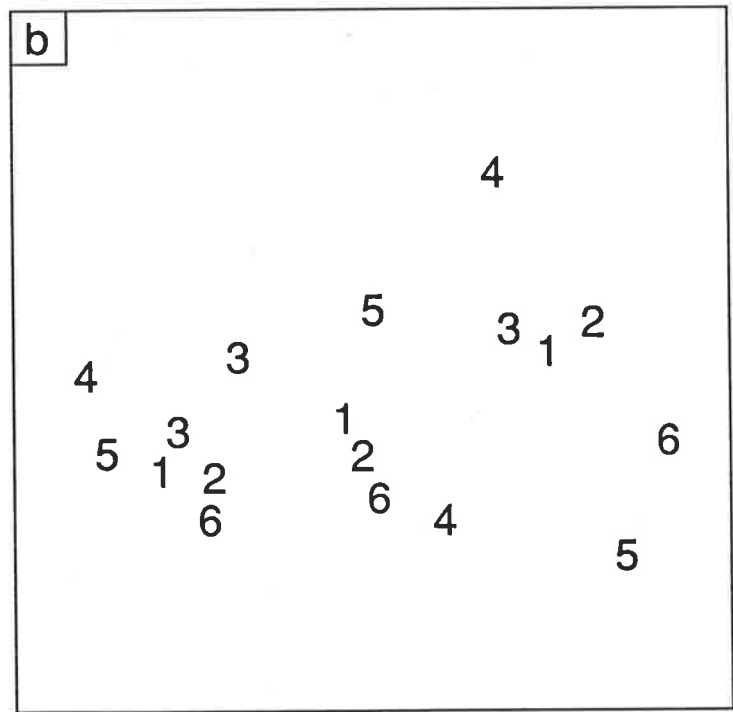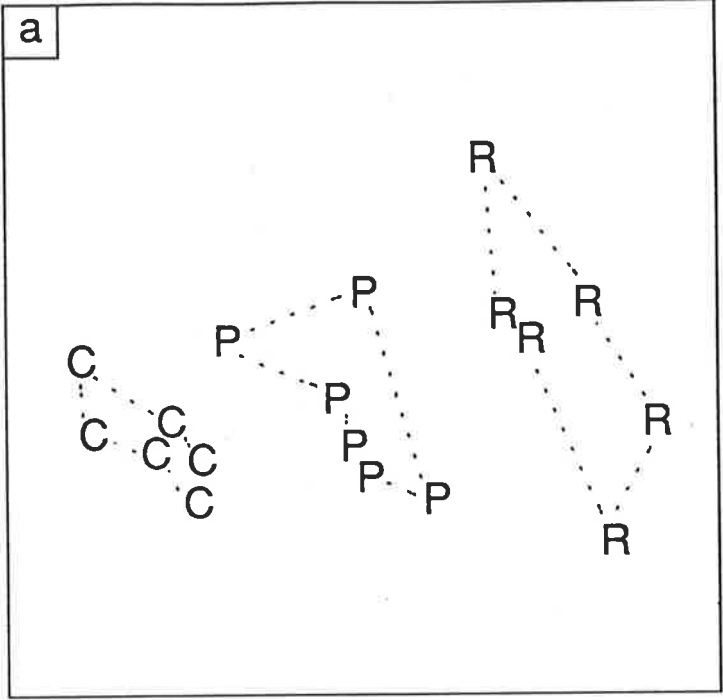
Fig. 8.5 Two-dimensional MDS ordination plots of epifaunal assemblages from Diana - Night based on biomass (stress = 0.073) labelled by a) habitat, and b) block. C = control; P = partly cut; R = removed
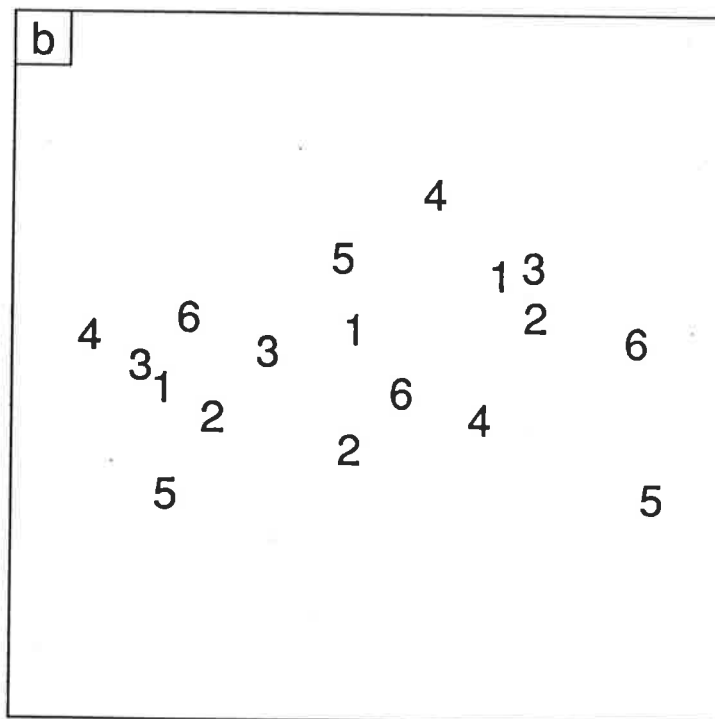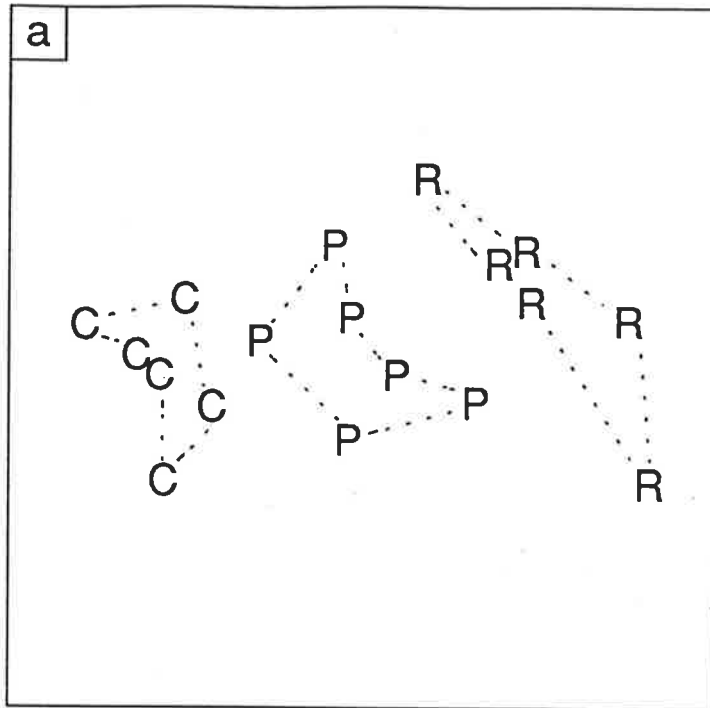
Fig. 8.6 Two-dimensional MDS ordination plots of epifaunal assemblages from Diana - Day based on abundance (stress = 0.073) labelled by a) habitat, and b) block. C = control; P = partly cut; R = removed
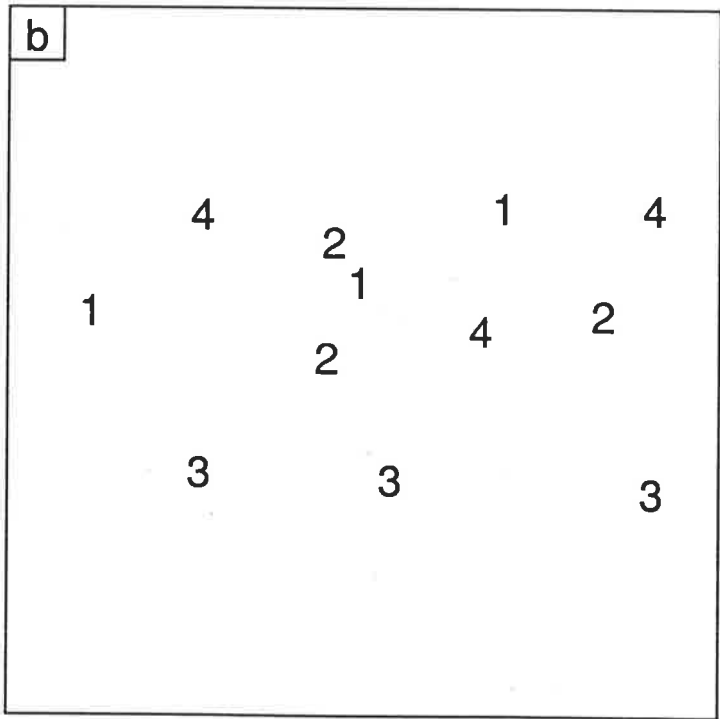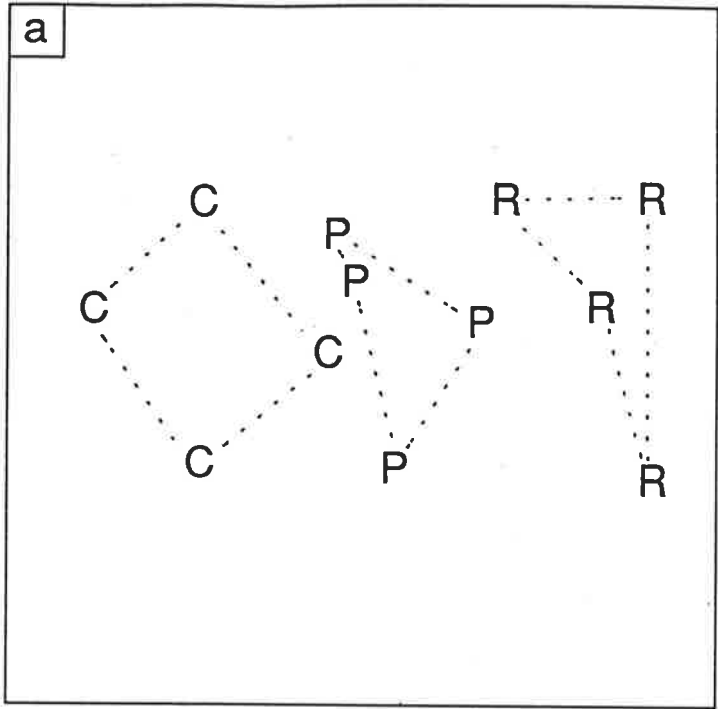
Fig. 8.7 Two-dimensional MDS ordination plots of epifaunal assemblages from Diana - Day based on biomass (stress = 0.042) labelled by a) habitat, and b) block. C = control; P = partly cut; R = removed
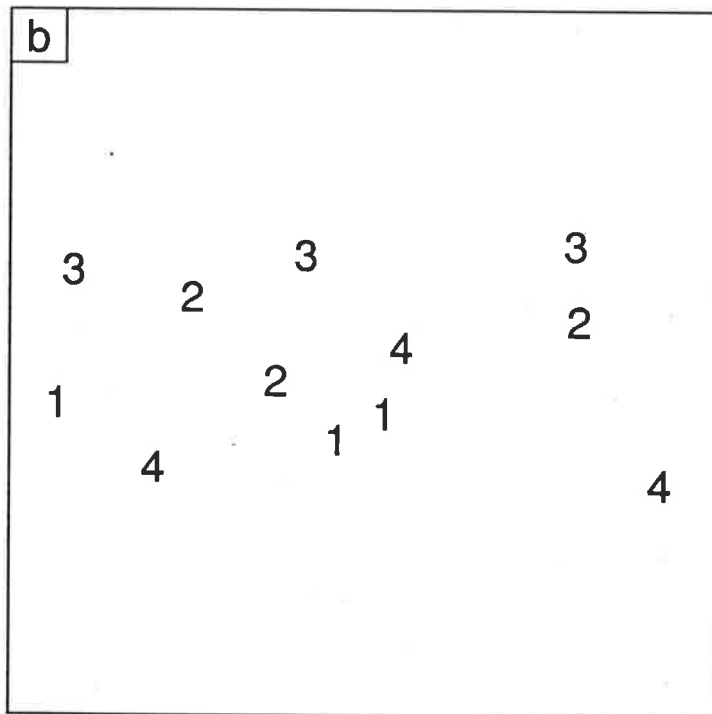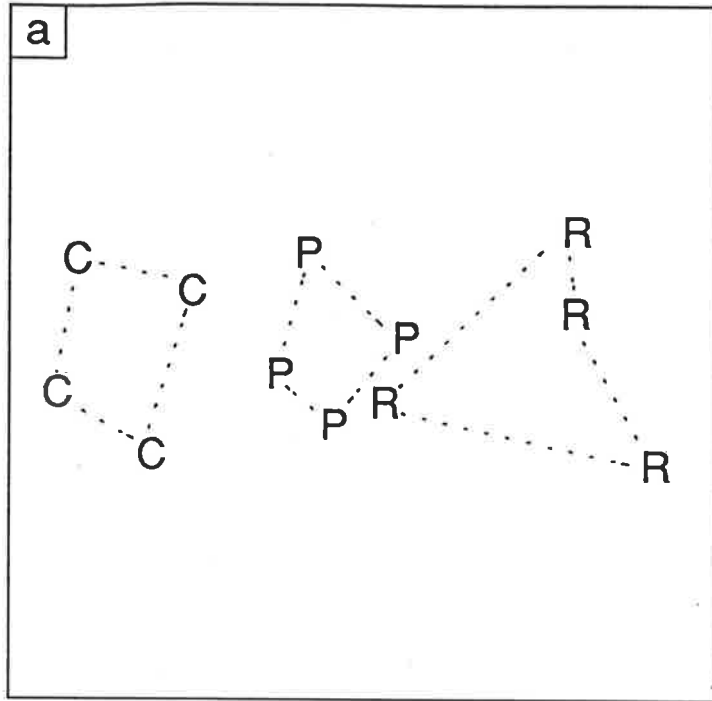
Fig. 8.8 Two-dimensional MDS ordination plots of epifaunal assemblages from all locations based on a) abundance (stress = 0.123) and b) biomass (0.124).
C = control; P = partly cut; R = removed
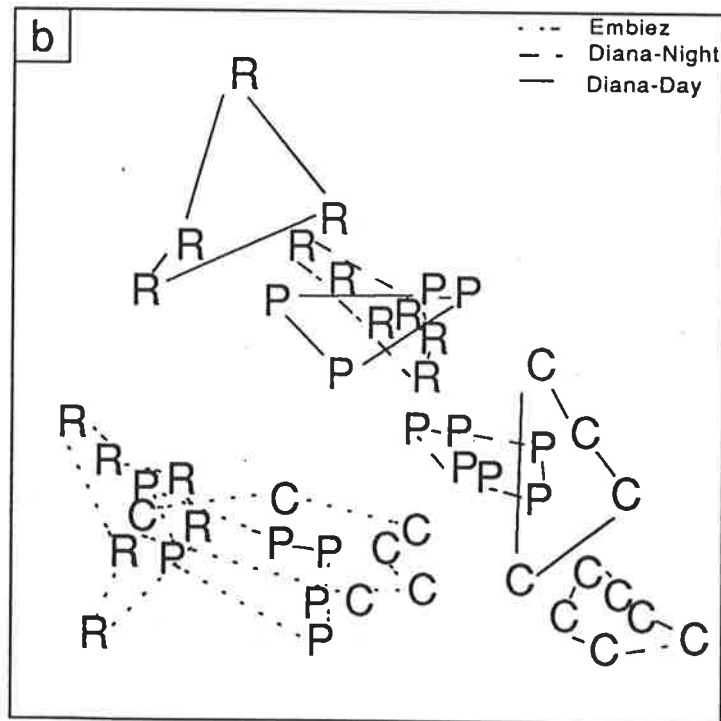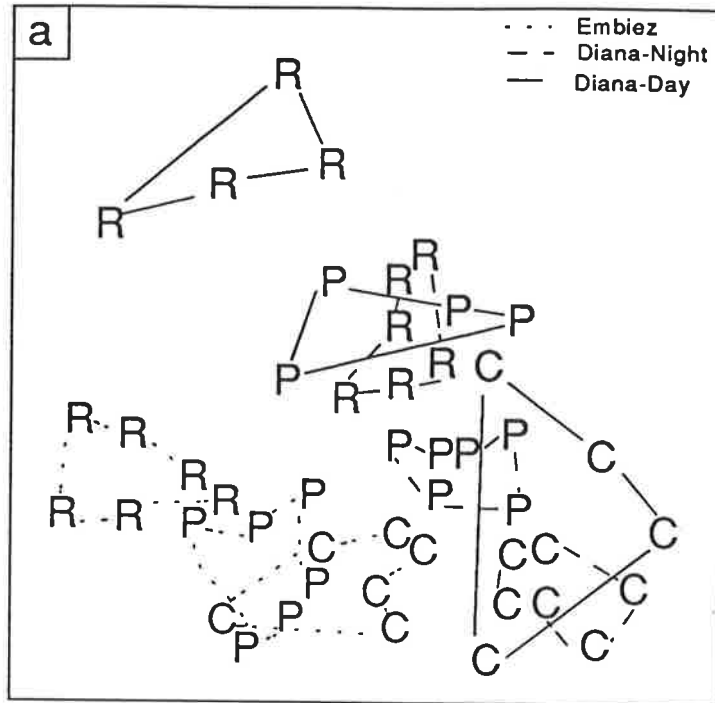
Chapter 9

The role of seagrass as preferred habitat for juvenile
Sillaginodes punctata: habitat selection or feeding?

9.1 Introduction

The two most common explanations for the greater abundance of fish in seagrass have been 1) that seagrass provides greater protection from predators (often larger fish) and 2) that seagrass provides more food, not directly to grazing fish but by supporting a diverse and abundant invertebrate fauna which can be consumed by carnivorous fish. Although many studies have shown a higher abundance of invertebrates associated with seagrass than with adjacent unvegetated patches (Howard *et al.* 1989), the role of seagrass in providing protection from predators is the model that has received most attention in recent times (Heck & Orth 1980; and see review by Bell & Pollard 1989).

Predation seems not to be the direct cause of greater fish abundances in seagrass compared with unvegetated habitat. A more strongly supported explanation is that of Bell & Westoby (1986b) in which fish select seagrass in preference to unvegetated areas. The same hypotheses have been tested using motile invertebrates, which are also more abundant in seagrass compared to unvegetated habitats. Experiments investigating the importance of seagrass to invertebrates as a source of protection from predators have tested between the two factors, protection from predators and habitat selection. The behavioural mechanism of habitat selection is assumed to be a response to the habitat itself, but the underlying advantage might result from any of several factors, including increased living space and food availability (Leber 1985). Movement to find food is not the same thing as habitat selection, but may result in occupation of habitat that supports more food.

As Bell & Westoby (1986a) point out, the results of their experiments may be explained in ways other than habitat selection by fish. Fish might, for example, be attracted to more abundant food in denser seagrass. A model not excluded by their results is that of small fish simply swimming (with or without pattern) until they find food, at which time they pause to eat it, and continue swimming. Under this scenario, referred to as the simple feeding model in this thesis, fish would be found more often where there is more food, regardless of seagrass density. This contrasts with the prediction from the habitat selection model that fish should be found in seagrass regardless of the amount of food available.

Adult *Sillaginodes punctata* spawn offshore, and post-larvae settle in shallow coastal areas (Jones *et al.* 1990). As early juveniles, they are much more common over eelgrass (*Zostera muelleri*) than over adjacent unvegetated patches (Chapter 3). This close association with eelgrass makes early juveniles of *S. punctata* ideal test animals for distinguishing between the predictions of the two models, habitat selection and simple feeding. Furthermore, in the experiment in Chapter 6 in which eelgrass was removed from large patches, *S. punctata* numbers matched invertebrate production more closely than presence or absence of eelgrass. The responses of *S. punctata* were therefore investigated in the laboratory, where food availability and vegetation could be controlled independently.

The contents of this chapter are substantially the same as those in Connolly (1994a) entitled "The role of seagrass as preferred habitat for juvenile *Sillaginodes punctata* (Sillaginidae, Pisces): habitat selection or feeding?", which is included as Appendix A.6.

9.2 Materials and Methods

Experiments were done in a tank, in which food availability was manipulated whilst fish were offered a choice between vegetated and unvegetated habitats. Half of the sandy bed of a 1 m diameter circular fibreglass tank was planted with eelgrass (*Zostera muelleri*) collected from the sublittoral fringe in Barker Inlet (described in Chapter 3). Sediment was washed from the roots of the eelgrass to remove fauna prior to planting, leaving clean, intact plants, which were soaked for eight hours in fresh water. This soaking killed associated fauna, but did not damage the eelgrass. Eelgrass was then planted into sterilised sand and the tank filled with 350 l of filtered seawater. This stand of eelgrass was similar to that at the collection site with respect to shoot density and shape, size, colour, texture and flexibility of leaves. The remaining half of the tank was left as bare sand. The absence of motile invertebrates in the tank was confirmed using a 95 μm sweep.

Mean leaf length of eelgrass was 107 mm (s.e. 3.7) and water depth was 450 mm. This left a large volume of water above the eelgrass canopy, a space in which *S. punctata* juveniles are often observed swimming in the field. Eelgrass appeared green and healthy throughout the study and was replanted in a different sector of the tank part way though each individual experiment. The tank was also rotated through a random number of degrees between trials within experiments.

Juvenile *S. punctata* were collected from Barker Inlet using a 1 mm mesh seine net two days before the beginning of each experiment. Mean fish length of all fish used in experiments (measured after experiment) was 35.8 mm (s.e. 0.37). These fish were collected from an area with patches of eelgrass and unvegetated habitat. The fish were allowed to acclimate to laboratory conditions in five holding tanks, each with live eelgrass and unvegetated patches. Fish were fed frozen brine shrimp (*Artemia* sp.) for

the first day and then live crustacea (their normal diet) collected from Barker Inlet for the remainder of their time in the holding tanks.

For all experiments, each trial began by placing five fish, one from each holding tank, into a clean, opaque tank containing no food, eelgrass or sand. After two hours, during which feeding was impossible, the five fish were released at the water's surface in the centre of the experimental tank. All fish immediately swam towards the bottom. In experiments requiring food, frozen brine shrimp were thawed and pipetted onto the sediment surface prior to fish release. For day experiments, an observer, motionless at the side of the tank on the boundary between the two habitats, recorded the habitat with which each fish was associated, every five minutes for the first 20 minutes, then every 15 minutes until 125 minutes after fish release. The height above sediment and distance to the side of the tank were also estimated. For night experiments, observations were made only at 10 minutes and 60 minutes, by briefly illuminating the tank with a spotlight.

All fish from four trials (i.e. 20 fish) in the day and night experiments in which food was offered were preserved for later inspection of their stomachs for the presence of brine shrimp.

Design, And Predictions From The Models

Two designs were used to test between the models: 1) food offered only in the unvegetated habitat and 2) food offered in neither habitat. Predictions from the two models for both designs are shown in Table 9.1.

Although early juvenile *S. punctata* do not school strongly, the position of a fish in the tank could have affected the positions of other fish. To avoid potential dependence of counts within a tank, in each trial at each observation time the habitat containing the

majority of fish scored a one and the other habitat a zero. These scores were summed for each time over the 25 trials of that experiment. Counts were then averaged over two periods (≤ 20 minutes and > 20 minutes) in which fish behaved quite differently in some experiments. The aim was to test the mean scores of each habitat (over the two periods) for departure from a 1:1 ratio. Data from night experiments were analysed using chi-square tests. Chi-square tests are valid only when based on counts drawn from a Poisson distribution (Kramer & Schmidhammer 1992). Counts within each time were tested for departure from a Poisson distribution using a runs test with two nominal categories (Zar 1984) and no departure from Poisson was detected in either of the experiments. It was possible to analyse data from day experiments using a t-test by using the multiple times to generate frequencies for each trial. This makes a more powerful test than the chi-square test (Kramer & Schmidhammer 1992). A one-sample t-test was used to determine whether fish were more often over one habitat by testing whether the average number of times the majority of fish were over a habitat differed from the expected average (based on a 1:1 ratio).

Fish near the side of the tank (within three centimetres) may have been exhibiting thigmotaxis (the habit of positioning themselves near objects) and may not have been choosing between habitats. The scoring within trials was recalculated after excluding these fish. Where the same number of fish was found over both habitats, each habitat scored 0.5.

For both day experiments, the heights of fish over the two habitats were averaged in each trial over all times, so that for each trial there was one number for each habitat. The habitats were compared using the numbers from the 25 trials.

9.3 Results

Design 1. Food In Unvegetated Habitat Only

Day

Many fish spent the first 15 - 20 minutes feeding over the unvegetated habitat. After 20 minutes, fish fed much less frequently, and tended to be in or over the eelgrass. Significantly more fish were found over the unvegetated habitat during the first 20 minutes, but the number of fish over eelgrass after 20 minutes was not significantly greater (Fig. 9.1). The results over the first 20 minutes support the simple feeding model. Results from after 20 minutes fit the predictions of neither model.

The same patterns of fish behaviour and distribution were evident when fish close to the side of the tank were excluded from calculations (Fig. 9.1). Fish were closer to the side of the tank much more often over unvegetated habitat, and the effect of removing these from calculations was to increase the relative frequency of occurrence over eelgrass habitat.

Night

Fish were positioned differently during the night compared to the day. At both observation times there were no obvious patterns (Fig. 9.1). This evidence fits the predictions of neither of the models and does not distinguish between them. Results were changed very little when fish close to the side of the tank were excluded from calculations (Fig. 9.1).

Design 2. Food In Neither Habitat

Day

Fish were consistently more common in eelgrass habitat than over unvegetated habitat over both periods, and the differences were significant (Fig. 9.1). No marked difference in fish behaviour was observed before and after 20 minutes. These results support the habitat selection model.

When fish close to the side of the tank were excluded, the relative frequency of occurrence in eelgrass was increased. The pattern was the same as when all fish were included (Fig. 9.1), again supporting the habitat selection model.

Night

As for Design 1, no departure from a 1:1 ratio was found at either 10 or 60 minutes (Fig. 9.1). The evidence supports the simple feeding model as a departure from a 1:1 ratio was not shown (Table 9.1). This support is, however, logically weak, being a situation in which a negative (non significant) result supports a positive conclusion (support for a model) (Toft & Shea 1983); the result is more useful after consideration of statistical power. Power calculations for the tests on fish positions are the same for both times. From observed values 14 : 11, which is an effect size of about 0.1 (Cohen 1988; equation 7.2.1), and with $\alpha = 0.05$, power = 0.1. If we require equal probabilities for Type I and II statistical errors ($\alpha = \beta = 0.05$, and power = 0.95), with n = 25, the minimum detectable effect size is 0.7, which equates to a departure from 1:1 of at least 21 : 4. The low power of the test limits the support that the result provides for the simple feeding model.

Results were the same when fish close to the side of the tank were excluded from calculations.

Stomach Analysis

16 of the 20 fish from the Day, Design 1 experiment had brine shrimp in their stomachs. In the night experiment, eight fish had brine shrimp in their stomachs. The stomachs of several of the fish from the day experiment were full to the point of being distended, a condition not observed during the analysis of hundreds of stomachs from *S. punctata* juveniles feeding in the wild (Chapter 4).

Distance Above Sediment

In both the Day, Design 1 experiment and the Day, Design 2 experiment fish were significantly higher above the sediment in the eelgrass than on the unvegetated side (Design 1: eelgrass, mean 56 mm (s.e. 1.7), unvegetated 28 mm (0.9), $p < 0.001$; Design 2: eelgrass 64 mm (1.4), unvegetated 32 mm (0.9), $p < 0.001$). The mean height in eelgrass was less than the top of the canopy, and only a few fish were observed above the canopy.

9.4 Discussion

The daytime distribution of *Sillaginodes punctata* did not completely support either of the two models, habitat selection or simple feeding. Rather, the results show that after fish had been unable to feed for a short period their distribution could be explained by the simple feeding model if food was present. Once satiated, however, fish showed no preference for either habitat, and neither model was supported. In the absence of food, their distribution could be explained by the habitat selection model.

Night experiments gave conflicting results. In Design 1, neither model was supported, as fish were evenly distributed in the tank. In Design 2, the same even distribution fits the predictions of the simple feeding model. The support is weak, however, because the test had little power to detect a departure from a 1 : 1 ratio. Even were a 1 : 1 ratio to be demonstrated, the simple feeding model is not the only explanation of an even distribution of fish. Taken together with the evidence from the Design 1 experiment, it seems that at night, although some of the fish ate food, they generally took little account of habitat or food.

Much of the rationale for the current experiments comes from the work of Bell & Westoby (1986a,c), especially the discounting of predation as the proximate cause of lower fish abundance in less dense seagrass. However, Bell & Westoby compared fish densities of dense and sparse eelgrass, unlike the present comparison between eelgrass and unvegetated habitat. Their overall model has two parts relevant to the present study: 1) fish larvae settle into seagrass beds, regardless of the density of seagrass, in much greater abundance than into adjacent unvegetated areas, and 2) juvenile fish then select dense in favour of sparse seagrass within the seagrass bed. Although *S. punctata* juveniles and the eelgrass beds of Barker Inlet are similar to the fish and eelgrass worked on by Bell & Westoby, in Barker Inlet fish move in over tidal flats on each tide, necessarily choosing anew each tide between eelgrass and unvegetated

habitat. In this system, comparisons both of differing densities of eelgrass and of eelgrass with unvegetated habitat are relevant. The model of Bell & Westoby explains two observations; the fact that more fish occur in seagrass and the fact that they are more abundant in dense seagrass. Both observations can, however, possibly be explained by the simple feeding model, since there is more food available in denser eelgrass than in sparser eelgrass, and more food also in sparser eelgrass than adjacent bare patches (Chapter 5, and Howard *et al.* 1989).

The models of habitat selection, simple feeding and protection from predators need not apply exclusively to all animals in seagrass. In tests comparing the importance of predation and habitat selection for invertebrates, different species gave different results (Leber 1985). Rozas & Odum (1988) concluded that both protection from predation and better feeding were important for the fish they studied in freshwater marshes, although the mechanism resulting in more fish being in vegetated patches was not determined. In tank experiments offering a choice between artificial seagrass and unvegetated substratum, juvenile walleye pollock (*Theragra chalcogramma* (Pallas)) were found more often over unvegetated habitat, except when fish were exposed to a predator model, when fish moved to the artificial seagrass (Sogard & Olla 1993). Sogard & Olla (1993) took no account of food availability.

The daytime responses of juvenile *S. punctata* differed depending on whether or not food was present. Even in the presence of abundant food, however, behaviour differed as time spent exposed to food increased, presumably resulting in a decreasing degree of hunger. In optimality modelling terms, responses depended on an environmental variable but also on the internal state of animals (Krebs & Kacelnik 1991). An appropriate further step in experimentation with *S. punctata* would be to examine the trade-offs fish make in deciding, for example, whether to search and where to search for food. The current experiments could also be combined with exposure to predators. Fish should be

offered choices not only between seagrass and unvegetated habitats but also between dense and sparse seagrass.

The experiments presented here were done in the laboratory because there is no way of presenting food independently of vegetation in the field. A feature of these experiments is that live eelgrass was used in the tank. However, several other factors may be importantly different from field conditions. The density of *S. punctata* in Barker Inlet ranges up to 2.5 fish/m^2, whereas in the tank the density was 7.1 fish/m^2. The size of the tank was small relative to the size of patches worked on by Bell & Westoby (1986a,c) and to those in the surveys (Chapter 3) and field experiments (Chapter 6) of this thesis. The observations that generated the models tested in this chapter may not hold for patch sizes as small as those in the tank. An alternative explanation for the increased abundance of fish in eelgrass in the tank is that, because water circulation was less vigorous than in the field, dissolved oxygen levels may have been higher on the eelgrass side during the day. Oxygen levels were not measured during the experiments.

Habitat selection by fish may be innate or learned. These two possibilities have not been separated here, since fish were collected after exposure to an area with eelgrass and unvegetated patches, and concomitant abundances and distributions of predators and prey. As well, fish were briefly exposed to the habitats and prey distribution of holding tanks.

In conclusion, the responses of early juvenile *S. punctata* support the simple feeding model when fish have not fed for a short time, but support the habitat selection model in the absence of food. *S. punctata* took little account of food availability or habitat type at night.

Table 9.1 Predicted results from the two models, habitat selection and simple feeding

✓✓ = majority of fish on this side of tank.
✓ = about half of fish on this side of tank.

Model	Design 1		Design 2	
	Eelgrass No food	Unvegetated Food	Eelgrass No food	Unvegetated No food
Habitat selection	✓✓		✓✓	
Simple feeding		✓✓	✓	✓

Fig. 9.1 Mean number of trials in which most fish were in eelgrass habitat. Circles are means for the periods shown as numbers in minutes on x-axis. Closed circles = all fish. Open circles = fish near side of tank excluded. Dashed line shows number of trials expected (12.5) under 1:1 relationship between eelgrass and unvegetated habitats. Statistical significance is shown by probabilities: ns > 0.05, * < 0.05, ** < 0.01, *** < 0.001, based on chi-square tests for night data and t-tests for day data, as explained in text. Probabilities are the same for open and closed circles except for Design 2, Day, ≤ 20 minutes, as shown

Mean no. of trials in which fish predominated in eelgrass

Design 1: Food in unvegetated habitat only

Design 2: Food in neither habitat
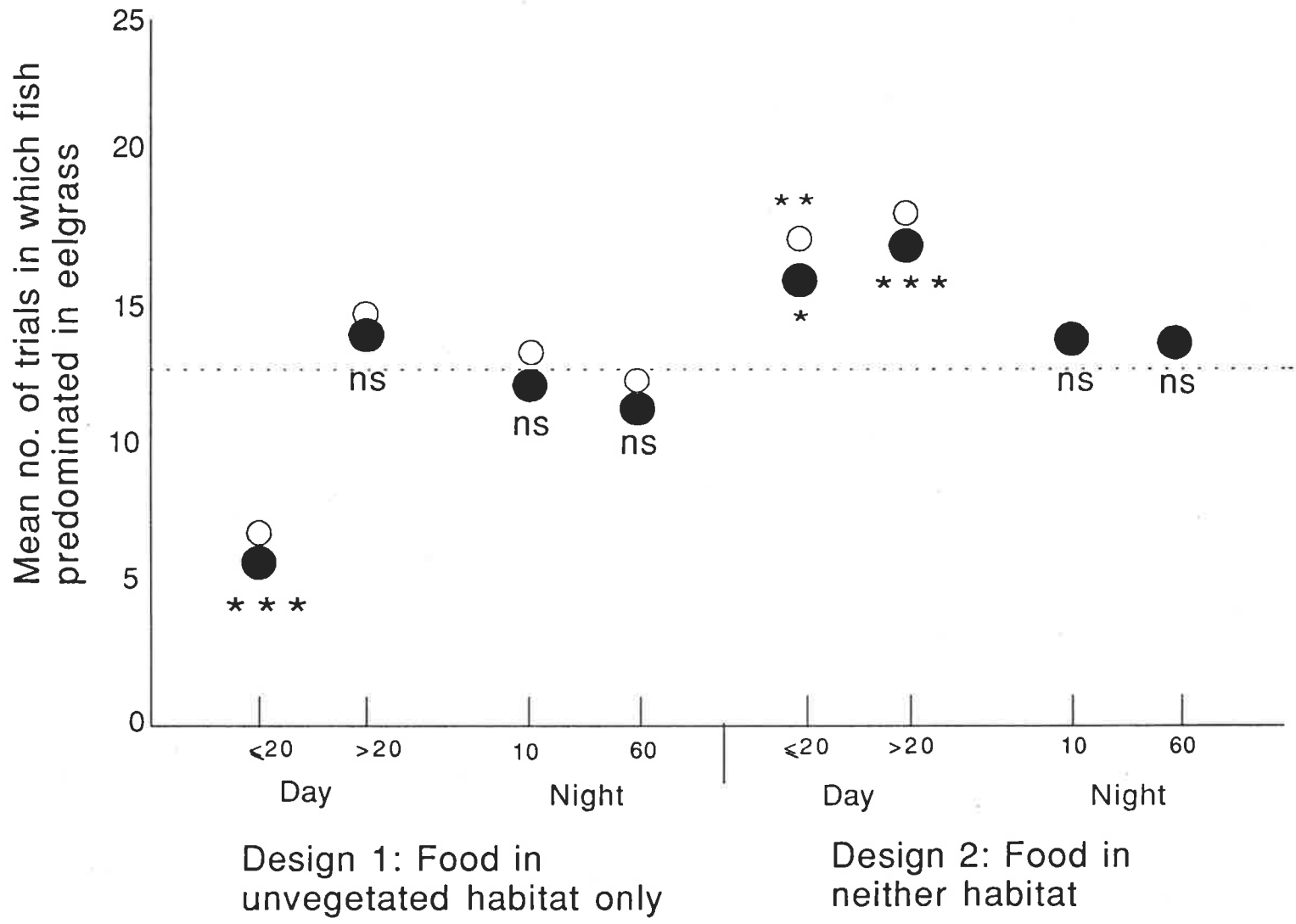
Chapter 10

Concluding discussion

The pattern of fish abundances typical of shallow, soft-substratum habitats worldwide has been confirmed for the first time in South Australian waters. Eelgrass areas of Barker Inlet had more species of fish, more individuals, and different assemblages of fish than unvegetated areas. These differences persisted through time. Prey availability was also greater in eelgrass than in unvegetated habitat. This is consistent with a model in which fish distributions are determined by food availability.

To determine whether it is the eelgrass itself that is important to fish or whether they are influenced by some environmental factor associated with eelgrass presence or absence, manipulative experiments are required in which fish are presented with eelgrass and unvegetated patches without the possibility of confounding environmental factors. Attempts to demonstrate the importance of seagrass *per se* to either fish or invertebrates have mostly involved the placement of seagrass mimics in unvegetated areas. Although this method has been useful in answering questions about dispersal, in the context of the present study aimed at assessing the likely impact of seagrass loss, seagrass removal experiments more closely match the questions posed. Although there are few examples of experiments in which seagrass cover has been reduced on a spatial scale large enough to affect fish in a measurable way, when tried it has been very informative. The experiments involving thinning and reduction in height of eelgrass by Bell & Westoby (1986a,c), for example, in conjunction with predator exclusion cages and evidence from surveys, encouraged a shift in emphasis in the literature from direct predation to preferential selection of habitat by fish. The disadvantages of manipulating seagrass cover are discussed in Chapter 6.

Habitat selection models implicate above-ground vegetation (canopy) as the part of seagrass important to fish. When eelgrass canopy was removed experimentally (Chapter 6), total fish numbers declined but not to levels found in adjacent patches unvegetated prior to the experiment. When pelagic species were excluded from analyses, total fish numbers were the same in patches of untouched eelgrass and patches cleared of eelgrass, and both of these habitats had many more fish than unvegetated patches. Numbers of *Sillaginodes punctata* were not reduced at all in patches cleared of eelgrass, but were lower in unvegetated patches. These data do not fit predictions from the habitat selection model described in Chapter 1, at least in the sense that fish select habitat based on canopy characteristics. The data also conflict with possible explanations (listed in Lewis (1984)) for the importance of seagrass involving the presence of physical structure usable as living space or the dampening of hydrodynamic forces. Given that eelgrass removal also caused epifaunal production to decline slightly but not to match the low levels found in unvegetated habitat, results are, however, consistent with predictions from the simple feeding model.

Some fish species increased in abundance when eelgrass was thinned experimentally in NSW by Bell & Westoby (1986a). To determine whether prey availability could explain this, more detailed analysis of the types of invertebrates actually accessible, physically and behaviourally, to the fish species concerned would be needed.

Bell & Westoby (1986b) found that although *within* a bed, denser seagrass supported more fish than sparse seagrass, fish numbers did not consistently match seagrass densities across a greater spatial scale involving a number of beds. Dense seagrass in some beds supported no more fish than sparse seagrass in other beds. This observation, along with the high rates of settlement into artificial seagrass placed in unvegetated areas, prompted Bell & Westoby to propose that fish are distributed patchily prior to settling, settle into seagrass rather than unvegetated habitat, and then re-distribute within a bed but not between beds. The higher number of fish in some beds may, however, also be consistent

with the explanation of fish distributions in terms of food availability, if the beds supporting higher numbers of fish have more abundant prey.

In the surveys of eelgrass and unvegetated habitats (Chapter 3), patches ranged in size from 500 m^2 to several hectares. At this scale, abundances of several species, including *S. punctata*, and of all species combined, were much higher in eelgrass, and fish assemblages from the two habitats were clearly different. In the field experiment (Chapter 6), comparisons of fish from "control" eelgrass and unvegetated habitat can be considered equivalent to comparisons made during August and October surveys. Abundances of *S. punctata* and all species combined were again higher in eelgrass than in unvegetated habitat, although the statistical significance of these differences was less convincing than in surveys. This could be partly because of the smaller sample size in the experiment than in the survey (n = 5 compared with n ≥ 7). The differences between eelgrass and unvegetated patches really do seem to be less than in surveys, however. As an example, more *S. punctata* were caught over unvegetated habitat during the experiment than at any period in the survey, even though the total unvegetated area netted during the experiment was considerably less. The marked difference in fish assemblages between eelgrass and unvegetated habitats found during the survey was also not evident during the experiment. These less pronounced differences probably reflect the smaller size of unvegetated patches in the experiment, which ranged from 30 m^2 to 120 m^2. Ferrell & Bell (1991) showed that fish assemblages in unvegetated habitat were more different from those in eelgrass (*Zostera capricorni*) at 100 m than at 10 m from eelgrass. The surveys of Chapter 3 involved unvegetated patches at the former scale, whilst the field experiment of Chapter 6 involved unvegetated patches at the latter scale.

Alternatively, the different collecting method used in the experiment could be responsible for the less obvious difference found between fish from eelgrass and unvegetated habitats. The qualitative similarity of results from the experiment using pop nets and the surveys using seine nets gives some confidence that differences in catches between seine

and pop nets are approximately the same in both eelgrass and unvegetated habitat, but the possibility remains that the effectiveness of the two methods of collection in the different habitats is dissimilar enough to cause the reduction in magnitude of differences between eelgrass and unvegetated habitat.

The laboratory experiment using *S. punctata* (Chapter 9) which enabled food and eelgrass to be offered independently of each other was conceived as a further test of the predictions from the two models, habitat selection and simple feeding. I felt that it was necessary to use a laboratory experiment for this test because it is impossible in the field to allow fish access to eelgrass and unvegetated habitat whilst excluding much smaller, highly mobile invertebrates which are attracted to eelgrass. The habitat patches offered in the tank, however, were much smaller than the patches of habitat sampled in the field experiment (Chapter 6) and surveys (Chapter 3). The Mediterranean work (Chapter 8) indicates that epifauna respond to changes in seagrass cover at the scale of 1 m^2, which is approximately that used in the tank experiment. Nevertheless, the juvenile *S. punctata* used in the tank experiment were larger than the epifauna dealt with in the Mediterranean experiment, and were very mobile. The spatial scale of the tank experiment was perhaps too small to make a convincing test between the two models, at least using *S. punctata*. More sedentary species such as syngnathids, even where these are larger than the *S. punctata* used in the experiment, may be more amenable to small scale laboratory experiments.

If results from the laboratory experiment (Chapter 9) are meaningful despite the small scale, then they provide some evidence that food availability can determine fish distribution. The importance of food availability was clear when fish had been unable to feed for a short time, but diminished when fish became satiated. In the sea, where food might never or rarely be as freely available to fish as it was in the laboratory experiment, fish may spend much of their time looking for food (i.e. to some extent, be less than satiated). Thus, the implication that food availability is a determinant of fish distribution

may well be applicable to non-laboratory scenarios. On the other hand, the fact that fish presented with eelgrass and unvegetated habitat tended to occur more often over eelgrass when no food was present anywhere in the tank indicates that the simple feeding model alone does not fully explain fish distribution. Selection of habitat, at least at this spatial scale, seems to be involved.

The close association of juvenile *S. punctata* with eelgrass shown here has not always been demonstrated in Victorian studies. In Western Port, juveniles were found over eelgrass soon after settling but at about 45 mm length were more common over unvegetated habitat, where they fed on ghost prawn larvae and to a lesser extent polychaetes (Robertson 1977). Early juvenile *S. punctata* were found more often over eelgrass in Port Phillip Bay but in Swan Bay, a smaller bay adjoining Port Phillip Bay, were more common in unvegetated patches amongst eelgrass (*Heterozostera tasmanica*) (Jenkins *et al.* unpubl. MS, a). Jenkins has suggested that distributional patterns of juvenile *S. punctata* are linked not with habitat *per se* but with food availability. No evidence of higher numbers of *S. punctata* in unvegetated habitat in Barker Inlet was found at any sampling period, although this may have occurred between sampling periods. At no period was there any noticeable leap in levels of prey availability in unvegetated patches of Barker Inlet comparable with the seasonal abundance of ghost prawn larvae reported by Robertson (1977).

The diet of older juvenile trumpeter whiting (*Sillago maculata maculata* Quoy & Gaimard) in Botany Bay, NSW, also comprised ghost prawns and polychaetes. Juveniles of this species, and of the other sillaginid species common in shallow waters in NSW, sand whiting (*Sillago ciliata* Cuvier), were found over both eelgrass (*Zostera*) and unvegetated sand (Burchmore *et al.* 1988).

Sillaginodes punctata spawn off-shore and larvae arrive in sheltered coastal waters several months later. Studies in the Barker Inlet region (Bruce & Short, unpubl. MS) and in Victoria (Jenkins & May 1994) have found that larvae are distributed patchily, both spatially and temporally, prior to settlement. This is consistent with part of the model of Bell & Westoby (1986b) explaining fish distributions over soft-substrata. In formulating hypotheses about the interaction between pre- and post-settlement processes in determining the distribution and abundance of juvenile *S. punctata* within a bay, Jenkins *et al.* (unpubl. MS, b) suggest that both the extent to which larvae are carried to parts of a bay and the amount of food available to newly settled juveniles are important. This is part of a recent trend towards synthesis of research emphasising pre-settlement and post-settlement processes as joint determinants of the eventual input to adult fish stocks (e.g. Levin 1994).

Epifaunal abundance and production were shown to decline in line with a reduction in eelgrass canopy. This result was obtained for partial reduction and total removal of canopy on a scale of 1 m² in the Mediterranean, and for total removal of canopy on a scale of 30 m² in Barker Inlet. The reduced epifaunal production resulting from partial reduction of canopy suggests that previous experiments looking at the effects on fish of altered levels of canopy cover fail to separate two factors: the response of fish directly to canopy changes and their response to changing prey availability. Epifaunal abundance and production in patches from which eelgrass canopy had been removed were not, however, as low as in unvegetated habitat. This evidence carries the same implications for explanations of the importance of seagrass to invertebrates as results described above did for its significance to fish.

Although the Barker Inlet region is technically an estuary, the outer reaches where my work was done are marine-dominated at all times of year, with salinities approximating those in the main body of Gulf St Vincent. Salinities in l'Etang de Diana and la Lagune du Brusc are also similar to those in the open Mediterranean Sea. I therefore consider that the findings presented here are applicable to shallow, sheltered coastal waters more generally, and that results can be appropriately incorporated with those from the extensive list of studies about fish and invertebrates in seagrass included in Chapter 1.

One of the most challenging problems with research to date using either artificial seagrass or manipulations of seagrass canopy has been to get the spatial scale large enough to have meaning in relation to actual areas of seagrass habitat being destroyed through human activities. Studies into the impacts on fish of losing hundreds of hectares of seagrass in a single area have always been mensurative (*sensu* Hurlbert 1984). Perhaps this is the only realistic way to gain information on the larger scale, in which case we are already on the right track in combining these hypothesis-forming surveys with smaller scale manipulations that provide useful information about the ecology and behaviour of juvenile fish.

The ultimate aim of the work in this thesis has been to contribute to the understanding of the role of seagrass as habitat for small fish, against the background of continuing destruction of seagrass habitat in all parts of the world. Reviews of the fauna associated with seagrass have already called attention to the need for conservation of seagrass areas (Kikuchi 1980, Pollard 1984). Here I have begun to separate the importance of seagrass from that of other factors that may be associated with seagrass, such as food availability, with a view to refining scientific advice to managers about the environmental conditions that affect fish.

Appendix

The following is a list of papers either published, in press or submitted for publication, corresponding to the chapters indicated.

A.1 Chapter 2. Comparison of fish catches from a buoyant pop net and a beach seine net in a shallow seagrass habitat. *Mar. Ecol. Prog. Ser.* 109: 305-309.

A.2 Chapter 3. A comparison of fish assemblages from seagrass and unvegetated areas of a southern Australian estuary. *Aust. J. Mar. Freshwat. Res.* 45 (in press).

A.3 Chapter 6. Removal of seagrass canopy: effects on small fish and their prey. *J. Exp. Mar. Biol. Ecol.* (in press).

A.4 Chapter 7. The effects of removal of seagrass canopy on assemblages of small, motile invertebrates. *Mar. Ecol. Prog. Ser.* (submitted).

A.5 Chapter 8. The effects of altering seagrass canopy height on small, motile invertebrates of shallow Mediterranean embayments. *Mar. Ecol. (Napoli).* (submitted).

A.6 Chapter 9. The role of seagrass as preferred habitat for juvenile *Sillaginodes punctata* (Cuv. & Val.) (Sillaginidae, Pisces): habitat selection or feeding? *J. Exp. Mar. Biol. Ecol.* 180: 39-47.

Throughout this thesis I have stressed the need to consider the statistical power and minimum detectable effect size of experiments, especially when considering non-significant test results. Many authors have made a clarion call for the consequences of making Type I and II statistical errors to be used in determining the ratio $\alpha : \beta$ (Fairweather 1991). During my candidature I submitted a manuscript entitled "Improved experimental procedure in ecology: what to set, what to forget" to *Australian Journal of Ecology* explaining an improved testing procedure in which the desired $\alpha : \beta$ ratio could be maintained after an experiment using an estimate of variability from the main experiment (rather than a pilot study). This manuscript was not accepted for publication, but during the review process, an unpublished manuscript by Bruce Mapstone (GBRMPA, Townsville) came to light; in it, he argued, *inter alia*, for the same concept in an environmental impact detection setting. Mapstone's paper, which is now being published (Mapstone, in press), gives a thorough theoretical account of the procedure that I also suggested. I think that my manuscript, which includes a worked example, can be recast as a useful intermediary between the statistical theory and the practical ecologist. I have included the manuscript as Appendix A.7.

A.1

NOTE

Comparison of fish catches from a buoyant pop net and a beach seine net in a shallow seagrass habitat

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ABSTRACT: A large (25 m²), remote-release buoyant pop net, floorless to permit normal behaviour by benthic fish, and a beach seine net were evaluated by comparing the catch from the 2 net types. Both nets were made of 1 mm mesh, aimed at catching fish from 10 to 100 mm long. The pop net caught more fish than the seine net, mainly because 9 times more *Favonigobius lateralis* were caught in the pop net. Apart from species such as *F. lateralis* that dwell on the sea bed, the seine net catches small fish as well as the pop net, and for some species is a faster, cheaper collection method for survey work. However, as seine net catches can misinform about rank order of abundances of species, its main use may be in collecting presence/absence data. The pop net gives a more accurate estimate of bottom-dwelling species, and is useful where fish need to be collected from small, defined areas such as experimental plots.

KEY WORDS: ■ · ■ · ■

Seagrass meadows provide important habitat for small fish (Pollard 1984) and most ecological studies of seagrass fauna include estimates of fish densities. Methods used to count fish include netting, poisoning and visual surveys. The composition of fish assemblages reported depends on the method of collection. Assemblages collected from the same meadow using 2 different methods (poisoning and trawling) can be more different than assemblages collected from different meadows with the same method (Gray & Bell 1986). Even the catches from different types of beam trawl from within one seagrass meadow vary in number of species and number of individuals (McNeill & Bell 1992).

Fish assemblages in eelgrass *Zostera muelleri* Irsmisch ex Aschers. meadows in shallow South Australian embayments have been surveyed using a small, fine mesh beach seine (Connolly in press). Data from seine netting is more informative when an estimate can be made of the catching efficiency of the net (Parsley et al. 1989), and to do this, a buoyant pop net was

designed with the aim of making a more complete catch. The seine net is also unwieldy when fish need to be collected from small, defined areas. The pop net permits collection of fish from such areas, for example from experimental plots in which habitat has been manipulated.

Buoyant pop nets usually consist of 4 mesh walls, depressed prior to release, and a mesh floor. Such traps can be lifted clear of the water to collect ensnared fish. Small pop nets (area 5.6 m²) have been used in vegetated backwaters of a river (Dewey et al. 1989) while a larger net (14.5 m²) was tested for estimating fish abundance associated with artificial structures in lakes (Larson et al. 1986). In the present study, a floorless pop net was designed with the same intention as the much smaller floorless net of Dewey et al. (1989); viz. to avoid the problem that any floor of mesh fine enough to catch the target fish (10 to 100 mm length in the present study) would alter the nature of the sea bed and might disrupt the feeding behaviour of benthic fish. The pop net is an alternative to the floorless lift net designed to collect fish from littoral marshes (Rozas 1992). The pop net design nets 4 times the area of the lift net and avoids the need for above-ground structures used to raise the net. This absence of above-ground structures may be especially important in more open, less densely vegetated habitats.

This paper compares fish catches from the pop net and seine net in shallow eelgrass meadows to determine the relative catching efficiencies of the 2 methods.

Materials and methods. The experiment was done in November 1991 on Torrens Island in the Barker Inlet — Port River estuary, South Australia. This shallow, marine-dominated estuary contains large intertidal flats (maximum tidal amplitude 2 m) supporting eelgrass *Zostera muelleri*, typically with a canopy height of 10 to 20 cm. Netting was done during the day on an incoming tide, at water depths between 40 and

100 cm. Sites were positioned at random along a 1 km stretch of coast and at each site both a pop and seine net were used simultaneously within 40 m of one another. The order of netting was chosen randomly. The aim was to compare the catch of the 2 methods. The assumption was made that with this experimental design, different catch rates would reflect different catching efficiencies of the net types.

The seine net used was 5 m long by 2 m high, of 1 mm diameter fibreglass mesh, and was weighted along the bottom with floats at the top. The net was pulled by 2 people, one at either end, for (a pre-measured) 20 m; the actual area netted was calculated over 10 pulls to be 84 m² (SE = 1.19).

The pop net consisted of 4 walls of the same material and mesh size used in the seine net, and was 5 m long by 1.4 m high (Fig. 1). The top of the mesh was sewn around lengths of 25 mm diameter PVC pipe, sealed at the ends for buoyancy. The bottom was weighted, and also pegged to the sea bed. The net was set at low tide when the sea bed was exposed. The netting was folded and sandwiched between the sea bed and the PVC pipe, which was pushed down so as to make the whole apparatus as nearly as possible flush with the sea bed. The top of the net was weighted with 8 flat concrete blocks of 10 kg each, so that when the net was covered with water it did not move until released. Each concrete block was attached by wire along the sea bed to 1 of 2 remote points, at least 10 m from the net. The net

Fig. 1. Pop net design. (a) Prior to release (diagram is representative only — actual number of blocks used was 8, 4 from each of the 2 stakes, which were situated diagonally to the net so that all sides received 2 blocks). (b) After release, with collecting net

remained in place for 1 d, being released on the following day's incoming tide. At release, 1 person moved slowly to each of the 2 remote points, and all blocks were simultaneously pulled away from the net. The top of the net surfaced within 2 seconds of release in water up to 1 m deep. The area netted was 25 m².

A solid-framed collecting net (Fig. 1), of the same mesh, was used to collect fish from within the pop net. The collecting net, which fits neatly inside the pop net, was pulled 3 times through the area with the pop net walls being held against the ends of the collecting net at all times. Fish were collected separately from each pull. This method removes fish immediately after net release, unlike the method of Rozas (1992) in which fish are collected in a pit on the receding tide. Although predation within nets was not reported as a problem in the densely vegetated marshes in which Rozas (1992) worked, predation by decapods, birds or other fish could result in losses on open eelgrass flats, and collecting fish immediately avoids this potential problem. The pursing together of the mesh sides to collect fish (Dewey et al. 1989), whilst useful for small nets, is not manageable over 25 m².

All fish were identified and the first 50 of each species from each pull of the seine net or the collecting net were measured. Lengths of individuals of common species were compared from the 2 net types by calculating a mean length for individuals within a net and testing differences over all nets using a paired t-test.

Recovery efficiency within pop nets was estimated using the method of Kneib (1991). Numbers from the 3 pulls of the collecting net tended to describe an exponential decay function, and a linear regression through the 3 points (using $\log_{10}$ transformed values of number of fish and number of pulls) predicted the number of pulls needed to remove all fish from the pop net. The total number of fish within the pop net was estimated by summing the predicted number of fish from all pulls. Efficiency was calculated as the proportion of the estimated total number of fish actually caught in each of the 3 pulls, counted cumulatively. For total fish numbers and long-finned goby Favonigobius lateralis (Macleay) numbers this procedure was done separately for each pop net. Individuals of King George whiting Sillaginodes punctata (Cuvier & Valenciennes) and small-mouthed hardyhead Atherinosoma microstoma Günther were uncommon in the second and third pulls of collecting nets, and Kneib's (1991) method was used on data averaged over all pop nets. The precision of the recovery efficiency value could therefore not be estimated for these 2 species. When the method was used on total fish and F. lateralis numbers summed over all pop nets, however, estimates of recovery efficiency were similar to the means of values from separate pop nets. This suggests that the recovery efficiencies esti-
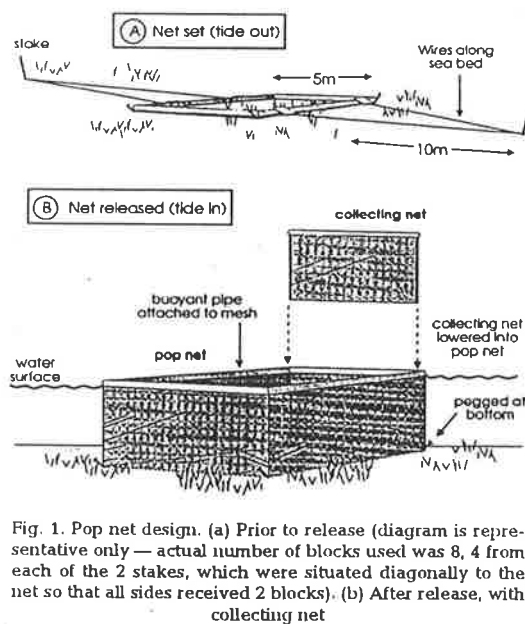
mated for *S. punctata* and *A. microstoma* are reliable. No *A. microstoma* were caught in the third pull of collecting nets in any pop net, so the regression for this species was calculated on $\log_{10}(x + 0.1)$ data. This procedure would tend to underestimate recovery efficiency.

Prior to the main experiment, 4 paired nettings were done on the same stretch of coast and the numbers of fish per m² (all species combined) were counted (Table 1). The estimate of the variability of differences (within pairs) between pop net and seine net catches was used to estimate the sample size required to attain desired probabilities of type I and II statistical errors for a specified effect size. I wanted a good chance of detecting a difference when seine net catches differed from pop net catches by more than 20 %. The consequences of the 2 error types were considered equally serious, implying $\alpha = \beta$. Taking into account the cost and effort involved in using pop nets and the high variability of fish densities, I believe that $\alpha = \beta = 0.05$ is appropriate. For an effect size of 0.76 fish m^{-2} (i.e. 20 % of 3.80, the mean catch of the pop net in Table 1), using Eq. (8.8) in Zar (1984) for a paired *t*-test, the required number of pairs is 17 or more.

Seventeen pairs were therefore used in the main comparison. After checking the normality of the distribution of differences using Lilliefors' (1967) test, data were analysed using a paired *t*-test. Although it was considered unlikely that the catch rate of the pop net would be lower than that of the seine net, a 2-tailed test was used so as not to preclude the possibility of testing the significance of any departure in that direction.

To determine why the effectiveness of the 2 net types might be different, underwater observations were made of fish behaviour before and after pop net release and from behind the seine net during seine netting.

Results. A total of 4991 fish of 15 species were counted during the study (Table 2). The number of fish caught in pop nets was significantly greater than that from seine nets (Table 3). Analysis of the more common species shows that the higher number of fish in pop nets reflects catches of the most common species, *Favonigobius lateralis*, which was caught much more often in pop nets (Table 3). Numbers of *Sillaginodes punctata* and *Atherinosoma microstoma* were not

Table 1. Results of preliminary study. Total fish numbers (indiv. m^{-2}) n = 4 pairs of nets

	Mean	SD
Pop net	3.80	
Seine net	1.70	
Differences	2.10	0.80

shown to be different from the 2 net types. In the case of these 2 species, the statistical power of the tests was low. The chance of detecting a difference in seine and pop net catches of 20 % of the mean pop net catch was 12 % for *S. punctata* and 7 % for *A. microstoma*.

Numbers of blue sprats *Spratelloides robustus* Ogilby provide no meaningful comparison of net types because all fish were caught in 1 seine pull. A total of only 10 bridled goby *Arenigobius bifrenatus* Kner. were caught during the study, but all of these were from pop nets, with individuals found at 4 different sites. The lack of any *A. bifrenatus* in seine catches, despite the area netted by seine nets being more than 3 times that netted by pop nets, is notable.

No significant differences were found in the lengths of fish from pop and seine nets for any of the 3 common species, *Sillaginodes punctata*, *Favonigobius lateralis* and *Atherinosoma microstoma* (Table 4). Numerous spat of *A. microstoma* (length 7 to 20 mm) were collected in several pop nets but were rarer in seine nets.

The proportion of fish in pop nets caught by each successive pull of the collecting net declined rapidly

Table 2. Number of individuals of main species caught during study. Pop net and seine net combined

	No. of fish	% of total
Favonigobius lateralis	3209	64.3
Sillaginodes punctata	1233	24.7
Atherinosoma microstoma	217	4.4
Spratelloides robustus	167[a]	3.3
Other species	165	3.3
All species total	4991	100

[a]All caught in 1 seine net

Table 3. Comparisons of pop net and seine net catches. Numbers in first 3 columns are means of 17 sites (fish m^2) For differences, pop net catch is greater than seine net except where difference is negative. 17 pairs used in *t*-tests

	Pop net	Seine net	Paired differences	Difference as % of pop	Probability from *t*-test
All species	6.318	1.615	4.703	74	<0.001
Favonigobius lateralis	5.414	0.636	4.778	88	<0.001
Sillaginodes punctata	0.584	0.690	−0.106	−18	0.462
Atherinosoma microstoma	0.125	0.080	0.045	36	0.423

Table 4. Comparison of lengths of fish from pop net and seine net. Numbers are means (in mm) from all nets of the mean length of fish within a net. Differences were tested with a paired *t*-test, using only pairs of nets in which at least 1 individual of the species was caught in both net types (this figure in column 'No. of pairs')

	Pop net	Seine net	No. of pairs	Probability from *t*-test
Favonigobius lateralis	28.58	29.54	17	0.109
Sillaginodes punctata	30.56	30.55	16	0.993
Atherinosoma microstoma	32.85	41.64	12	0.178

for all species combined and for the common species (Table 5). Note, however, that the decline in the proportion of *Favonigobius lateralis* caught in successive nets was less steep than for *Sillaginodes punctata* and *Atherinosoma microstoma*. Recovery efficiencies were very high for *S. punctata* and *A. microstoma*, with virtually all *S. punctata* and all *A. microstoma* recovered by the third pull. Recovery efficiencies for *F. lateralis*, and since this was the most abundant species therefore also for all species combined, were lower (Table 5).

Underwater observations of pop nets gave no evidence of fish being more common around the net or blocks prior to release. *Favonigobius lateralis* and juvenile *Sillaginodes punctata* usually swam alone but sometimes showed weak schooling behaviour. *F. lateralis* always remained on the sea bed, while juvenile *S. punctata* tended to swim just over the top of the eelgrass canopy. *Atherinosoma microstoma* schooled strongly and swam near the water surface.

After net release, but prior to use of the collecting net, individuals of the above 3 species behaved as if they were not aware of being trapped, and swam within the 25 m² confine. Larger fish, such as adult smooth toadfish *Torquigener glaber* (Freminville) (up to 70 mm long), > 1 yr old yelloweye mullet *Aldrichetta forsteri* (Valenciennes) (to 80 mm), and > 1 yr old sea garfish *Hyporhamphus melanochir* (Valenciennes) (to 130 mm) were uncommon, but sometimes swam around the edge of the enclosure.

When trapped in a small volume of water at the end of a collecting net pull, *Sillaginodes punctata* and *Atherinosoma microstoma* were observed swimming near the surface, whereas *Favonigobius lateralis* remained near the sea bed.

Observers of seine nets saw some larger, fast swimming fish (unidentified) swim out of the ends of the net. Smaller schooling fish such as *Atherinosoma microstoma* sometimes swam along in front of the net, but were caught as the net-pullers moved together at the end of a run, closing off any escape. Observations of fish in vegetated habitats in shallow water are, of course, not quantitative, but no fish were seen escaping under the net.

Discussion. The pop net caught more fish than the seine net, mainly because 9 times more *Favonigobius lateralis* were caught in the pop net. *F. lateralis* individuals remained on the sea bed at all times, and not only avoided capture by the seine but also had a lower recovery efficiency within the pop net than other common species. Although no *F. lateralis* were seen escaping under the seine, this is the most likely place of escape, as small fish could fit into the shallow depressions in the sea bed over which the bottom rope of the seine would pass. Observations of the seine being used in meadows of seagrass species having a more erect habit (e.g. *Posidonia australis* Hook.) have clearly shown that small, bottom-dwelling fish do escape under the net, which glides through the sea-

Table 5. Comparisons of catches from 3 pulls of collecting net in pop nets. E% = recovery efficiency, calculated as described in text (numbers are means, with standard errors in parentheses; no estimates of precision are possible for *S. punctata* and *A. microstoma*). C% = percentage of total pop net catch caught in collecting net (calculated as % of total catch of individual pop net; means shown)

	Pull 1		Pull 2		Pull 3	
	E%	C%	E%	C%	E%	C%
All species	50.1 (7.6)	61.1	61.8 (7.9)	24.9	67.8 (7.6)	14.0
Favonigobius lateralis	50.0 (9.0)	56.8	60.3 (9.2)	27.4	65.3 (9.0)	15.8
Sillaginodes punctata	83.3	88.6	95.2	5.9	98.8	5.5
Atherinosoma microstoma	99.1	97.9	100	2.1	100	0

grass but not actually along the sea bed. During the testing of a beach seine net in a reservoir, fish species known to be associated with the bottom escaped more often over coarse sediment, presumably by getting under the net via irregularities in the bottom (Parsley et al. 1989). The possibility remains that *F. lateralis* is actually attracted to the pop net area, perhaps towards extra feeding opportunities provided by the disturbance of sediment whilst setting the net, violating the assumption that approximately equal numbers of fish would be in the vicinity of both net types. However, the evidence against this possibility is that observers did not detect any increased abundance of *F. lateralis* near the net prior to release, and that *F. lateralis*, even when trapped within the pop net, avoided the collecting net more often than the other common species. *Arenigobius bifrenatus*, which is also intimately associated with the sea bed and at times even burrows in the sediment, also seemed to avoid capture by the seine net.

Sillaginodes punctata, which swims mid water, seems to be caught equally well by both net types. Furthermore, most of the individuals of this species were caught in the first pull of the collecting net within the pop net. The numbers of *Atherinosoma microstoma*, which swims near the water surface, were also not shown to be different from the 2 net types, and very nearly all of the individuals of this species were caught in the first pull of the collecting net. Although no significant difference was shown in the mean length of *A. microstoma* from pop and seine nets, observations indicate that the seine net may catch *A. microstoma* spat less effectively than the pop net.

For general survey work in shallow embayments the seine net, which is much faster and cheaper to use than the pop net, can be considered a relatively accurate method for collecting small fish other than species that remain intimately associated with the sea bed (and possibly also spat less than 20 mm long). The seine net would also be useful for comparing numbers of a single species, such as *Sillaginodes punctata*, from different locations. Since data from the seine net can misinform about the rank order of abundances of species, those data are most reliably treated as presence/absence data for surveys of fish assemblages.

Most areas of eelgrass in South Australia are emergent or nearly so at low tide, permitting use of the pop net. Where there is particular interest in species that are not well caught by the seine, or where accurate collection of fish from relatively small, defined areas is required, the pop net described in this paper is a useful new design. The pop net, as presented here, would also be useful subtidally for the collection of fish species that remain within the seagrass canopy when disturbed. The relatively unsophisticated release mechanism was designed to be robust in fast flowing

This note was submitted to the editor

water which sometimes carries large quantities of drift algae. It also overcomes most of the problems caused by anglers (Larson et al. 1986), and during this study all net releases were successful. The range of recovery efficiencies within pop nets from 65 % for *Favonigobius lateralis* to 100 % for *Atherinosoma microstoma* approximately matches that for fish in Rozas' (1992) lift net. The main advantage of the pop net presented here is that it collects fish from larger areas than most other designs without losing portability. Kneib's (1991) flume weir collects fish reliably from an even larger area (100 m^2) but is best used for repeated sampling of the same site. The method of fish retrieval from the pop net worked well in eelgrass, but would be less effective in taller, more robust vegetation, where the collecting pits of Rozas (1992) would be more effective.

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A comparison of fish assemblages from seagrass and unvegetated areas of a southern Australian estuary

R. M. Connolly

Abstract

Assemblages of small fish from eelgrass (mainly *Zostera*) and unvegetated patches in a shallow, marine-dominated estuary were compared over one year as a preliminary step towards finding the consequences of eelgrass loss to small fish. There were more species and more individuals at eelgrass sites at every sampling period. Multivariate analysis (MDS ordination) of assemblages showed distinct grouping of eelgrass and unvegetated sites. The statistical significance of groupings was tested using an analysis of similarities (ANOSIM) randomisation routine. The cryptic syngnathid *Stigmatopora nigra* and juvenile whiting, *Sillaginodes punctata*, the species of greatest economic importance in the estuary, were predominantly over eelgrass, while the flounder *Rhombosolea tapirina* was usually caught at unvegetated sites. *Atherinosoma microstoma*, the most abundant species, was more common over eelgrass at 2 dates, but had similar abundances over both habitats at other dates. The limitations of survey work caused by possible associations between the presence of vegetation and environmental factors have been partially offset by interspersion of sites and by measurement of water temperature and salinity. Secondarily to habitat differences, fish assemblages were weakly grouped according to distance of sites from open water.

Introduction

Seagrass meadows in many parts of the world support large numbers of juvenile fish and provide a nursery habitat for many commercially important species (Pollard 1984). Unvegetated areas adjacent to seagrass meadows have different fish assemblages, usually with fewer fish and fewer species (Bell and Pollard 1989). Assemblages associated with seagrass consist mainly of small, inconspicuous species and juveniles of larger species, whereas the fauna of unvegetated areas is characterised by adults of large, mobile fish and species protected by schooling behaviour or camouflage against sediments (Bell and Pollard 1989).

The Barker Inlet/Port River region is a sheltered, marine-dominated estuary comprising extensive intertidal areas with either eelgrass (*Zostera, Heterozostera*) cover or no vegetation. The estuary has very high abundances of juveniles of commercially important fish species (Jones 1984) and mainly for this reason has been declared an aquatic reserve.

The estuary is almost surrounded by the city of Adelaide (population > one million people), and is consequently subjected to many types of pollution, viz : treated sewage, stormwater, agricultural and horticultural run-off, spilt oil, thermal effluent, shipping, altered flow regimes and fishing. Further extensive urban development along the foreshore is planned (OPUD 1992). Toxic dinoflagellate blooms (Hallegraeff *et al.* 1988), loss of mangroves (*Avicennia marina* (Forsk.)) to clearing (Talbot 1982), and build-up of the macroalga *Ulva* sp. (Connolly 1986) have all been recorded, but changes in eelgrass cover within the estuary are not documented. Since the installation of Adelaide's main sewage outfall just north of the Barker Inlet/Port River estuary, six hundred hectares of intertidal eelgrass have been lost adjacent to the outfall (Shepherd *et al.* 1989).

Within the estuary itself, various sized patches with and without eelgrass occur. Despite the factors contributing to the presence or absence of eelgrass being unknown, it is very likely that eelgrass cover has been and will be altered by human activities. The aim of this survey was to compare assemblages of

small fish from eelgrass and unvegetated areas as a preliminary step to finding the consequences of eelgrass loss to fish communities, and in particular to stocks of commercial importance.

Methods

Site selection

Unvegetated and eelgrass areas (mostly *Zostera muelleri* Irsmisch ex Aschers., occasionally *Heterozostera tasmanica* (Martens ex Aschers.)) of the Barker Inlet - Port River region (138° 30' E, 34° 45' S) were mapped in January 1990 (Fig. 1). Excluding the region south of Inner Beacon (because it is grossly affected by warm water effluent from a power station (Jones, unpublished data)), there were seven eelgrass and eight unvegetated areas. At each of five sampling periods between January 1990 and February 1991, 20 sites were selected for sampling, ten in each habitat. In some sampling periods, the actual number of sites sampled was less than the 20 selected because of the limited time of correct tidal height to sample (see sample sizes in Table 2).

The spatial distribution of areas of the two habitats was predetermined by the state of the estuary. There was, however, some interspersion of habitats, so that the situation of having all or most of one type of habitat at one end of the estuary, for example, was avoided. Within the patches of habitat, choice of sites was random subject to the restrictions that 1) each of the designated areas of both habitats received at least one sample and 2) within a patch of habitat, no two sampling sites were less than 200 m apart. The order in which sites were sampled was randomised, with the proviso that at least two sites of each habitat were sampled on each day. Where sites were covered with the macroalga *Ulva* sp., sampling was abandoned.

The above-ground biomass was measured at each eelgrass site in January 1990 only. At each site, three squares of 625 cm^2 were harvested and dried at 60° C for two days. The mean above-ground biomass for all eelgrass sites was 146 g dry weight/ m^2 (s.e. = 20.7, n = 9 sites).

Fish Sampling

Fish were sampled at all sites using two different sized beach seine nets which were operated sequentially, 30 m apart, pulled perpendicular to and towards the shore for (a pre-measured) 20 m. The small net was 5 m long with mesh size 1.4 mm, and the large net was 22 m long with mesh size 6 mm. The actual area netted was calculated over 10 pulls to be 84 m^2 (s.e. 1.19) for the small net and 347 m^2 (s.e. 7.80) for the large net. All fish were identified and counted, and *Sillaginodes punctata* (Cuvier and Valenciennes) (King George whiting), representing the most important commercial species, were also measured. Numbers from the two nets were combined, as both nets together constitute the sampling unit.

All netting was done at or just after the daytime low tide. All sites could not be sampled on one date, since it is important to take all nettings at a similar tidal state. Sampling was therefore spread over four consecutive days chosen for similarity of tides. The actual day on which sites were sampled was randomised, and all samples were treated as temporally equivalent. That is, variation between days within sampling periods was not analysed. In June 1991, the seine nets were used at night to catch *S. punctata* as part of a dietary study, and the numbers of *S. punctata* from those nettings are reported as an indication of diel activity. This sampling was done at or just after low tide over three nights between 2300 and 0100 hrs.

Water temperature and salinity

Water salinity and especially temperature (given the warm water discharges in the south of the estuary) could influence fish distributions and could also be associated with the presence of eelgrass. Water temperature was measured at each site at the time of netting, at 30 cm depth in water 60 cm deep. Water samples for salinity analysis were taken at the same time and place. The Practical Salinity Scale of 1978 (PSS 78) has been used in this paper.

Data Analysis

Comparisons between unvegetated and eelgrass areas of total abundances and abundances of the key species are straightforward applications of the Mann-Whitney U-test. The number of fish at eelgrass sites in January 1990 was tested for association with the above-ground biomass estimate of each site using Spearman's rank test. A comparison of assemblages (all species together) from eelgrass and unvegetated sites suggests a multivariate analysis of variance (MANOVA). The assumption of multivariate normality, however, is likely to be grossly violated by present data, the fish samples being characterised by small numbers with many zeros. A non-parametric analogue with no assumption of normality is the analysis of similarities (ANOSIM) which has the added advantage over MANOVA of being able to detect a difference not only in location of samples but also in spread (Clarke 1993). ANOSIM compares ranked similarities between and within groups selected *a priori* (here eelgrass and unvegetated habitats) using a randomisation test for significance. At each sampling period, assemblages from the two waterways, Barker Inlet and Port River, were also compared using a two-way crossed ANOSIM with habitat (eelgrass or unvegetated) as the second factor. This analysis determines whether assemblages from the two waterways differ after accounting for habitat differences. All ANOSIM tests involved 5000 simulations using the Primer package from Plymouth Marine Laboratory, U.K.

Non-metric multidimensional scaling (MDS) is an ordination technique that uses the same matrix of ranked similarities as ANOSIM; it displays samples in low (usually two) dimensional space while retaining as nearly as possible the similarity rankings between samples.

For comparisons of fish assemblages between unvegetated and eelgrass sites, raw counts were transformed using $x^{.25}$ to emphasise the distribution of less common species in the analysis. The transformation $x^{.25}$ gives slightly more emphasis to less common species than log x in cases such as this where counts are small (Clarke 1993). The Bray-Curtis similarity coefficient is used throughout, as a meaningful and robust measure (Clarke 1993).

Analysis of the similarity matrix used in MDS and ANOSIM has also been used to highlight the species making the largest contribution to between-group differences (Clarke 1993).

The association of environmental variables with patterns in biotic data can be measured by correlating the ranked similarity matrices of the environmental and biotic data (Clarke and Ainsworth 1993). At each sampling period, the association between fish assemblages and the environmental variables 1) water temperature, 2) salinity, and 3) distance to open water were measured using the weighted Spearman's coefficient recommended by Clarke and Ainsworth (1993). Distance to open water was measured as the shortest distance by sea from sites to gulf waters unprotected by islands or shoals. Distances ranged from 1.0 to 9.1 km.

All comparisons (univariate and multivariate) have been done for each period separately, because the fauna changes over time within both habitats as juveniles of larger species move to deeper water or grow too large to be caught by the nets. An MDS ordination has also been performed on data from all periods combined, and a two-way crossed ANOSIM was used to test differences between periods and habitats.

Results

Number of Species

A total of 36 species were caught during the survey. More species were caught at eelgrass sites than unvegetated sites at all sampling dates (Fig. 2).

Number of individuals

A total of 13871 fish were caught, 9866 (71%) from eelgrass sites and 4005 (29%) from unvegetated sites.

The number of individuals caught at eelgrass and unvegetated sites is shown for the ten most common species from both eelgrass and unvegetated sites in Table 1. Comparisons of catches at eelgrass and unvegetated sites are shown (Fig. 3) as mean number per site (small and large net combined), separately at each sampling date, for a) total catch (all species), b) *Sillaginodes punctata* c) *Atherinosoma microstoma* Günther and d) all species except *A. microstoma* and *Spratelloides robustus* Ogilby. Results of Mann-Whitney U-tests for differences between catches from eelgrass and unvegetated sites are listed in Table 2 for all dates.

There were more fish at eelgrass sites than at unvegetated sites at all dates (Fig. 3 a), and only at August 1990 was the difference not significant (Table 2). In February 1991 the mean numbers of fish at both eelgrass and unvegetated sites were very high because of an extraordinarily large number of *Pelates sexlineatus* (Quoy and Gaimard) (striped perch) at eelgrass sites and *A. microstoma* (hardyheads) at eelgrass and unvegetated sites. The above-ground biomass of eelgrass at eelgrass sites was not correlated with total fish numbers in January 1990 (Spearman's rank test: $p > 0.1$).

Sillaginodes punctata (King George whiting)

The number of *S. punctata* at eelgrass sites was significantly greater than at unvegetated sites at all dates (Table 2, Fig. 3 b). Nearly all fish were caught in large nets in the first half of 1990 and 1991, and in small nets in the second half of 1990. This highlights not only the different size selectivity of the two nets used, but also, in conjunction with the median lengths of *S. punctata* shown in Table 3, the growth of year classes. *S. punctata* spawns in April (Jones *et al.* 1990), is first caught in the estuary about mid-year, and after another year is too large to be caught by the nets used in this study.

At night in June 1991 there were significantly more *S. punctata* per site over eelgrass (mean = 6.8 fish, n = 9 sites) than over unvegetated areas (mean = 0.8 fish, n = 5 sites) (Mann-Whitney U-test of medians: $p = 0.03$)

Median lengths of *S. punctata* from eelgrass and unvegetated sites have been compared at dates where enough fish were caught at unvegetated sites to permit a reasonable comparison (Table 3). Even at these dates sample sizes were much larger at eelgrass sites, however the variance and degree of skewness and kurtosis were similar across habitats, so test results can be regarded, cautiously, as meaningful. *S. punctata* were longer in the unvegetated habitat at all dates, significantly so at January 1990 and February 1991.

Atherinosoma microstoma (small-mouthed hardyhead)

A. microstoma was the most abundant species at both eelgrass and unvegetated sites (all dates combined). Only at two dates were significantly more caught in eelgrass, while similar numbers were caught at eelgrass and unvegetated sites on three dates (Table 2 and Fig. 3 c).

All species except *A. microstoma* and *Spratelloides robustus*

If *A. microstoma*, which is numerically dominant and at some dates does not show a strong pattern of greater abundance over eelgrass, is excluded and the numbers of all other species are combined, then numbers are greater at eelgrass sites than at unvegetated sites at all dates (Table 2, Fig. 3 d). *Spratelloides robustus* (blue sprat) occurred infrequently but was also excluded because it is similar in size and behaviour to *A. microstoma*.

Stigmatopora nigra Kaup (wide-bodied pipefish)

166 individuals of *S. nigra* were caught over the survey period. 13 of these were caught in the large net and can be excluded because this species can swim easily through the mesh of the large net and is only caught when it is entrapped by algal fronds. Of the 155 fish caught in small nets, 153 were over eelgrass and two were over the unvegetated habitat.

Rhombosolea tapirina Güenther (greenback flounder)

This species, of which only juveniles were caught, was found mainly at unvegetated sites. They are effectively caught in both small and large nets, and of the total of 53 individuals taken over the survey period, 12 (23%) were from eelgrass sites and 41 (77%) were from unvegetated sites.

Multivariate analysis

The differences between eelgrass and unvegetated sites in species richness and abundances of several common species suggest differences between assemblages of these habitats. Two-dimensional ordination plots show, at every period, strong grouping of eelgrass and unvegetated sites (Fig. 4). Assemblages of the two habitats were significantly different at all periods as judged by the ANOSIM results (Table 4). Of the species contributing most to differences in assemblages associated with the eelgrass and unvegetated habitats (Table 4), *Sillaginodes punctata* predominated. No differences were found at any period between assemblages of the two waterways, Barker Inlet and Port River.

Water temperature and salinity did not match the biotic data. At all periods, the distance from sites to open water was the only environmental variable having any importance in matching the patterns in fish assemblages. Nor did any combination of the three environmental variables match the biotic data better than distance to open water alone. However, even distance to open water was only weakly matched, with correlations between matrices of fish assemblages and distance ranging from 0.10 to 0.34. There is currently no test for significance of these correlations but the values are low (Clarke and Ainsworth 1993). A simple overlay of distance to open water onto MDS plots of fish assemblages (Fig. 5) reveals that for all periods except August 1990 one site, with the smallest distance to open water, is far from any other site. In all cases, this site is from the unvegetated patch at the extreme north-western end of the sampling region. In August 1990, no fish were caught at the site in this unvegetated patch and the sample was therefore excluded from the MDS ordination. When the site is included, the correlation between similarity matrices of fish assemblages and distance to open water is 0.21. After excluding the site (as in the plots in Figs 4 & 5), the correlation drops to - 0.01. This evidence, taken together with the overlays at other periods, suggests that the weak correlation of distance to open water and fish assemblages is due mainly to this one unvegetated patch which is near open water and has a peculiar fish assemblage, persistent over time. This patch with greatest exposure to open water is characterised by having very low fish catches, always including at least one *Rhombosolea tapirina*. Any importance of distance to open water in determining fish assemblages seems to be in separating the patch most exposed to open water from other areas rather than causing a gradual change along the length of the estuary.

When all periods are combined, the distinction between eelgrass and unvegetated sites remains the overwhelming difference (Fig. 6). After accounting for differences between habitats, sampling periods are, however, also different (Two-way ANOSIM, factor "period", $p < 0.001$). Differences over time are due to either the sporadic occurrence of large numbers of one or two species or fluctuations in the number of individuals of species present in the estuary at all periods. The relative position of sites over time is different within the two habitats (Fig. 6). Within the unvegetated habitat, periods are quite evenly spaced. Within the eelgrass habitat, January 1990, April 1990 and February 1991 are separated from August 1990 and October 1990. This is mainly the result of large catches of *Pelates sexlineatus* at the first three dates.

Water temperature and salinity

Water temperatures and salinities differed among dates, with both showing marked seasonality. Mean temperatures and salinities were, however, very similar at eelgrass and unvegetated sites (Table 5). No significant differences were found in either temperature or salinity between the two habitats using Mann-Whitney U-tests.

Discussion

The fish assemblages of eelgrass and unvegetated areas were distinctly different, and these differences persisted over time. Differences between habitats were as found in other researchers' comparisons (Bell and Pollard 1989, and references therein). The eelgrass habitat was typified by the syngnathid *Stigmatopora nigra*, a small species with cryptic habit, and juveniles of *Sillaginodes punctata*, while *Rhombosolea tapirina*, a flounder with extreme modifications for camouflage against a sand/mud background, was found mostly in unvegetated areas. *Atherinosoma microstoma* is a small species that schools, and might have been expected to be mostly over unvegetated habitat. However, this fish feeds near the surface of the water, at least during the day, and has little to do with the sediment, vegetated or not; this may explain why large numbers of this species were caught over both habitats.

Changes in fish assemblages over the duration of the study were due to seasonal fluctuations in abundances of several species. The common fish of the estuary are either permanent or temporary residents (see definitions in Bell and Pollard 1989). Species which are small as adults such as *A. microstoma*, *F. lateralis*, *Stigmatopora nigra* and *Kaupus costatus* are permanent residents. Seasonal fluctuations in abundance of these species result from short-term (often annual) recruitment and mortality. The temporary residents are larger species that recruit seasonally and move elsewhere later. *S. punctata*, for example, spawns offshore, and planktonic larvae settle to a benthic existence in the estuary from June to August. Fish move out of the estuary to deeper waters after two to three years (Jones *et al.* 1990). The size of species caught over time reflects the growth of these fish. In this study, the largest size of *S. punctata* captured was 140 mm (about 1 year old) in April 1990. Failure to catch larger fish in this study, however, can be attributed to the smallness of the two nets, rather than to the departure of larger *S. punctata* to deeper waters.

Different parts of an estuary can have different fish assemblages regardless of vegetation (Bell and Pollard 1989), which could lead to spurious associations of faunas with vegetation types. Fortunately thorough interspersion of the two habitats was possible in this survey, limiting the likelihood that the different assemblages were simply a result of different locations. Differences in fish assemblages between eelgrass and unvegetated habitat can also depend on how far unvegetated sites are from eelgrass (Ferrell and Bell 1991). The differences shown in the present study are clear even though the distance of unvegetated sites from eelgrass varied widely. Distance to open water explained some of the pattern in assemblages, especially the distinctive assemblage of the most exposed patch. Since this patch was unvegetated, the comparison of habitats would have been influenced by the peculiarity of the assemblage there. However, the difference between eelgrass and unvegetated sites evident in the ordination plots was consistent across the estuary as a whole at all periods. No difference was detected between fish assemblages of the two waterways, Barker Inlet and Port River. This is despite Port River being a major shipping lane and having being modified by dredging, wharf building and reclamation of its shores. The fauna of Port River may not have been greatly affected by human activities because eelgrass, which is a relatively fast growing, colonising plant, has persisted. The similarities of the fauna of the two waterways could also be taken to imply that both waterways have been affected by human activities.

In any comparison of fauna from different habitats, demonstrated differences between habitats are potentially attributable to differences in the effectiveness of the method of capture. In the present study, for example, fish in unvegetated areas, or perhaps certain sizes of fish in unvegetated areas, may have been more easily able to avoid capture. The best evidence that demonstrated differences in faunal abundances between habitats are not due simply to different capture efficiencies would be a similar result using another method of capture. Comparisons of fish from eelgrass and unvegetated habitat in the Barker Inlet region using a buoyant pop net, a method of ensnaring fish over a 25 m^2 area (Connolly 1994), showed the same pattern of greater abundance over eelgrass for *Sillaginodes punctata* and all species combined (Connolly, in press). The comparison using a pop net was done only at one time of year and used unvegetated patches smaller than those in the present survey, but nevertheless provides some evidence that differences in fish abundances described here are not simply a sampling artefact.

Eelgrass presence was not associated with water temperature or salinity, so these factors are unlikely to be the cause of the different fish assemblages. Even secondarily to habitat differences, temperature and salinity were not associated with any faunal differences. This may simply be a result of the extent of the

survey region, which included only relatively open, well-mixed waters. Temperatures in this study were similar to those measured by Jones (unpublished data) outside the region influenced by hot water effluent entering the southern part of the estuary, and the comparison presented here should be considered representative only of the areas north of the southern limit to sampling shown in Fig. 1.

This comparison of habitats is a necessary step in confirming that eelgrass in the Barker Inlet/Port River estuary is important as habitat for small fish and in particular for juveniles of commercially important species. Absence of eelgrass may be correlated with one or more other factors, not measured in this study, which cause the fish distributions reported here; experimentally manipulating eelgrass densities can distinguish between absence of eelgrass and other factors. The importance of eelgrass probably lies either in the protection it offers from predators (larger fish), or the greater abundance of associated food (mostly small invertebrates). Either or both of these factors may not be directly causal, but may be the ultimate cause of evolution of habitat selection shown by fish in these shallow areas (Bell and Westoby 1986). Manipulative experiments have been done in the estuary to clarify the role of eelgrass as habitat for small fish (Connolly, in press).

Of most significance to fisheries management, especially in Gulf St Vincent, is the close association of *Sillaginodes punctata* with eelgrass. *S. punctata* accounts for nearly half the value of inshore scalefish landings in South Australia (Anon. 1992). Robertson (1977) found that small *S. punctata* in Westernport, Victoria, used unvegetated areas at some times of the year; there is no evidence of that in the Barker Inlet/Port River region, at least during the day. Some species have very different distributions at night compared to day (Robertson 1980; Bell and Pollard 1989) and, apart from data for June 1991, this study does not address that possibility. Sampling in June 1991 showed that *S. punctata* were over eelgrass more than unvegetated patches at night also. The greater median length of *S. punctata* at unvegetated sites at several times of the year raises the possibility of size-selective mortality, at one or more times of year. Many other alternative explanations exist, however, and this presents a line for further investigation.

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Table 1. Number of individuals of the ten most common species (all dates combined) in eelgrass and unvegetated habitat.

First ten species were most common at eelgrass sites. Final four species are those in the most common ten species at unvegetated sites not included in the first ten species. Numbers in parentheses are percentages of total number in that habitat.

Species name	Eelgrass	Eelgrass rank	Unvegetated	Unvegetated rank
Atherinosoma microstoma Small-mouthed hardyhead	4046 (41.0)	1	2333 (58.3)	1
Sillaginodes punctata King George whiting	2291 (23.2)	2	87 (2.2)	5
Pelates sexlineatus Striped perch	1823 (18.5)	3	10 (0.2)	10
Favonigobius lateralis Long-finned goby	559 (5.7)	4	417 (10.4)	3
Aldrichetta forsteri Yelloweye mullet	435 (4.4)	5	909 (22.7)	2
Stigmatopora nigra Wide-bodied pipefish	166 (1.7)	6	2 (0.0)	14.5
Hyporhamphus melanochir Sea garfish	125 (1.3)	7	40 (1.0)	7
Kaupus costatus Deep-bodied pipefish	78 (0.8)	8	5 (0.1)	12.5
Haletta semifasciata Blue weedy whiting	58 (0.6)	9.5	0	9.5
Heteroclinus perspicillatus Common weedfish	58 (0.6)	9.5	0	9.5
Spratelloides robustus Blue sprat	3 (0.0)	25.5	100 (2.5)	4
Rhombosolea tapirina Greenback flounder	12 (0.1)	15.5	41 (1.0)	6
Sillago schomburgkii Yellowfin whiting	12 (0.1)	15.5	27 (0.7)	8
Arripis georgianus Tommy rough	2 (0.0)	27.5	12 (0.3)	9
Other species	198 (2.0)		22 (0.5)	
Total	9866		4005	

Table 2. Results of Mann-Whitney U-tests for differences in abundance between eelgrass and unvegetated sites.

Probabilities: significance criterion = 0.05. Non-significant comparisons marked "ns".

Sampling period	Number of sites eelgr : unveg	All species	*Sillaginodes punctata* (King George whiting)	*Atherinosoma microstoma* (Hardyhead)	All species except *A.microstoma*, *S.robustus*
Jan 90	9:11	0.037	0.001	0.183 ns	0.004
Apr 90	10:10	0.003	0.001	0.041	0.019
Aug 90	8:7	0.298 ns	0.001	0.601 ns	0.015
Oct 90	8:7	0.003	0.008	0.024	0.005
Feb 91	10:9	0.050	0.019	0.205 ns	0.003

Table 3. Comparisons of median length of *Sillaginodes punctata* at eelgrass and unvegetated sites.

Habitats: e = eelgrass, u = unvegetated. Mann-Whitney U-test probabilities: significance criterion = 0.05. Non-significant comparisons marked "ns".

Sampling period	Habitat	Number of fish	Median length (mm)	Mann-Whitney U-test result
Jan 90	e	310	80	<0.001
	u	13	110	
Apr 90	e	210	110	not tested
	u	4	130	
Aug 90	e	202	24.5	not tested
	u	2	25.5	
Oct 90	e	221	32	0.060 ns
	u	19	35	
Feb 91	e	169	90	0.002
	u	19	110	

Table 4. Multivariate comparisons of eelgrass and unvegetated assemblages.

ANOSIM probabilities: all comparisons are significant at 0.05 level. Contributing species: two main species only. A.f = *Aldrichetta forsteri*, A.m = *Atherinosoma microstoma*, H.m = *Hyporhamphus melanochir*, P.s = *Pelates sexlineatus*, S.n = *Stigmatopora nigra*, S.p = *Sillaginodes punctata*.

Sampling period	Probability from ANOSIM test	Main contributing species
Jan 90	< 0.001	S.p, A.m
Apr 90	< 0.001	S.p, H.m
Aug 90	< 0.001	S.n, S.p
Oct 90	0.008	S.p, A.f
Feb 91	< 0.001	S.p, P.s

Table 5. Water temperature and salinity at eelgrass and unvegetated sites separately at each sampling period.

Habitats: e = eelgrass, u = unvegetated. Temperature measured in degrees Celsius. Salinity measured using the Practical Salinity Scale of 1978 (PSS 1978). Numbers are means with standard errors in parentheses.

Sampling period	Habitat	Temperature	Salinity
Jan 90	e	22.7 (0.46)	38.5 (0.22)
	u	22.8 (0.44)	38.3 (0.37)
Apr 90	e	21.9 (0.39)	38.1 (0.15)
	u	22.8 (0.40)	38.3 (0.18)
Aug 90	e	15.2 (0.61)	35.3 (0.18)
	u	14.6 (0.86)	35.3 (0.14)
Oct 90	e	19.1 (0.80)	36.1 (0.31)
	u	18.5 (0.63)	36.5 (0.19)
Feb 91	e	27.7 (0.40)	37.8 (0.16)
	u	27.4 (0.23)	38.0 (0.17)

Figure captions

Fig. 1. Map of survey region and habitat location.

Fig. 2. Total number of species from eelgrass and unvegetated habitats caught at each sampling period.

Fig. 3. Mean number of fish per site from eelgrass and unvegetated habitats: (a) all species, (b) *Sillaginodes punctata*, (c) *Atherinosoma microstoma*, (d) all species except *A. microstoma* and *Spratelloides robustus*. Lines = standard error (of mean per site, small and large net combined).

Fig. 4. Two-dimensional MDS ordination plots of fish assemblages showing habitat differences: (a) January 1990 (stress value (Kruskal's formula 1) = 0.088), (b) April 1990 (0.104), (c) August 1990 (0.098) - one unvegetated site at which no fish were caught is excluded, (d) October 1990 (0.129), (e) February 1991 (0.195).

Fig. 5. Overlay of distance from site to open water onto MDS ordination plots of Fig. 4. Diameter of circle is proportional to distance. Smallest circle in any plot = 1.0 km, largest = 9.1 km.

Fig. 6. Two-dimensional MDS ordination plot of fish assemblages over all periods combined. Stress = 0.197. Sites of a given habitat (eelgrass or unvegetated) at a given period have been combined and plotted at their centroid. Periods are indicated by numbers: 1 = January 1990, 2 = April 1990, 3 = August 1990, 4 = October 1990, 5 = February 1991.

a

b
Survey area
Gulf
St Vincent
Adelaide

AUSTRALIA

c
Beacon
Saint Kilda
Outer Beacon
Section Bank
Barker Inlet
Outer Harbour
Inner Beacon
Gulf
St Vincent
Torrens Island
Southern
Edge of
Survey
Area
Power
Station
Garden
Island
Angas Inlet
Port
River
North
Arm

Legend:

0   1
km

N

Shallow sand flat
Unvegetated

Land

Shallow sand flat with
eelgrass cover
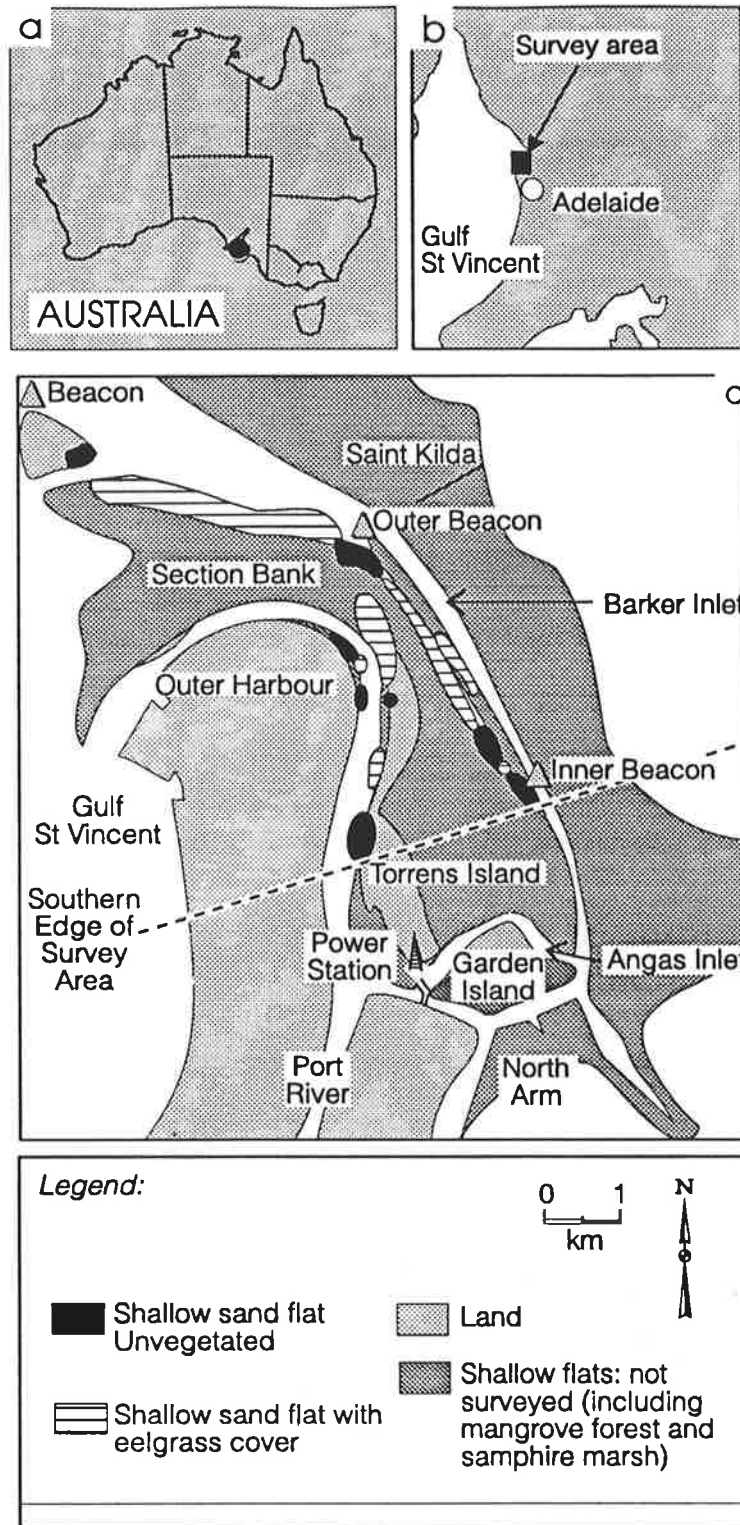
Shallow flats: not
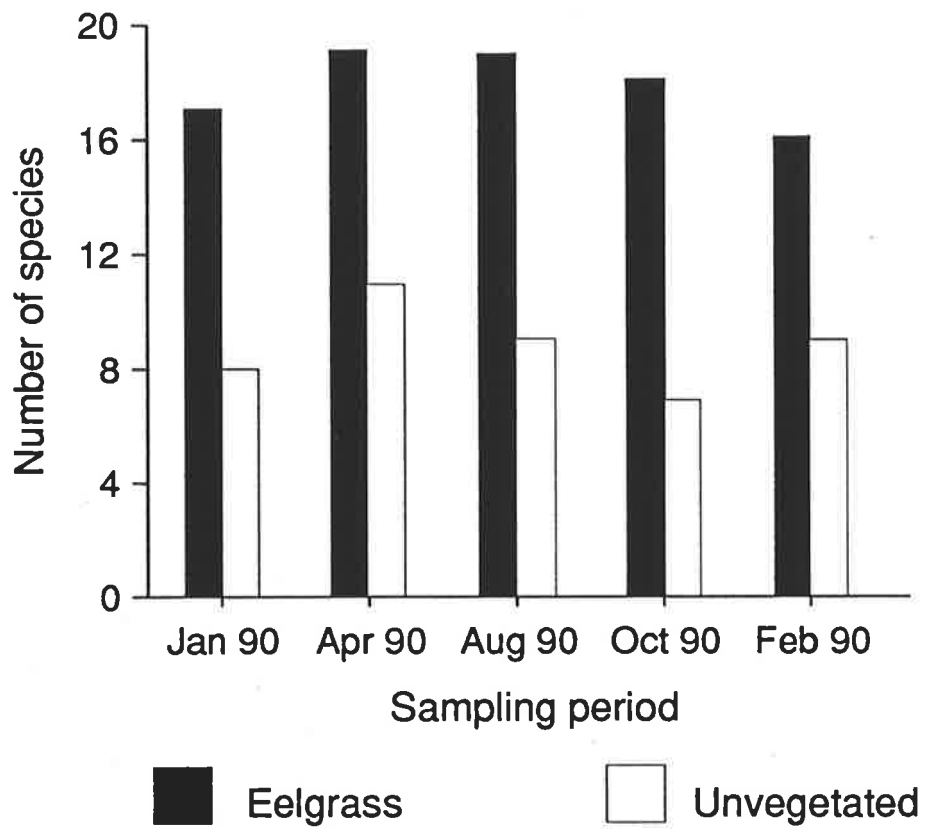surveyed (including
mangrove forest and
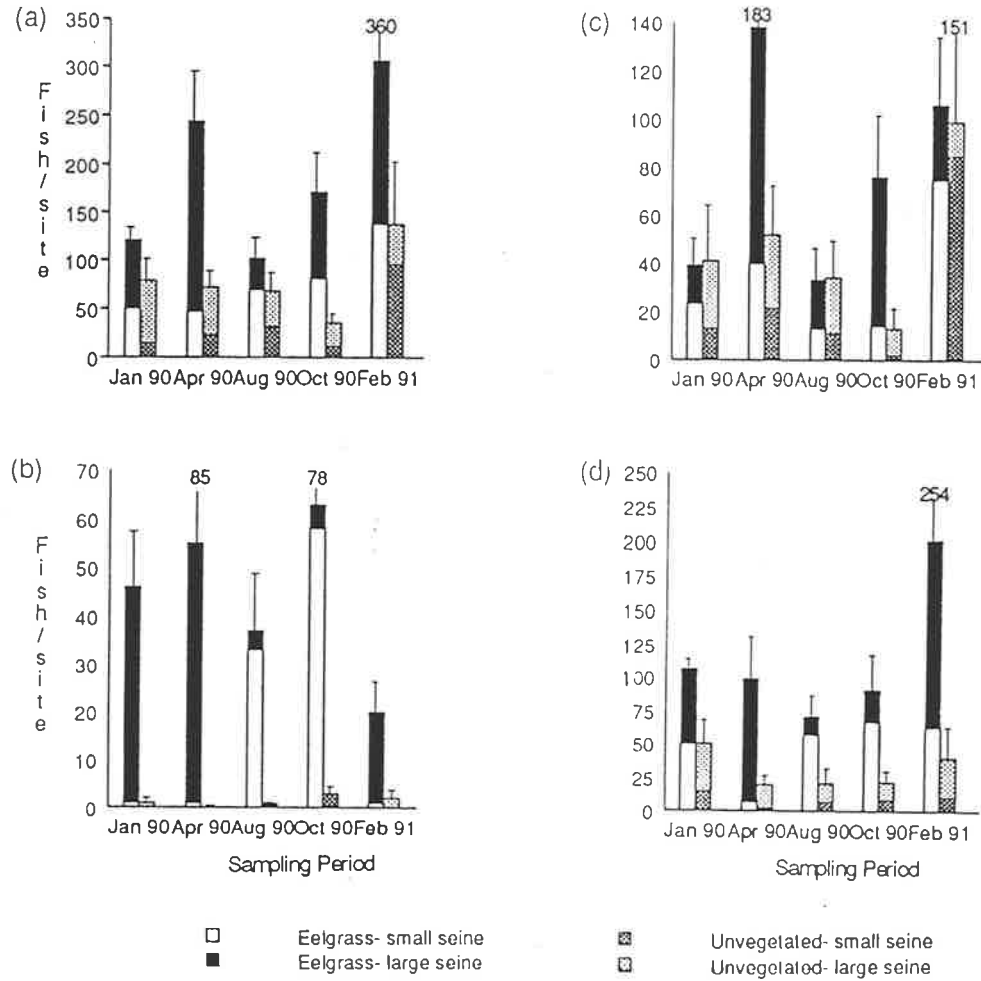samphire marsh)

Fig. 1
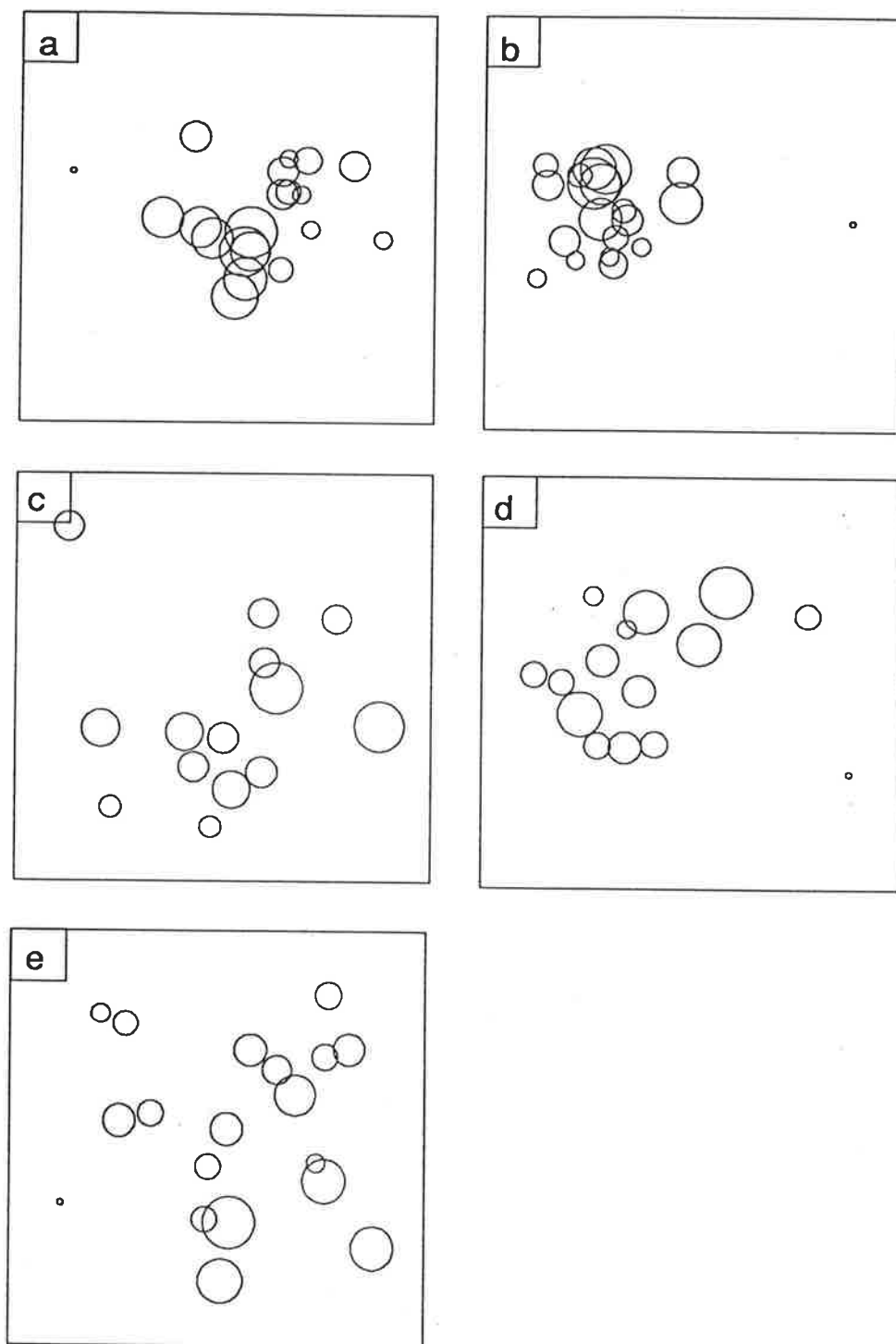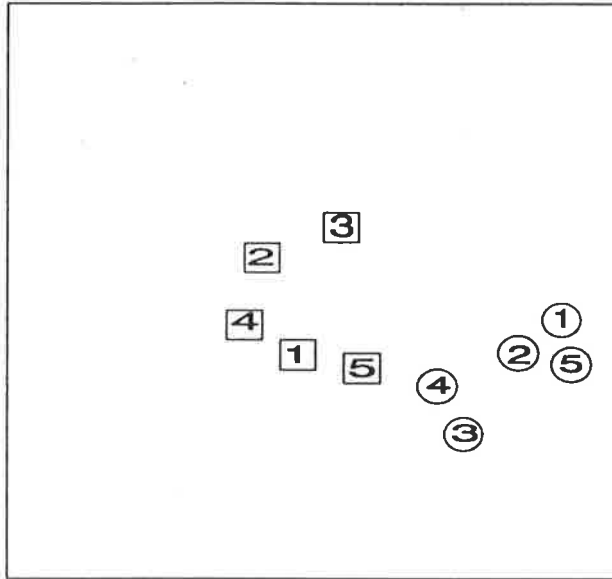
Fig. 2

Fig. 3

Fig. 4

Fig. 5

○ Eelgrass

□ Unvegetated

Fig. 6

A.3

Removal of seagrass canopy: effects on small fish and their prey

Rod M Connolly

Abstract

In an experiment in a southern Australian estuary, patches of seagrass canopy were removed to test the importance of the canopy to fish in areas where all other factors were known to be consistent with seagrass presence. The total number of fish was slightly lower in patches cleared of seagrass than in patches of undisturbed seagrass, but was not as low as in unvegetated patches. The benthic habitat was expected to be especially important to non-pelagic species, yet their numbers, and those of the most important commercial species, *Sillaginodes punctata*, were not lower in patches cleared of seagrass, despite being lower in unvegetated patches. The disturbance associated with removing seagrass was simulated and was not found to affect fish numbers. The diet of all fish caught consists mainly of invertebrates associated with the seagrass canopy and sediment surface (epifauna). Epifaunal abundance and production were highest in seagrass patches, lowest in unvegetated patches and intermediate in patches cleared of seagrass. Patterns of fish abundance did not provide evidence of the importance of seagrass canopy in attracting increased fish abundances compared with unvegetated areas but were consistent with a model stressing the importance of prey availability in the role seagrass plays as habitat for small fish.

Introduction

Seagrass meadows in many parts of the world support large numbers of juvenile fish and provide a nursery habitat for many commercially important species (Pollard, 1984). The importance of seagrass is implied by reports of declining seagrass cover being matched by declining fish catches in, for example, King George whiting (*Sillaginodes punctata* (Cuvier & Valenciennes)) (Bell & Pollard, 1989). Unvegetated areas adjacent to seagrass meadows have also been shown to have different fish assemblages, usually with fewer fish and fewer species (Bell & Pollard, 1989). The difference between fish assemblages of seagrass and unvegetated areas is, however, only an association. Environmental factors (e.g. a water quality variable) concomitant with, or resulting in, seagrass absence may also be the cause of differing fish assemblages.

Attempts to demonstrate the importance of seagrass have mostly involved the construction of patches of artificial seagrass in unvegetated areas. The question posed is: what is the effect on fish of placing seagrass mimic in positions having all other factors consistent with absence of seagrass? An alternative is to remove seagrass from areas where it is naturally occurring. The question them becomes: what is the effect on fish of removing seagrass from positions having all other factors consistent with seagrass presence? This more closely matches the question: what is the effect of seagrass loss on fish? The disadvantages of seagrass removal are firstly that regrowth necessitates either a short-term experiment or repeated removal, and secondly that seagrass removal is irresponsible except when working with species that recover quickly.

The importance of seagrass probably lies either in the protection it offers from predators (larger fish), or in the greater abundance of associated food (mostly small invertebrates). Bell & Westoby (1986b) manipulated seagrass densities in field experiments and used predator exclusion cages to show that small fish were more common in denser seagrass regardless of predator presence/absence. They showed convincingly that low fish numbers in patches with less dense seagrass cover were not due to increased predation, and concluded that small fish select habitat. As Bell & Westoby (1986a) point out, their

results may be explained in ways other than habitat selection by fish. Fish might, for example, be attracted to more abundant food in denser seagrass. Food abundance was not measured by Bell & Westoby (1986a,b). The lower abundance of fish in unvegetated areas is, according to the model of Bell & Westoby (1986a,b), the result of fish choosing to settle in seagrass beds in preference to adjacent unvegetated areas.

The aim of the present study was to determine the effects on small fish distribution of removing above-ground vegetation (seagrass canopy). If the seagrass canopy is important, for whatever reason, then patches from which the vegetation has been removed should support fewer fish and different fish assemblages than seagrass patches. Moreover, if the seagrass canopy is the important difference between seagrass and unvegetated habitat, then fish assemblages associated with patches from which the seagrass canopy has been removed should match assemblages from patches which were unvegetated prior to the experiment. If small fish are less abundant in unvegetated patches because they do not settle there then, as predicted above, the numbers of fish in patches cleared of seagrass should match the number from areas unvegetated prior to the experiment. If, on the other hand, small fish are attracted to seagrass directly to feed upon more abundant prey (as proposed, for example, by Edgar & Shaw, 1993), then the number of fish in patches cleared of seagrass should match prey abundance and production associated with the modified habitat and will not necessarily be the same as fish numbers from areas unvegetated prior to the experiment.

Materials and Methods

The Barker Inlet/Port River region (138° 30' E, 34° 45' S) is a sheltered, marine-dominated estuary comprising extensive intertidal areas with either eelgrass (*Zostera, Heterozostera*) cover or no vegetation. A comparison of assemblages of small fish from eelgrass and unvegetated areas has demonstrated the typical differences described above (Connolly, 1994a). The estuary is strongly tidal, typically with two tides per day, with a maximum tidal amplitude of 2 m, and fish occupying the lower intertidal zone must choose anew the habitat over which they swim on every incoming tide. The experiment was situated in an area dominated by *Zostera muelleri* Irsmisch ex Aschers., a fast growing, colonising species. The experiment was done in September 1991, and was timed to coincide with the seasonal recruitment into the estuary of juveniles of the most important commercial fish species, *Sillaginodes punctata*, which accounts for nearly half the value of inshore scalefish landings in South Australia (Anon., 1992).

Fish were collected from the following four habitats (treatments) marked as 5.5 x 5.5 m squares:
1) eelgrass in natural state (control = C),
2) eelgrass removed by cutting with shears at the sediment surface whilst emergent on low tides (removed = R),
3) eelgrass uncut, but with equivalent time and effort spent at site mimicking cutting (procedural control = P), and
4) unvegetated mudflat (unvegetated = U).

Six eelgrass sites were assigned to each of the first three treatments in a randomised block design. That is, one replicate of each of the first three treatments was assigned at random to six randomly selected areas (blocks) along a 1 km stretch of shore. The unvegetated treatment could not be randomly assigned. Instead, the nearest unvegetated site to the block occurring at the same height in the intertidal was selected as the unvegetated patch. The blocked design guaranteed interspersion, which is important because of the potential patchiness of fish abundances.

Patches were prepared over several days, and fish were collected 14 days later. This was a short enough interval to avoid eelgrass regrowth. The order in which patches were prepared and therefore netted was chosen so that on any day only one patch within a block was netted, so as to avoid disturbance of nearby patches. During the experiment the netting schedule was disrupted by inclement weather and attempts to collect fish from one block were abandoned in a bid to return to schedule. Fish were collected only from a 5 x 5 m square in the centre of each patch, avoiding the edges of habitats. Fish were netted using a buoyant pop net released in water depths from 40 - 100 cm on an incoming daytime tide. The pop net was designed to collect fish neatly from experimental plots, a situation for which more conventional

seine netting is too unwieldy (Connolly, 1994b). All fish were identified and counted. Species considered to be pelagic (*Atherinosoma microstoma* Günther - Atherinidae, *Arripis georgianus* Valenciennes - Arripidae and *Spratelloides robustus* Ogilby - Clupeidae) were excluded from some analyses.

The amount of food available to fish within each patch was estimated by sampling the small, motile invertebrates (epifauna) associated with the eelgrass canopy and sediment surface. Epifauna, especially crustaceans and polychaetes, are the predominant food of virtually all of the fish species, including juvenile stages of larger species, normally caught with small nets in the shallow waters of the Barker Inlet - Port River region (Connolly, unpublished data). Three randomly placed collections were made within each patch on the day prior to fish collection. Invertebrates were collected using a 95 μm mesh net with a 25 x 25 cm opening, following the method of Sergeev *et al.* (1988) in which the net is placed rapidly over the canopy onto the sediment before dragging shut the net opening along the sediment surface. Invertebrates were later separated into sieve size classes of 2 mm, 1 mm, 500 μm, 250 μm, 125 μm and 75 μm before being identified to major taxa and counted. Numbers of very abundant taxa were counted from random subsamples with the aim of counting between 50 and 200 individuals of each taxon per sieve size in any sample. Nematodes and foraminifera were excluded from this study because they are taken rarely or not at all by the fish species caught. Ash-free dry weights (AFDW) were calculated by converting abundances for each taxon for each sieve size using Edgar's (1990) equation, $\log B = a + b.\log S$ (where B = AFDW (mg), S = sieve size (mm) and a and b vary depending on broad taxonomic category). This permits estimation of epifaunal production using Edgar's (1990) equation, $P = 0.0049.B^{0.80}.T^{0.89}$, relating production (P, μg/day) to sample AFDW (B, μg) and water temperature (T, °C).

The surface area of eelgrass leaves within all patches that supported eelgrass prior to the experiment was estimated before setting up the experiment and again on the day after fish collection. Leaf area was calculated for each patch from measurements of the number of leaves per 400 cm^2 quadrat, and the length and width of ten leaves, at five randomly selected sites. Prior to the experiment, leaf area did not differ between patches selected for the three treatments involving eelgrass (C: 1.54 m^2 leaf area/ m^2 sediment surface; P: 1.31; R: 1.39; ANOVA: p = 0.651). After removal, the leaf area within patches of treatment R was reduced almost to zero, whilst patches of P remained similar to patches of C (C: 1.55; P: 1.46; R: 0.02; ANOVA: p < 0.001; Tukey's HSD pairwise comparisons: C P R).

Data Analysis

The number of fish (all species combined and key species separately) from the four habitats were compared using a randomised block analysis of variance (ANOVA); this is equivalent to a mixed model, two-way ANOVA without replication, in which "habitat" is the fixed factor and "block" the random factor. Results of the significance test for effects of block have been reported, but should be treated cautiously since they depend on the untested assumption that the interaction effect is small (Zar, 1984). Furthermore, the intention of allocating treatments to blocks was to guarantee interspersion of treatments rather than to search for differences in fish abundances along the coast. However, by removing the variance due to block, a more sensitive test for differences amongst habitats is made than would be the case with a simple one-way ANOVA. Significant ANOVA results were followed by Tukey's HSD pairwise comparisons between habitat means. *Atherinosoma microstoma* is a species that schools strongly, unlike the other species analysed. This behaviour results in large fluctuations in number per net since catch rates are either zero or, if a school happens to be caught, in the order of 100 individuals. $\log_{10}(x + 1)$ transformation failed to render data normal, and *A. microstoma* numbers were therefore analysed using Friedman's non-parametric equivalent to the ANOVA described above (Zar, 1984). Invertebrate abundance and production in the four habitats were analysed in the same way as fish abundances, after averaging the three values from each patch. For both fish and invertebrates, sample variances increased with increasing means, and analyses were performed on $\log_{10}$ transformed data after checking that the transformation increased homoscedasticity. Pearson's r test was used to detect association between fish abundances and epifaunal production by patch. Significance levels are 0.05 throughout.

Fish assemblages from the four habitats were compared using an analysis of similarities (ANOSIM), which is a non-parametric analogue to a multivariate analysis of variance (MANOVA) without the

assumption of multivariate normality. ANOSIM has an additional advantage over MANOVA in being able to detect differences between groups without any need for assumptions of constant spread within each group (Clarke, 1993). A two-way ANOSIM without replication, equivalent to the univariate ANOVA described above, was used to test for differences amongst habitats and blocks (Clarke & Warwick 1994). The ANOSIM tests involved 5000 simulations using the PRIMER package from Plymouth Marine Laboratory, U.K.

The relationships amongst assemblages from each patch are graphically represented using non-metric multidimensional scaling (MDS), which is an ordination technique that uses the same matrix of ranked similarities as ANOSIM. MDS displays samples in low (usually two) dimensional space while retaining as nearly as possible the similarity rankings between samples.

For comparisons of fish assemblages among the four habitats, raw counts were transformed using $x^{0.25}$ to emphasise the distribution of less common species in the analysis. The transformation $x^{0.25}$ gives slightly more emphasis to less common species than $\log (x + 1)$ in cases such as this where counts are small (Clarke, 1993). The Bray-Curtis similarity coefficient was used, as a meaningful and robust measure (Clarke, 1993).

Results

Fish

A total of 2170 fish of 11 species were caught during the study, with 504 individuals of the three species categorised as pelagic. The mean number of individuals of each species and for all species together from each habitat is shown in Table I.

*** Table I about here

Total fish abundance in habitat P was greater than in habitat U. Fish abundances in habitats C and R were not different from each other and were intermediate between the other two habitats (ANOVA: Habitat - $p = 0.034$, Block - $p = 0.654$; Tukey's: $\underline{P \ \underline{C \ R} \ U}$).

Excluding pelagic species, more fish were caught in habitats C, P, and R than in U. Differences between catches in the first three habitats were not significant (ANOVA: Habitat - $p = 0.003$, Block - $p = 0.232$; Tukey's: $\underline{R \ P \ C}$ U).

Abundances of *Sillaginodes punctata* were higher in habitat R than in U. Abundances in habitats P and C were not different from each other and were intermediate between the other two habitats (ANOVA: Habitat - $p = 0.022$, Block - $p = 0.051$; Tukey's: $\underline{R \ \underline{P \ C} \ U}$).

Comparisons for *Favonigobius lateralis* (Macleay) and *Atherinosoma microstoma* detected no significant differences in abundance amongst habitats (*F. lateralis* - ANOVA: Habitat - $p = 0.498$, Block - 0.182; *A. microstoma* - Friedman's: Habitat - $p = 0.316$, no test for block). These non-significant results are more meaningful if the statistical power of the tests is examined. Power is the complement of β, which is the probability of making a Type II statistical error (i.e. when a test fails to reject a false null hypothesis). Power is related to α, the probability of making a Type I statistical error (i.e. when a test rejects a true null hypothesis), sample size and effect size. Effect size is defined as the minimum departure from the null situation able to be detected with the power specified. This is usually set as the minimum departure of biological interest. In the case of the fixed factor in a randomised block ANOVA, effect size can be specified as the difference between the two most extreme means (Zar, 1984). For the test amongst means of *F. lateralis* abundance, I consider it important to detect a departure from the null in which one treatment has a mean 50% lower than other treatments. An example of this effect size for *F. lateralis* would be the following means (units are fish/net): C = P = R = 50, U = 25. On $\log_{10}$ data this translates to: C = P = R = 1.7, U = 1.4. The chance of detecting a difference amongst habitats

in mean abundance of *F. lateralis* with the effect size specified above was 0.22 ($\beta = 0.78$) (Equ. 13.33; Zar, 1984). No formal power calculations are possible on the Friedman's non-parametric test of *Atherinosoma microstoma* abundances, but it is possible to apply the known power efficiency of Friedman's test compared with the equivalent ANOVA (0.76 for 4 treatment means; Zar, 1984) to an estimate of what power would have been if an ANOVA had been applied to the data. The effect size for *A. microstoma* would be similar to that for *F. lateralis* and degrees of freedom are identical, but variance is larger and therefore power would be something less than the figure of 0.22 for *F. lateralis*. This figure would be reduced further upon application of the power efficiency factor (multiply estimated power of ANOVA by 0.76), and the best estimate of power to detect a difference amongst median abundances of *A. microstoma* is therefore considerably less than 0.2. The low power in tests of *F. lateralis* and *A. microstoma* abundances suggests that, although no differences were detected, it should not be concluded that there are no biologically important differences amongst abundances of these species. Rather, the test results demonstrate a need for increased numbers of patches.

No clear differences between habitats are discernible in the ordination plots showing relationships amongst fish assemblages from each patch for all fish species (Fig. 1a) and non-pelagic species only (Fig. 2a). Statistical comparisons of fish assemblages found no significant differences amongst habitats whether or not pelagic species were included (ANOSIM: Factor Habitat; All fish - $p = 0.098$; Pelagic species excluded - $p = 0.328$). Ordination plots including (Fig. 1b) and excluding (Fig. 2b) pelagic species show some signs of grouping according to block, but results of ANOSIM tests are not significant (ANOSIM: Factor Block; All fish - $p = 0.592$; Pelagic species excluded - $p = 0.115$). No formal power calculations are currently possible with the ANOSIM method, but the small number of replicate patches serves as a reminder that a Type II error is possible.

*** Figs 1 and 2 about here

Epifauna

A total of 40082 invertebrates were caught and placed into 19 taxa, 12 crustacean and 7 other categories. Invertebrate abundance was higher in habitats C, P and R than in U. No difference was found between habitats C and P, but R had lower abundance than C (Table II). (ANOVA: Habitat - $p < 0.001$, Block - $p = 0.759$; Tukey's: $\underline{\underline{C\ P}\ R}\ U$).

*** Table II and Fig. 3 about here

Total epifaunal production was greater in habitat C than in U, and in P and R was intermediate between and not significantly different from C and U (Table II). (ANOVA: Habitat - p 0.014, Block - $p = 0.646$; Tukey's: $\underline{C\ P\ R}\ U$).

Neither total fish abundance nor total non-pelagic fish abundance were correlated with epifaunal production of patches (All species: Pearson's $r = 0.249$, $p = 0.291$; Pelagic species excluded: $r = 0.279$, $p = 0.233$). For a specified effect size of $r = 0.5$, the power of Pearson's correlation test was 0.64 ($\beta = 0.36$) (Cohen, 1988).

The relationship between mean fish abundance and mean epifaunal production by habitat is perhaps of greater importance than the search for a correlation between fish abundance and epifaunal production by patch. The relationship between mean fish abundance and mean epifaunal production by habitat is contrasted in Fig. 3 with the relationship between mean fish abundance and mean seagrass cover (leaf area) in the four habitats. Total abundance of all fish species matches epifaunal production rather than seagrass cover, and this is true also when pelagic species are excluded, although fish abundances then match epifaunal production less closely.

Discussion

The differences in fish catches from patches of the four habitats were not in assemblage composition but in overall fish abundance and abundance of *Sillaginodes punctata*. The main difference was between habitat U and the other three habitats. Independent surveys of eelgrass and unvegetated patches in the region also show lower total fish abundances and fewer *S. punctata* over unvegetated habitat (Connolly, 1994a). Surveys also show unequivocal differences between assemblages of the two habitats at all times of year, yet these were not evident in this study. The patch sizes of unvegetated habitat in the present study were slightly smaller than the smallest patches netted during survey work, and this difference in scale may explain why assemblage differences between undisturbed eelgrass and unvegetated patches were not apparent in the current study. Ferrell & Bell (1991) have shown that the distance of unvegetated sites from eelgrass affects how different the fish assemblages are from those of adjacent *Zostera* beds. The total area netted in this experiment was only about one third of the area netted with seine nets during each survey period. Less common species were therefore less likely to be caught during this experiment, and so species typical of a habitat without being abundant there, such as the syngnathid *Stigmatopora nigra* Kaup and the odacid *Haletta semifasciata* (Valenciennes) from eelgrass habitat, were not caught. The number of *Favonigobius lateralis* was similar at all habitats. This species was also found in similar numbers over eelgrass and unvegetated habitat during survey work. *F. lateralis* individuals are intimately associated with the seabed and even in eelgrass areas tend to occur in bare patches between clumps of eelgrass. The fish are well camouflaged when they are over sediment.

Total fish numbers tended to be lower over habitat U than over the other habitats, and these differences were clearer when pelagic species were excluded. A similar pattern was found for numbers of *Sillaginodes punctata*. The disturbance associated with eelgrass removal (habitat P) on its own had no marked effect on fish numbers.

If the eelgrass canopy itself is the characteristic of eelgrass habitat important in attracting an increased abundance of small fish compared with adjacent unvegetated areas, then fish numbers should have declined in the treatment from which eelgrass was removed. In this experiment fish numbers over habitat R were a little lower than in habitat P, but did not match the much lower number found in habitat U. Moreover, when considering only non-pelagic species, for which benthic habitat was expected to be especially important, numbers over habitat R were not lower than in habitats C and P. It must be concluded that over the length of this experiment, removal of eelgrass canopy did not cause fish to distribute themselves in a way consistent with the predictions of a model in which the eelgrass canopy alone is of major importance to small fish.

Two other possible explanations for the failure of fish numbers to fulfil expectations need examining. Firstly, the duration of the experiment was short relative to the seasonal settlement patterns of fish, and longer term manipulations of habitat, provided that they deal adequately with seagrass regrowth, may allow time for changes in physical factors such as sediment grain size affected by the presence of seagrass. As a test of the importance of seagrass canopy *per se*, however, the duration of this experiment was satisfactory, because fish were forced away from the area on every low tide, and could be expected to redistribute themselves semi-diurnally. Secondly, since patches of habitat U did not receive the disturbance inflicted on patches of R during eelgrass removal, the greater abundance of fish in habitat R compared with that in habitat U could be the result of the difference in degree of disturbance. Another treatment in which unvegetated patches received the disturbance of simulated eelgrass removal could have been used. The same disturbance in eelgrass patches did not alter fish numbers, generating some confidence that disturbance was not important when comparing habitat R with habitat U; that possibility has not, however, been altogether removed.

Epifaunal abundance and production were lowest in habitat U, intermediate in habitats P and R, and highest in habitat C. If fish are directly attracted to seagrass areas by the higher levels of epifaunal production, rather than selecting seagrass habitat *per se* and as a consequence gaining access to the greater abundance of prey, then fish abundance in the treatments of this experiment should match epifaunal production. Although no correlation between fish abundance and epifaunal production was demonstrated by patch, mean fish abundances by habitat did match epifaunal production when pelagic

fish species were included. When pelagic species were excluded, fish abundances by habitat matched epifaunal production less closely, but still more closely than the match with seagrass cover.

The evidence from this experiment does not support a model in which small fish select seagrass habitat because of the presence of seagrass canopy. The evidence better supports, but does not alone demonstrate, the importance of food in the role of eelgrass as habitat for increased numbers of fish compared with unvegetated habitat.

Acknowledgements

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Table I

Fish abundance by habitat. Numbers for each habitat are means, with 95% confidence limits in parentheses (confidence limits are antilog values of confidence limits calculated using $\log_{10}$ transformed data and residual variance from ANOVA, and are therefore shown only for taxa analysed using ANOVA) (n = 5). Total abundance is number of individuals.

	Control	Procedural control	Removed	Unvegetated	Total abundance	% of all fish
Favonigobius lateralis	37.2 (61,23)	56.8 (93,35)	49.6 (81,30)	26.4 (43,16)	850	39
Sillaginodes punctata	45.0 (74,27)	40.6 (66,24)	51.8 (85,31)	14.2 (23,8)	758	35
Atherinosoma microstoma	62.2	29.8	0.4	5.0	487	22
Kaupus costatus	1.2	1.6	0.8	0.2	19	1
Tetractenos glaber	1.2	0.8	0.6	0.8	17	1
Spratelloides robustus	0.4	2.8	0	0	16	1
Gymnapistes marmoratus	0.6	1.4	0.2	0.4	13	1
Heteroclinus perspicillatus	0.8	0.2	0	0	5	< 1
Rhombosolea tapirina	0	0	0.2	0.4	3	< 1
Arripis georgianus	0	0	0	0.2	1	< 1
Hyporhamphus melanochir	0.2	0	0	0	1	< 1
All species combined	148.8 (239,96)	134.0 (207,87)	103.6 (160,67)	47.6 (74,31)	2170	

Table II

Epifaunal abundance and production by habitat. Numbers are means with confidence limits, calculated as described in Table I, in parentheses (n = 6).

	Control	Procedural control	Removed	Unvegetated
Abundance	903 (1263,646)	794 (1110,568)	381 (533,273)	48 (67,34)
Production	170 (258,112)	126 (191,83)	86 (131,57)	50 (76,33)

Figure captions

Fig. 1. Two-dimensional MDS ordination plot of fish assemblages, all species included:
a) by habitat, C = Control, P = Procedural control, R = Removed, U = Unvegetated, and
b) by block. Stress value (Kruskal's formula 1) = 0.162.

Fig. 2. Two-dimensional MDS ordination plot of fish assemblages, pelagic species
excluded: a) by habitat, lettering as for Fig. 1, and b) by block. Stress value (Kruskal's
formula 1) = 0.136.

Fig. 3. Relationship of total number of fish and total number of non-pelagic fish (fish/net)
to epifaunal production (μg/day) and seagrass cover (m^2 leaf area/m^2 sediment area) by
habitat. All symbols represent means, with common scale. Lines are included to make
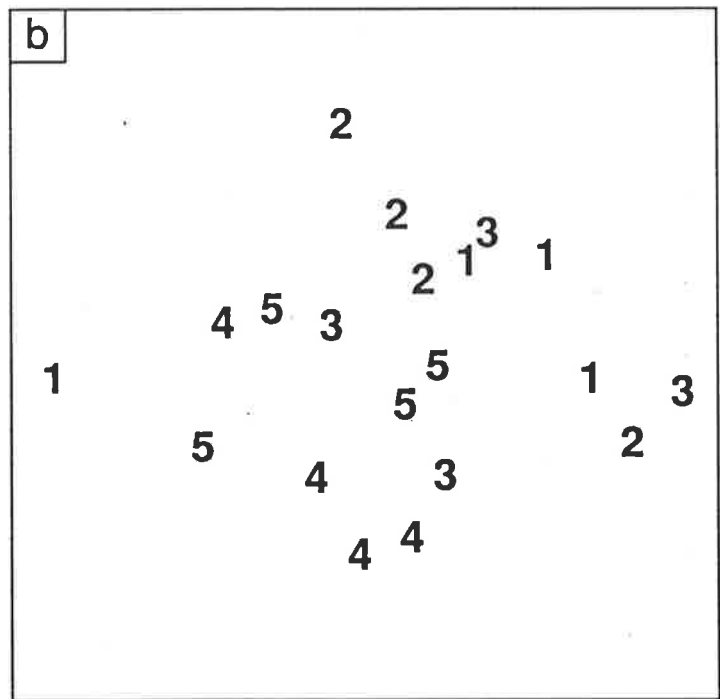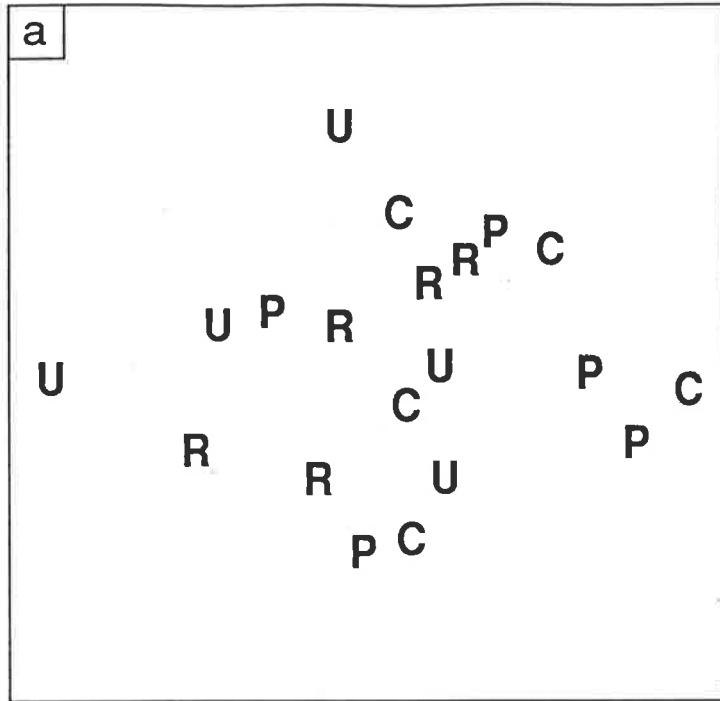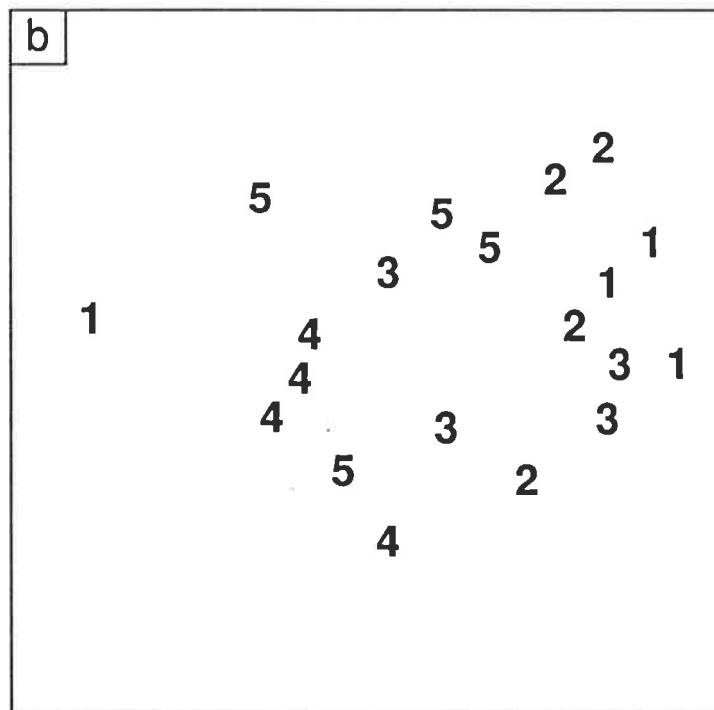patterns clear and do not imply that measurements are possible between habitats.
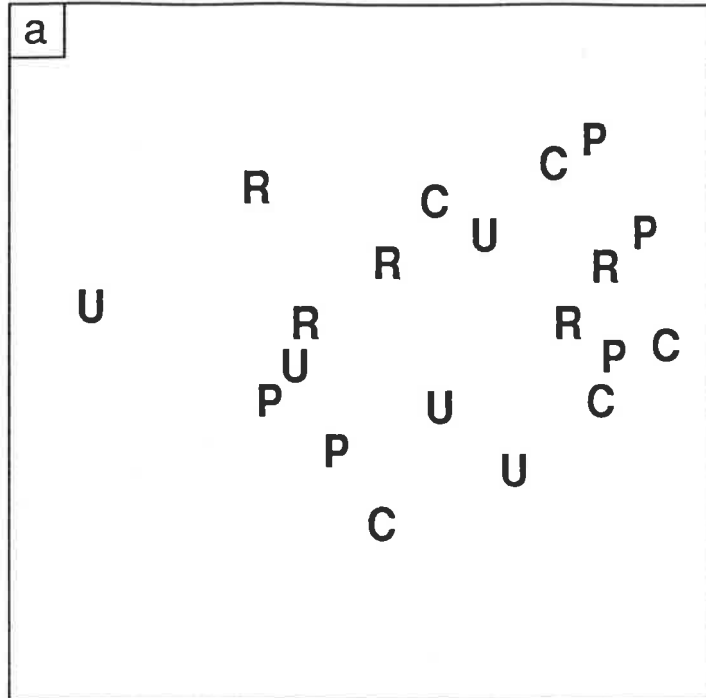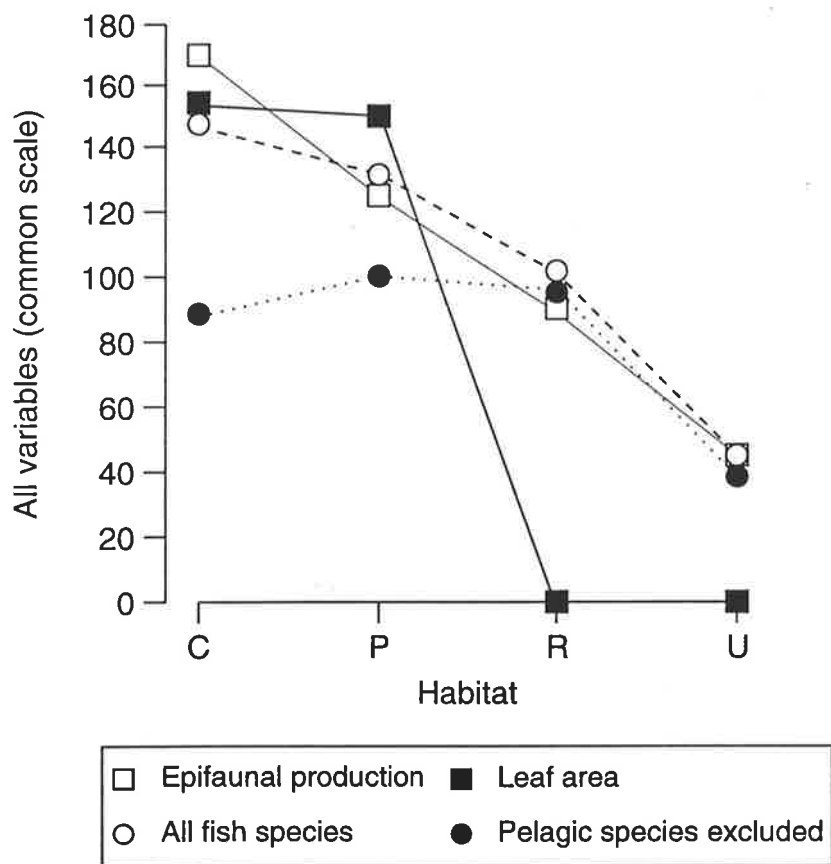
Fig. 1

Fig. 2

Fig. 3

A.4

The effects of removal of seagrass canopy on assemblages of small, motile invertebrates

Rod M Connolly

Abstract

To test the importance of seagrass canopy to epifaunal invertebrates in a southern Australian estuary, patches of the short, fine-leaved seagrass *Zostera muelleri* were cleared of canopy. All other factors were known to be consistent with seagrass presence, and a procedural control was used to measure any effects of the method used to remove seagrass. Effects on epifauna were measured as changes in abundance and biomass of key taxa and in total production, and as differences amongst assemblages, tested using an analysis of similarity (ANOSIM) randomisation routine. Removal of seagrass canopy had a weak but detectable effect on epifauna over and above the slight effect caused by the disturbance concomitant with seagrass removal. Epifauna associated with habitat from which seagrass had been removed did not, however, match that from areas unvegetated prior to the experiment. The epifauna from these previously unvegetated areas were characterised by low abundance and biomass of several key taxa, apart from one group, cumaceans, which were far more common in this habitat. The results suggest that the overriding importance of *Z. muelleri* to epifauna is not simply the presence of seagrass canopy, and explanations of the higher abundance of epifaunal invertebrates in vegetated compared to unvegetated habitats based merely on the presence of seagrass canopy are not supported.

Introduction

The abundance of small, motile invertebrates associated with seagrass is usually greater than that associated with adjacent unvegetated patches (Orth et al. 1984). This difference is more obvious for epifauna (animals associated with the leaf and sediment surfaces) than infauna (animals buried within the sediment) (Howard et al. 1989). Abundances of small fish are also greater in seagrass areas than in adjacent unvegetated areas (Bell and Pollard 1989), and seagrass meadows are thought to provide nursery areas for juveniles of many commercially important species (Pollard 1984). Epifauna, especially crustaceans, are the predominant prey of most small fish associated with seagrass beds, including juveniles of commercially important species (Klumpp et al. 1989).

Epifaunal assemblages associated with adjacent patches of different species of seagrass are often more similar than those associated with patches of the same species of seagrass separated by large distances (Howard et al. 1989). Artificial seagrass placed in unvegetated areas near natural seagrass beds attract a fauna similar to that in the natural beds (Howard et al. 1989, Edgar 1990b). Howard et al. (1989) suggest that the type of seagrass may be less important than the presence of seagrass. Larger epifaunal invertebrates (macrofauna, defined as animals retained on 0.5 mm mesh) are, however, capable of selecting amongst different densities of seagrass (Leber 1985). Bell & Westoby (1986b) manipulated seagrass densities in field experiments and used predator exclusion cages to show that decapods were more common in denser seagrass regardless of the presence or absence of predators. They showed convincingly that low decapod numbers in patches with less dense seagrass cover were not due to increased predation, and concluded that decapods select habitat. Stoner (1980) demonstrated that amphipods can also detect and respond to differences in canopy density. The high mobility of epifaunal invertebrates, even in their adult stages, enhances their ability to exercise behavioural selection for seagrass of differing densities. Although less experimental work has been done on smaller epifaunal invertebrates (Howard et al. 1989) (meiofauna, defined as animals passing through 0.5 mm mesh but

retained on 0.1 mm mesh), harpacticoid copepods are known to colonise artificial seagrass placed near natural seagrass beds (Bell & Hicks 1991).

The Barker Inlet - Port River region of South Australia is a shallow, marine-dominated estuary comprising extensive intertidal areas with either eelgrass (*Zostera, Heterozostera*) cover or no vegetation. Abundances of small fish are much greater in eelgrass patches than over unvegetated patches (Connolly, 1994), as are abundances of epifauna (Connolly, unpubl. data). Both meio- and macro-epifauna, especially crustaceans and polychaetes, are a major component in the diet of most fish species caught in the estuary (Connolly, unpubl. data). In the present study, therefore, the aim was to sample epifauna of all sizes. The health of eelgrass in the Barker Inlet - Port River region is threatened by many of the human activities causing seagrass decline in other sheltered coastal areas (such as nutrient input, changed drainage regimes, and land reclamation for urban development (Walker & McComb 1992)). North of the estuary, adjacent to Adelaide's main sewage outfall, a strip of intertidal eelgrass almost equal in area to the entire area of eelgrass within the estuary has been lost (Shepherd et al. 1989).

Attempts to demonstrate the importance of seagrass have mostly involved the construction of patches of artificial seagrass in unvegetated areas. The question posed is: what is the effect on fauna of placing artificial seagrass in positions having all other factors consistent with absence of seagrass? An alternative is to remove seagrass from areas where it is naturally occurring. The question then becomes: what is the effect on fauna of removing seagrass from positions having all other factors consistent with seagrass presence? This more closely matches the question: what is the effect of seagrass loss on fauna? The disadvantages of seagrass removal are firstly that regrowth necessitates either a short-term experiment or repeated removal, and secondly that seagrass removal is irresponsible except when working with species that recover quickly.

The aim of the present study was to determine the effects on epifaunal abundance and community composition of removing above-ground vegetation (seagrass canopy). If the seagrass canopy is important, for whatever reason, then patches from which the vegetation has been removed should support fewer invertebrates and different invertebrate assemblages than seagrass patches. Moreover, if the presence of the seagrass canopy is the important difference between seagrass and unvegetated habitat, then invertebrate assemblages associated with patches from which the seagrass canopy has been removed should match assemblages from patches which were unvegetated prior to the experiment.

Materials and Methods

The experiment was done in South Australia in the Barker Inlet - Port River region (138° 30' E, 34° 45' S) in September 1991. The estuary is strongly tidal, typically with two tides per day, and with a maximum tidal amplitude of 2 m. The experiment was sited low in the littoral zone, with all plots at a similar level. The area is dominated by *Zostera muelleri* Irsmisch ex Aschers., a fast growing, colonising species.

The small, motile invertebrates associated with the eelgrass canopy and sediment surface (epifauna) were collected from the following four habitats (treatments) marked as 5.5 x 5.5 m squares:

1) eelgrass in natural state (control = C),
2) eelgrass removed by cutting with shears at the sediment surface whilst emergent on low tides (removed = R),
3) eelgrass uncut, but with equivalent time and effort spent at the site mimicking cutting (procedural control = P), and
4) unvegetated mudflat (unvegetated = U).

Six eelgrass patches were assigned to each of the first three treatments in a randomised block design. That is, one replicate of each of the first three treatments was assigned at random to six randomly selected areas (blocks) along a 1 km stretch of shore. The unvegetated treatment could not be randomly assigned. Instead, the nearest unvegetated patch to the block occurring at the same height in the intertidal was selected as the unvegetated patch. The blocked design guaranteed interspersion, which is important because of the potential patchiness of epifauna.

Patches were prepared over several days, and epifauna was sampled 13 days later. This was a short enough interval to avoid eelgrass regrowth. The order in which patches were prepared and therefore sampled was chosen so that on any day only one patch within a block was netted, so as to avoid disturbance of nearby patches.

Epifauna were collected from three randomly placed sites within each patch, subject to the restriction that a 0.5 m wide strip around the perimeter of the patch was avoided. Collections were made on the daytime rising tide in water depths between 30 and 50 cm. A 95 μm mesh net with a 25 x 25 cm opening was used, following the method of Sergeev et al. (1988) in which the net is placed rapidly over the canopy onto the sediment. Whilst the net is held in place, shears are slipped under the net and seagrass, where present, is cut level with the sediment surface. In habitats without seagrass, the same action was taken, ensuring that the sediment surface was ruffled as it was where seagrass was present. The net opening was then slipped off its frame and dragged shut along the sediment surface. Animals were later separated into sieve size classes of 2 mm, 1 mm, 500 μm, 250 μm, 125 μm and 75 μm before being identified to major taxa and counted. Numbers of very abundant taxa were counted from random subsamples with the aim of counting between 50 and 200 individuals of each taxon per sieve size in any sample. Taxa are listed in Table 1. Motile epifauna is the predominant food of nearly all fish associated with shallow seagrass beds (Klumpp et al. 1989), and total epifaunal abundances from this experiment have also been used in an attempt to explain effects of canopy removal on small fish (Connolly in press). Nematodes, however, are typically not a component of fish diets, and were therefore treated separately in analyses. Nematode numbers are presented here but have been excluded from estimates of total epifaunal abundance. Ash-free dry weights (AFDW) were calculated by converting abundances for each taxon for each sieve size using Edgar's (1990a) equation, logB = a + b.logS (where B = AFDW (mg), S = sieve size (mm) and a and b vary depending on broad taxonomic category). This permits estimation of epifaunal production using Edgar's (1990a) equation, P = $0.0049.B^{0.80}.T^{0.89}$, relating production (P, μg/day) to sample AFDW (B, μg) and water temperature (T, °C).

***Table 1 about here

The surface area of eelgrass leaves within all patches that supported eelgrass prior to the experiment was estimated before setting up the experiment and again after sampling of epifauna. Leaf area was calculated for each patch from measurements of the number of leaves per 400 cm^2 quadrat, and the length and width of ten leaves, at five randomly selected sites. Prior to the experiment, leaf area did not differ between patches selected for the three treatments involving eelgrass (C: 1.54 m^2 leaf area/ m^2 sediment surface; P: 1.31; R: 1.39; ANOVA: p = 0.651). After removal, the leaf area within patches of treatment R was reduced almost to zero, whilst patches of P remained similar to patches of C (C: 1.55; P: 1.46; R: 0.02; ANOVA: p < 0.001; Tukey's HSD pairwise comparisons: C P R).

Data Analysis

Epifaunal assemblages (described both by abundance and biomass (AFDW)) from the four habitats were compared using an analysis of similarities (ANOSIM), which is a non-parametric analogue to a multivariate analysis of variance (MANOVA) without the assumption of multivariate normality. ANOSIM has an additional advantage over MANOVA in being able to detect a difference not only in location of samples but also in spread (Clarke, 1993). ANOSIM compares ranked similarities between and within groups selected a priori using a randomisation test for significance. Patches were treated as a nested factor (patch) within the main factor (habitat). This nested ANOSIM tested whether assemblages differed amongst the four habitats by treating the three samples from each patch as a single collective estimate of the fauna from the patch. After a significant difference was detected using this global ANOSIM test, the same technique was employed to test pairwise differences between habitats. All ANOSIM tests involved 5000 simulations using the PRIMER package from Plymouth Marine Laboratory, U.K.

The relationships amongst assemblages from each patch are graphically represented using non-metric multidimensional scaling (MDS), an ordination technique that uses the same matrix of ranked similarities as ANOSIM. MDS displays samples in low (usually two) dimensional space while retaining as nearly as possible the similarity rankings between samples. The ordinations presented were done on data averaged over the three samples within each patch to simplify presentation and make habitat

groupings clearer. Ordinations were also done using all 72 individual samples, and habitat groupings were very similar to groupings using averaged data.

For comparisons of epifaunal assemblages, raw counts were transformed using $x^{0.25}$ to emphasise the distribution of less common taxa in the analysis. The Bray-Curtis similarity coefficient was used throughout, as a meaningful and robust measure (Clarke, 1993).

Analysis of the similarity matrix used in MDS and ANOSIM has also been used to highlight taxa making a large contribution to between-group differences (Clarke, 1993).

The association of environmental variables with patterns in biotic data can be measured by correlating the ranked similarity matrices of the environmental and biotic data (Clarke & Ainsworth 1993). The association between epifaunal assemblages and the position of a patch along the shore was measured using the weighted Spearman coefficient (ρ_w) recommended by Clarke and Ainsworth (1993). The position of a patch was described as the distance from the first patch at one end of the experiment. For this analysis the data from each sample within a patch were averaged to give just one assemblage per patch.

The abundance and biomass of epifauna (all taxa combined and key taxa separately) and total epifaunal production from the three habitats were compared using nested analysis of variance (ANOVA). Patches were treated as a nested factor (patch) within the main factor (habitat). Where habitat differences were significant, Tukey's HSD pairwise comparisons of habitat means used variance estimates from replicate patches, not from samples nested within patches. Sample variances increased with increasing means, and all univariate analyses were performed on $\log_{10} x$ transformed data (or $\log_{10} (x + 1)$ where zeros occurred) after checking that the transformation increased homoscedasticity. Significance levels are 0.05 throughout this paper.

Results

Two-dimensional ordination plots showed a very similar pattern on both abundances (Fig. 1a) and biomasses (Fig. 1b). Assemblages from patches of habitat U were grouped separately from those of other habitats. Differences amongst the other three habitats were less obvious. Assemblages from habitats C and P overlapped considerably, and those from habitat R, whilst overlapping with C and P, tended to group more distinctly and be positioned closer to habitat U. The global ANOSIM test for differences amongst habitats detected significant differences using both abundance and biomass data (Table 2). On abundance data, assemblages from habitat U were different from those of all other habitats, and no differences were detected amongst habitats C, P and R. On biomass data, assemblages from habitat U were different from all other habitats, habitats C and P were not separate, and habitat R was different from C but not from P (Table 2). The ANOSIM results confirm the patterns evident in ordination plots, except that habitat R was found to be intermediate between habitat U and habitats C and P on biomass data only. Assemblages differed amongst patches within habitats using both abundance and biomass data.

***Figs 1 & 2 and Table 2 about here

The correlation between similarities in epifaunal assemblages and in positions of patches along the shore was close to zero for both abundance ($\rho_w = 0.03$) and biomass ($\rho_w = 0.04$). Although no formal test of this correlation is currently available, these values are extremely low (Clarke & Ainsworth 1993) and provide evidence that there was no gradient in epifauna along the shore. A simple overlay of the position along shore of each patch onto the ordination plot of epifaunal assemblages (Figs 2 a,b) shows no obvious pattern and supports the view that epifauna did not change systematically along the shore.

When nematodes were included in the analysis, habitat groups on ordination plots were not noticeably altered. The results of ANOSIM pairwise comparisons using biomass data showed the same differences described above for biomasses, and using abundance data the pattern of differences upon inclusion of nematodes became the same as that for biomass data. On both abundance and biomass data, nematodes were the major contributor to differences between several pairs of habitats. The order of importance of

other taxa important in differentiating habitats was unchanged upon the inclusion of nematodes in the analysis. Cumaceans remained the most important taxon distinguishing habitat U from the other habitats.

***Table 3 about here

Mean abundances in each habitat of total epifauna and of key taxa contributing to differences amongst assemblages are shown in Table 3 along with ANOVA and Tukey results. Total abundance was not significantly different between habitats C and P, and was lower in habitat R and lowest in U. Of individual taxa, numbers of harpacticoids and gastropods were not significantly different in habitats C, P and R, but were lower in U. Amphipod numbers were lowest in U, not significantly different in C and P, and in R were intermediate between U and C but not significantly different from P. Polychaete numbers showed the same pattern except that they were not significantly lower in U than in R. The only significant difference in tanaid numbers was between C and U. Numbers of calanoids and ostracods did not differ significantly amongst habitats. Cumaceans were rare in habitats C, P and R, and common in U. Abundances of all taxa combined and several individual taxa differed between patches within habitats; the habitat differences described above, however, were evident over and above differences amongst patches. The overwhelming trend (except for cumaceans) is of abundances being similar in habitats C and P but lower in R and lowest in U.

Nematodes were more numerous than all other animals combined, and abundances showed the same trend evident in total epifaunal abundances. Nematode numbers were highest in habitats C and P, intermediate in R and lowest in U.

Mean biomasses in each habitat of total epifauna and of key taxa contributing to differences amongst assemblages are shown in Table 4. Total biomass tended to decrease from habitat C to P to R to U. Biomass in habitat C was higher than in R and U but was not significantly different from P. P was higher than U and intermediate between, but not significantly different from, C and R. R was intermediate between, but not significantly different from, P and U. The same differences were found for biomasses of polychaetes and nematodes. Amphipod biomasses were different only between C and U, with P and R being similar to each other and intermediate between C and U. Harpacticoids showed a similar pattern but the biomass in R was not significantly different either from C and P or from U. For tanaids, calanoids, ostracods and gastropods no differences were detected amongst biomasses from the four habitats. Cumacean biomass was low in habitats C, P and R, and was high in U. Total epifaunal biomass was not found to differ amongst patches within habitats, but differences were detected for several individual taxa. The general pattern evident in abundance data of similarity between habitats C and P, with R intermediate and U lowest is also present in biomass data but the trend is weaker. The main difference between abundance and biomass data is that the gap between habitat U and the other habitats is less obvious in biomass data. The narrowing of this gap suggests that the mean biomass of individuals was greater in habitat U than in other habitats. The mean biomass of individuals in each sample was calculated by dividing the total biomass of a sample by the total number of individuals in the sample. The mean biomass of individuals was highest in habitat U (C: mean = 18.1 μg, (s.e. = 3.6); P: 14.2 (2.2); R: 15.3 (4.8); U: 24.2 (4.8)) although differences amongst habitats were not significant when tested using a nested ANOVA (Habitat: $p = 0.4$ ns). Nor did individual biomasses differ significantly amongst patches within habitats (Patch: $p = 0.17$ ns).

***Table 4 about here

Total epifaunal production and crustacean production differed amongst habitats as follows: Total production: ANOVA, Habitat: $p < 0.001$, Patch: $p = 0.101$, Tukey comparisons: $\underline{\text{C} \ \underline{\text{P} \ \text{R}} \ \text{U}}$; Crustacean production: ANOVA, Habitat: $p = 0.007$; Patch: $p = 0.024$, Tukey comparisons as for total production. Production (total and crustacean) in habitat C was higher than in R and U but was not significantly different from P. P was higher than U and intermediate between, but not significantly different from, C and R. R was intermediate between, but not significantly different from, P and U.

Discussion

Expectations based on published surveys showing higher epifaunal abundance, biomass and production associated with seagrass patches compared to adjacent unvegetated patches (Orth et al. 1984) were fulfilled in the present study by the marked differences found between undisturbed (control) eelgrass plots and plots unvegetated prior to the experiment (unvegetated). The abundance and biomass of individual taxa were mostly lower in unvegetated patches, the striking exception being cumaceans, which were more abundant and had higher biomasses in unvegetated patches.

Effects of the disturbance associated with eelgrass removal were weak relative to effects of removing eelgrass. No effects of disturbance alone were detected on assemblages, using either abundance or biomass data. Of the important taxa analysed separately, some were found to have lower abundances and biomasses in disturbed (procedural control) plots than in undisturbed (control) plots. These differences, however, were typically smaller than those between disturbed eelgrass (procedural control) plots and plots from which eelgrass had been removed. That is, the removal of eelgrass had a detectable effect on epifauna over and above any effects of disturbance associated with eelgrass removal.

Removal of eelgrass canopy altered assemblages, rendering them more similar than those from intact eelgrass to assemblages from unvegetated habitat. Removal of eelgrass canopy also decreased the abundance and biomass of several key taxa. The overall effect of removing the canopy was to alter the fauna in the direction of that from unvegetated patches. Removal of canopy did not, however, cause the fauna to match that from previously unvegetated habitat. Assemblages from unvegetated habitat were clearly different from all other habitats, and abundances and biomasses of most of the key species were obviously lower than in other habitats. Cumaceans, which were abundant in habitat unvegetated prior to the experiment, were rare or absent in other habitats.

The results show that the eelgrass canopy does have some importance to epifauna, but that the eelgrass canopy itself is not the only difference, and is not the overriding difference, between patches with and without eelgrass.

The two most commonly invoked explanations for the greater abundance of epifauna associated with vegetated habitats are that seagrass provides protection from predation or a greater abundance of food. The role of seagrass in providing protection from predators has received most attention in recent times (Heck & Orth 1980, and see review by Bell & Pollard 1989). The work of Bell & Westoby (1986b) demonstrated that, for the macrofauna they studied, lower abundance in less dense eelgrass was not due simply to predators eating the target species. Macrofauna were rarer in less dense eelgrass regardless of the presence or absence of predators. Bell & Westoby (1986b) suggested that macrofauna select denser eelgrass (and pointed out that predation may have been the ultimate selective agent for this behaviour). The same habitat selection behaviour can be used to explain the greater abundance of epifauna in general in vegetated compared to unvegetated habitats. Epifauna may preferentially select vegetated habitat. It has been shown that macrofauna (Bell & Westoby 1986b, Virnstein & Curran 1986, and review in Howard et al. 1989) and meiofauna (Bell & Hicks 1991) move around over the temporal (two weeks) and spatial (tens of metres) scales used in the present study. The tidal water flow in the Barker Inlet - Port River region increases the chance that invertebrates moved about during the experiment, and that their abundances reflected preferences. The results of the present experiment therefore do not support the model of animals selecting vegetated over unvegetated habitats based on the presence of canopy. Bell & Westoby (1986a) point out alternative explanations for the greater abundance of macrofauna in denser seagrass, for example that macrofauna may be attracted to higher abundances of food. The possibility that epifauna are more abundant in vegetated than in unvegetated habitats because they are attracted to higher food abundance has not been tested in the present study. Although removal of eelgrass may have lessened the amount of food available to epifauna (food includes any or all of the following: detritus, bacteria, microscopic algae, and perhaps some of the smaller invertebrates themselves), food availability was not measured.

Other explanations for the greater abundance of epifauna in vegetated habitats are (as listed by Lewis (1984)): 1) the presence of physical structure usable as living space, 2) dampened hydrodynamic forces, 3) increased number of microhabitats, and 4) greater stabilisation and deposition of sediment. Results of the present experiment exclude 1) and 2) as plausible possibilities as these explanations are reliant on

the immediate presence of above-ground vegetation. The number of microhabitats available to epifauna would have been greatly reduced by the removal of seagrass canopy. All the different heights in the canopy, and positions among shoots, are removed along with the canopy. There may be some difference in the number of microhabitats, however, between patches from which seagrass was removed and unvegetated patches, because of the presence of the root/rhizome mat in the former habitat. For this reason, the failure of the fauna in these two habitats to match does not necessarily exclude explanation 3) above. Removal of seagrass canopy should render sediment deposition similar to that experienced in unvegetated patches. However, the stability of sediments is very likely to be affected by the retention of the seagrass root/rhizome mat in patches from which canopy was removed, and in any case it may take time after the removal of canopy before sediment becomes similar to that of unvegetated areas. Results therefore do not exclude the possibility that differences in the epifauna from vegetated and unvegetated habitats are caused by differences in sediment characteristics. Another explanation, one not previously considered in the literature, is that epifauna are more abundant in vegetated habitats simply because the canopy causes them to swim more slowly there. Results of the present experiment discount this explanation.

An alternative explanation for the results of this experiment is that, since patches unvegetated prior to the experiment did not receive the disturbance inflicted on patches from which eelgrass was removed, the difference in degree of disturbance may have caused the failure of the fauna of the two habitats to match. Another treatment in which unvegetated patches received the disturbance of simulated eelgrass removal could have been used. The same disturbance in eelgrass habitat had only a weak effect on epifauna, and this generates some confidence that disturbance was not important when comparing the two habitats without eelgrass canopy; the possibility that degree of disturbance was the important difference between these two habitats has not, however, been altogether removed.

The differences amongst the epifauna from the four habitats lay in the abundance or biomass of taxa, not in the presence or absence of taxa (except for cumaceans). This result may reflect the gross clumping of species and possibly functional groups into single, higher taxa, so that changes in the fauna at these levels would not have been detected. Nevertheless, Warwick (1988) showed that multivariate analyses at family level reproduced very closely the results obtained at species level, and even analyses at the level of phylum generally agreed surprisingly well with those at lower taxonomic levels. Warwick suggests that for some purposes analyses based on higher taxa may more closely reflect the information of interest than those based on species. In any case, the significant differences detected amongst epifauna from different habitats demonstrates that the taxa used in this study were adequate for examination of the general question posed about the effects of canopy removal on epifauna.

Results from the present experiment apply only to daytime distributions of epifauna. In another experiment examining the effects on epifauna of manipulating seagrass canopy height (Connolly & Butler, unpubl. data), epifauna were collected during both the night and day. Abundances and biomasses of key taxa and of all taxa combined were higher at night than during the day, and this is typical of seagrass systems (e.g. Howard 1987). The effects of manipulating canopy height, however, were similar at both night and day.

Epifaunal assemblages, and abundances and biomasses of some key species, differed from patch to patch even within habitats. These differences were not correlated with position along the shore, and at this stage must be considered as unexplained variability.

In summary, removing eelgrass canopy had a weak but detectable effect on epifaunal assemblages and on the abundance and biomass of some taxa. The epifauna associated with habitat from which eelgrass was removed did not, however, match that from areas unvegetated prior to the experiment. This result suggests that the overriding importance of eelgrass to epifauna is not, at least over short periods, simply the presence of seagrass canopy. The evidence therefore supports models in which differences between the epifauna of vegetated and unvegetated habitats are not directly linked to the presence of seagrass canopy.

Acknowledgements

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Table 1. List of taxa into which animals were grouped.

Abbreviations shown are those used in Table 2.

	Crustacea		Non-crustacea
	Caridea	Pol	Polychaeta
	Mysidacea	Gas	Gastropoda
Amp	Amphipoda - Gammaroidea		Bivalvia
	Amphipoda - Caprellidea		Ophiuroidea
Tan	Tanaidacea		Actiniaria (Anemones)
	Isopoda		Ascidiacea larvae
Cum	Cumacea		Nematoda
Har	Copepoda - Harpacticoida		
	Copepoda - Cyclopoida		
Cal	Copepoda - Calanoida		
	Copepoda - nauplii (unidentified)		
Ost	Ostracoda		

Table 2. Results of ANOSIM comparisons amongst epifaunal assemblages.

Results are probabilities. Pairwise tests are for differences between pairs of habitats: C = control, P = procedural control, R = removed, U = unvegetated. Significance level for each pairwise comparison is 0.0083 so that overall significance level for six comparisons is 0.05 (ns = not significant). Contributing taxa are those making a consistently large contribution to differences between samples from the two habitats, listed in order of decreasing importance.

Variable	Global ANOSIM result	Pairwise ANOSIM results	Main contributing taxa
Abundance	Habitat < 0.001	C,P 0.593 ns	Amp, Cal, Pol
	Patch < 0.001	C,R 0.017 ns	Tan, Gas, Har
		C,U 0.002	Cum, Pol
		P,R 0.128 ns	Ost, Gas, Har
		P,U 0.002	Cum, Har, Pol
		R,U 0.002	Cum, Har, Cal
Biomass	Habitat < 0.001	C,P 0.517 ns	Pol, Gas, Amp, Tan
	Patch < 0.001	C,R 0.006	Gas, Har, Tan
		C,U 0.002	Har, Pol, Cum
		P,R 0.056 ns	Gas, Cal, Har
		P,U 0.002	Cum, Har
		R,U 0.002	Cum, Har

Table 3. Abundances of total epifauna and key taxa in each habitat.

Numbers are means (individuals/net) with standard errors in parentheses. ANOVA results are probabilities; ns = not significant. Tukey results are for comparisons between pairs of habitats, and show significant differences as letters not grouped by underlining: C = control, P = procedural control, R = removed, U = unvegetated.

	C	P	R	U	ANOVA results Habitat	Patch	Tukey results
All taxa combined	903 (152)	794 (132)	381 (56)	148 (21)	< 0.001	0.016	<u>C P</u> R U
Amphipods	16 (2)	10 (2)	6 (1)	2 (0)	< 0.001	0.019	<u>C P R</u> U
Tanaids	3 (1)	3 (1)	2 (1)	0 (0)	0.017	0.082 ns	<u>C P R U</u>
Cumaceans	1 (1)	1 (0)	0 (0)	12 (2)	< 0.001	0.198 ns	U <u>C P R</u>
Harpacticoids	590 (101)	613 (122)	282 (35)	105 (18)	< 0.001	0.001	<u>P C R</u> U
Calanoids	5 (2)	11 (3)	6 (2)	2 (1)	0.3 ns	< 0.001	
Ostracods	142 (106)	32 (17)	29 (20)	3 (1)	0.17 ns	0.001	
Polychaetes	118 (19)	102 (28)	43 (8)	22 (8)	< 0.001	0.003	<u>C P R</u> U
Gastropods	15 (5)	9 (5)	8 (3)	1 (0)	0.03	0.001	<u>C P R</u> U
Nematodes	1446 (187)	1487 (326)	350 (60)	97 (20)	< 0.001	0.063 ns	<u>C P R</u> U

Table 4. Biomasses of total epifauna and key taxa in each habitat.

Numbers are means (mg) with standard errors in parentheses. ANOVA results are probabilities; ns = not significant. Tukey results are for comparisons between pairs of habitats, and show significant differences as letters not grouped by underlining:
C = control, P = procedural control, R = removed, U = unvegetated.

	C	P	R	U	ANOVA results Habitat	Patch	Tukey results
All taxa combined	17.8 (7.9)	10.8 (2.0)	8.9 (5.4)	3.8 (1.0)	< 0.001	0.112 ns	C P R U
Amphipods	2.4 (0.8)	1.1 (0.2)	0.5 (0.2)	0.3 (0.1)	< 0.018	0.012	C P R U
Tanaids	0.4 (0.1)	0.3 (0.3)	0.4 (0.2)	0.0 (0.0)	0.04 ns	< 0.001	
Cumaceans	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.7 (0.4)	< 0.001	0.123 ns	U C P R
Harpacticoids	2.6 (0.5)	2.8 (0.7)	1.1 (0.2)	0.5 (0.3)	< 0.001	0.001	P C R U
Calanoids	0.1 (0.0)	0.1 (0.0)	0.0 (0.0)	0.0 (0.0)	0.18 ns	0.001	
Ostracods	0.5 (0.4)	1.1 (1.1)	2.9 (2.9)	0.0 (0.0)	0.7 ns	0.5 ns	
Polychaetes	4.1 (0.8)	2.0 (0.5)	1.2 (0.3)	0.3 (0.1)	< 0.001	0.039	C P R U
Gastropods	5.3 (4.4)	1.0 (0.4)	2.4 (1.7)	0.5 (0.4)	0.26 ns	0.363 ns	
Nematodes	9.7 (2.5)	8.6 (2.7)	6.9 (5.4)	1.7 (1.5)	< 0.001	0.108 ns	C P R U

Figure captions

Fig. 1. Two-dimensional MDS ordination plot of epifaunal assemblages, averaged for the three samples within each patch, based on: a) abundances (stress = 0.143), b) biomasses (stress = 0.150). C = control, P = procedural control, R = removed, U = unvegetated.

Fig. 2. Overlay of position of patch along shore onto MDS ordination plots in Fig. 1: a) abundance data, b) biomass data. Diameter of circle is proportional to distance along shore. Smallest circle = 0 m, largest = 900 m.
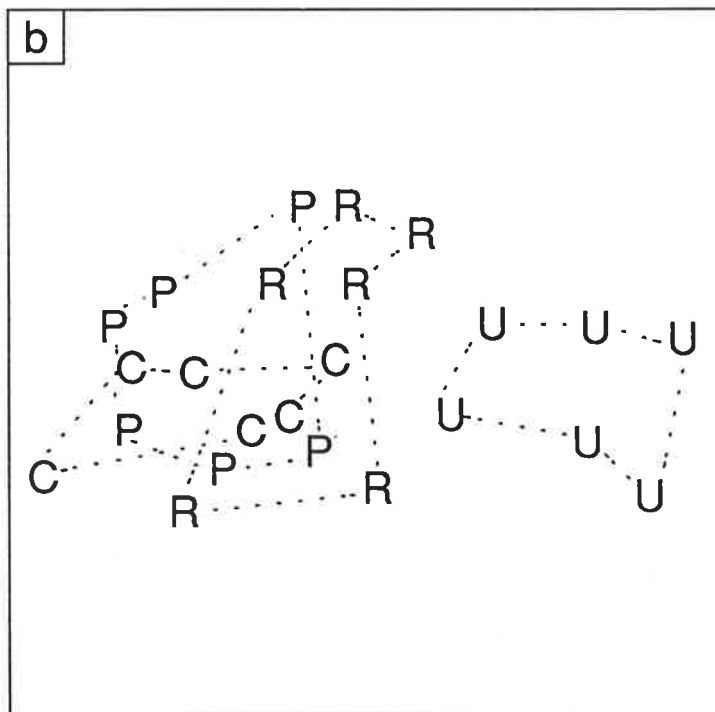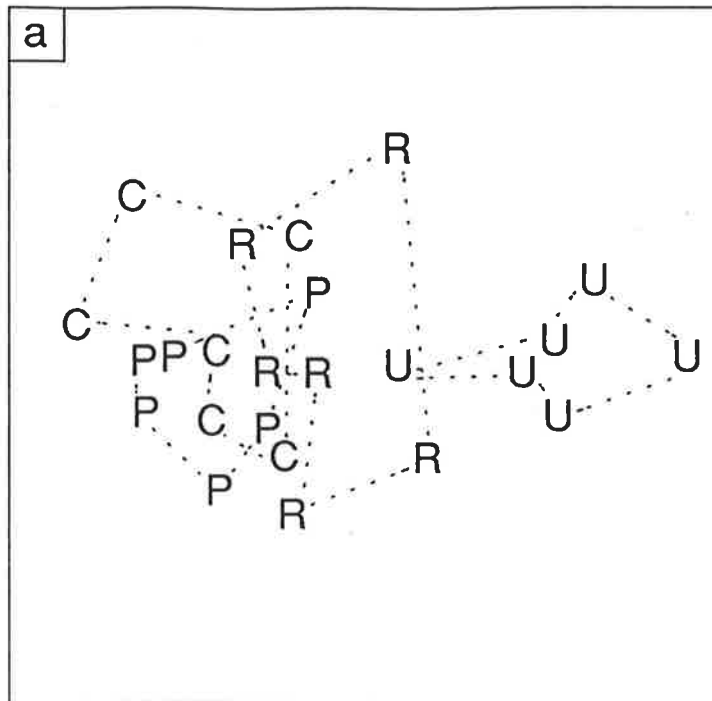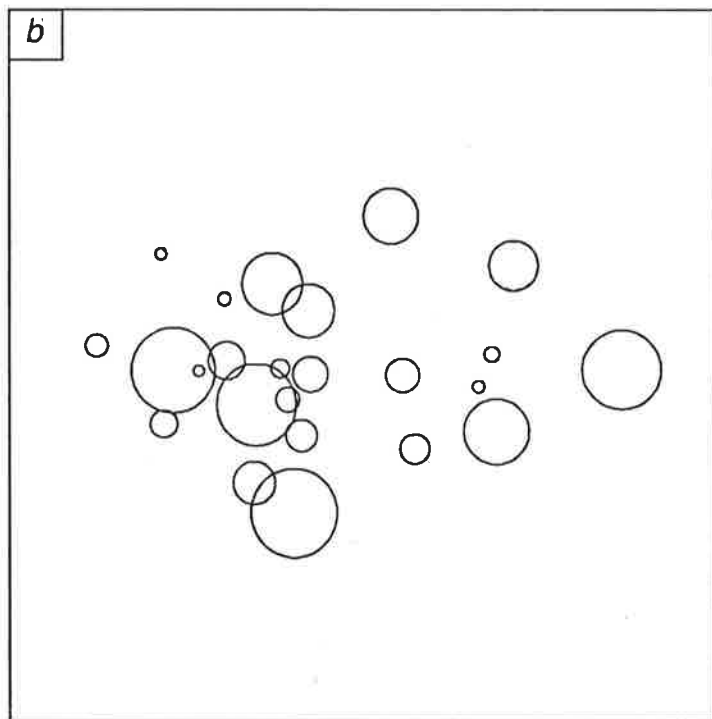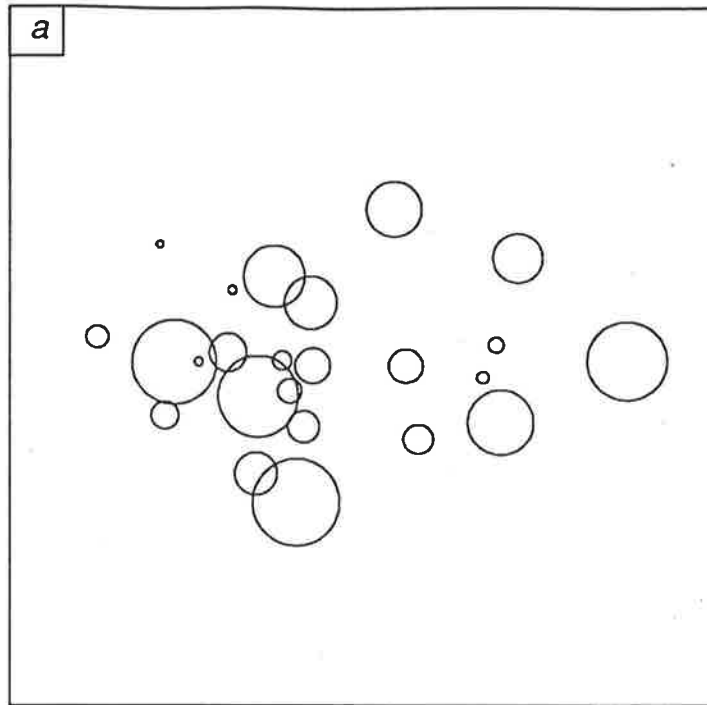
Fig. 1

Fig. 2

A.5

The effects of altering seagrass canopy height on small, motile invertebrates of shallow Mediterranean embayments

Les effets des changements de l'hauteur de l'herbier des phanérogames marines sur les petits invertébrés mobiles des embaiements peu profonds en Méditerranée

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Abstract

The height of seagrass was manipulated in experimental plots in meadows of the fine-leaved seagrass *Cymodocea nodosa* at two sites in the Mediterranean Sea, la Lagune du Brusc, Iles des Embiez near Toulon, and l'Etang de Diana on Corsica. Epifauna (small motile invertebrates associated with the seagrass canopy or sediment surface) was collected at night, and during the day at Diana, from three treatments: full seagrass canopy, reduced canopy, and canopy removed entirely. Although epifaunal assemblages from the two sites were different, habitat modification had the same effect at both when analysed using multivariate ordinations. Abundance and biomass of total epifauna and of key taxa, and total epifaunal production, were all reduced in line with decreasing seagrass cover at both sites. The effects of habitat modification on epifauna collected during the day were of the same type but of greater magnitude, both on assemblages and on total abundance and biomass. At both sites and at night and day the fauna of plots from which seagrass had been removed tended to be dominated by heavier animals than the fauna of plots with full canopy. Epifauna form the major dietary component of small fish inhabiting shallow, sheltered embayments. The results are therefore consistent with a model in which reduced abundance of fish associated with reduced seagrass canopy is explained by a reduction in food availability.

Résumé

L'hauteur de l'herbier de *Cymodocea nodosa* était manipulé dans des carrés expérimentaux à deux sites en Méditerranée, la Lagune du Brusc, Ile des Embiez, près de Toulon, et l'Etang de Diana, Corse. L'épifaune (petits invertébré mobiles associés avec la marquise de l'herbier ou avec la surface du sédiment) étaient prelèvés par nuit, et par jour à Diana, de trois traitements: marquise entière, marquise réduite, et marquise enlevée totalement. Malgré que les assemblages des épifaunes étaient différentes aux deux sites, la modification de l'habitat avait le même effet quand analysé avec des ordonnations multivariés. L'abondance et la biomasse de l'épifaune entière, et des taxa clées, même que la production totale de l'épifaune, étaient réduits selon la couverture de l'herbier à chaque site. Les effets de la modification de l'habitat sur l'épifaune prélevée pendant la journée étaient de la même forme mais d'une magnitude plus grande, même sur les assemblages que sur l'abondance et la biomasse. Aux deux sites, par nuit et jour, la faune des carrés sans marquise montraient une tendance d'être plus dominé par des animaux lourds que les carrés avec marquise entière. L'épifaune fait la partie majeure du nourriture des petits poissons qui habitent les embaiements abritées et peu profondes. Ces résultats sont, ainsi, compatibles avec un modèle qui attribue la réduction d'abondance des poissons, qui est associée avec une réduction de la marquise de l'herbier, à une réduction du niveau de la nourriture.

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Problem

Seagrass meadows in many parts of the world, including the Mediterranean Sea, support large numbers of juvenile fish and provide a nursery habitat for many commercially important species (POLLARD, 1984). Unvegetated areas adjacent to seagrass meadows have different fish assemblages, usually with fewer fish and fewer species (BELL & POLLARD, 1989). Seagrass meadows are declining in area due to pollution and land reclamation around the world, and the loss of seagrass cover has been correlated with declining fish catches (for example of King George whiting (*Sillaginodes punctata*) in Victoria, Australia (BELL & POLLARD, 1989)).

The importance of seagrass to small fish probably lies either in the protection it offers from predators (larger fish), or in the abundance of associated food (mostly small invertebrates). When a canopy of *Zostera capricorni* was shortened in experimental plots, fish abundance declined, as did the abundance of many individual species. Thinning the canopy also caused several species to become less abundant, although three species increased in abundance (BELL & WESTOBY, 1986a). BELL & WESTOBY (1986c) manipulated seagrass densities in field experiments and used predator exclusion cages to show that small fish were more common in denser seagrass regardless of the presence or absence of predators. They showed convincingly that low fish numbers in patches with less dense seagrass cover were not due to increased predation, and concluded that small fish select habitat. According to the model of BELL & WESTOBY (1986b), completely unvegetated areas have different fish assemblages and fewer fish as a result of fish choosing to settle in seagrass beds in preference to adjacent unvegetated areas. As BELL & WESTOBY (1986b) point out, their results may be explained in other ways. Fish might, for example, be attracted to more abundant food in denser seagrass. There is typically more food associated with seagrass beds than with adjacent unvegetated areas (HOWARD et al., 1989). Food abundance was not measured by BELL & WESTOBY (1986a, 1986b, 1986c). When artificial seagrass units were placed in unvegetated areas the number of fish settling matched the number in natural seagrass beds and not the number in unvegetated areas (BELL et al., 1987). This may be explained by selection of habitat, but since artificial seagrass also attracts small invertebrates (e.g. VIRNSTEIN & CURRAN, 1986), the amount of food associated with artificial seagrass is also higher than in adjacent unvegetated areas.

BELL & WESTOBY (1986b) developed the following general model to explain comprehensively the distribution of small fish in shallow, sheltered embayments: 1) fish larvae are distributed patchily when ready to settle, 2) fish select seagrass of any density rather than unvegetated areas when settling to a benthic existence, and 3) fish redistribute themselves within a bed by selecting denser seagrass but do not, at least in the short term, move across large, unvegetated expanses to other beds. The observations leading to points 2 and 3 above, however, are also consistent with an alternative model in which fish simply swim until they find food, pause to eat it, then swim again. Under this model, abundances of small fish match prey abundance.

Small fish associated with seagrasses are predominantly carnivorous, epifaunal invertebrates forming the major part of their diet (KLUMPP et al., 1989). Crustaceans are the most important prey. Epifaunal, but not infaunal, polychaetes and molluscs are also taken (KLUMPP et al., 1989). Dietary studies on fish from seagrasses in the Mediterranean Sea (CASABIANCA & KIENER, 1969; BELL & HARMELIN-VIVIEN, 1983; KHOURY, 1984) and Black Sea (DUKA, 1978) support these general patterns.

The aim of this study was to manipulate the seagrass canopy in experimental plots to determine whether alterations to canopy height and surface area would be matched by changes in epifauna. This was done with a view to confirming the possibility that fish abundances can be explained by food availability.

Material and Methods

***Fig 1 about here

1. Experimental design and sampling

Experiments were done in shallow, sheltered embayments in la Lagune du Brusc near the shore of l'Ile des Embiez and l'Etang de Diana on Corsica (Fig. 1), where the dominant vegetation is the fine-leaved seagrass *Cymodocea nodosa*. These sites are referred to below as Embiez and Diana respectively. The small motile invertebrates associated with the seagrass canopy and sediment surface (epifauna) were collected from the following three habitats (treatments) marked as 1 m x 1 m plots:

1) seagrass uncut (control = C),
2) seagrass canopy cut to one third of original height (partly cut = P), and
3) seagrass canopy removed entirely (removed = R).

Seagrass was cut using hand shears and was shaken vigorously in the water before being removed to minimise the amount of epifauna carried away from the plot. The disturbance associated with cutting was simulated in control plots by spending an equivalent time mimicking cutting.

At each location, six sites were assigned to each of the treatments in a randomised block design. That is, one replicate of each treatment was assigned at random to six areas (blocks) strung along a 300 m stretch of coast at Diana and placed in a 0.25 ha area adjacent to the coast at Embiez. At Diana an additional four replicates of each treatment were set up for collection of epifauna during the day. All other sampling was done immediately after dusk. The blocked design guaranteed interspersion, which is important because of the potential patchiness of epifauna. All plots were in water between 30 and 70 cm deep. During the experiments the water height fluctuated 12 cm at Embiez and 2 cm at Diana.

Epifauna was collected using a 150 μm mesh net with a 25 x 25 cm opening following the method of SERGEEV *et al.* (1988) in which the net is placed rapidly over the canopy onto the sediment before dragging shut the mouth of the net along the sediment surface. Samples were taken two days after the setting up of treatments, and the order in which plots were sampled was randomised. One sample was taken approximately in the centre of each plot. Animals were later separated into sieve size classes of 2 mm, 1 mm, 500 μm, 250 μm, 125 μm and 75 μm before being identified to major taxa and counted. Numbers of very abundant taxa were counted from random subsamples with the aim of counting between 50 and 200 individuals of each taxon per sieve size in any sample. Twenty-two taxa were used, 13 crustacean and 9 others (Table 1). Nematodes and foraminifera were excluded from this study because they are typically not an important component in the diet of small fish inhabiting seagrass meadows (KLUMPP *et al.*, 1989). Ash-free dry weights (AFDW) were calculated by converting abundances for each taxon for each sieve size using EDGAR'S (1990) equation, $\log B = a + b \log S$ (where B = AFDW (mg), S = sieve size (mm) and a and b vary depending on broad taxonomic category). This permits estimation of epifaunal production using EDGAR'S (1990) equation, $P = 0.0049 \, B^{0.80} \, T^{0.89}$, relating production (P, μg/day) to sample AFDW (B, μg) and water temperature (T, °C).

***Table 1 about here

The length and surface area of seagrass leaves in each plot were estimated prior to cutting and after epifauna collection. Leaf area was calculated for each plot from measurements of the number of leaves per 100 cm^2 quadrat, and the length and width of ten leaves, at three randomly selected places. All vegetation was *Cymodocea nodosa* except for occasional *Zostera noltii* plants at Diana.

2. Data analysis

Epifaunal assemblages (described both by abundance and biomass (AFDW)) from the three habitats were compared using an analysis of similarities (ANOSIM), which is a non-parametric analogue to a multivariate analysis of variance (MANOVA) without the assumption of multivariate normality. ANOSIM has an additional advantage over MANOVA in being able to detect a difference not only in

location of samples but also in spread (CLARKE, 1993). ANOSIM compares ranked similarities between and within groups selected *a priori* (here the three habitats) using a randomisation test for significance. After a significant difference has been detected using this global ANOSIM test, the same technique is employed to test pairwise differences. Assemblages from the three "locations", Embiez, Diana-Night and Diana-Day, were also compared using a two-way crossed ANOSIM with habitat as the second factor. This analysis determines whether assemblages differed amongst the locations after accounting for habitat differences. All ANOSIM tests involved 5000 simulations using the PRIMER package from Plymouth Marine Laboratory, U.K.

The relationships amongst assemblages from each patch are graphically represented using non-metric multidimensional scaling (MDS), an ordination technique that uses the same matrix of ranked similarities as ANOSIM. MDS displays samples in low (usually two) dimensional space while retaining as nearly as possible the similarity rankings between samples.

For comparisons of epifaunal assemblages, raw counts were transformed using $x^{0.25}$ to emphasise the distribution of less common taxa in the analysis. The Bray-Curtis similarity coefficient was used throughout, as a meaningful and robust measure (CLARKE, 1993).

Analysis of the similarity matrix used in MDS and ANOSIM has also been used to highlight the taxa making the largest contribution to between-group differences (CLARKE, 1993).

The abundance and biomass of epifauna (all taxa combined and key taxa separately) and total epifaunal production from the three habitats were compared using analysis of variance (ANOVA) with Tukey's HSD pairwise comparisons following significant ANOVA results. Differences between the three "locations" in the above variables were compared using a two-factor ANOVA with habitat as the second factor. Sample variances increased with increasing means, and all univariate analyses were performed on $\log_{10} x$ transformed data (or $\log_{10} (x + 1)$ where zeros occurred) after checking that the transformation increased homoscedasticity. Significance levels are 0.05 throughout this paper.

The relationship between biomass and abundance for habitats at each location is shown in cumulative dominance plots of both variables (Abundance - Biomass Comparisons). This graphic technique has been used to detect the effects of disturbance or pollution on marine benthic fauna (WARWICK, 1986), but in its general form provides information about the degree to which fauna is dominated by fewer, larger animals or many smaller ones.

Results

1. Ile des Embiez

Leaf lengths in the three habitats prior to cutting were: C, mean = 161 mm (s.e. = 8.7); P, 152 (9.9); R, 167 (8.6), and at the time of epifauna collection were: C, 171 (8.8); P, 56 (2.3); R, 18 (2.0). Surface areas prior to cutting were: C, mean = 7.26 m^2 leaf area/m^2 sediment surface area (s.e. = 0.63); P, 6.24 (0.95); R, 7.04 (0.16), and after epifauna collection were: C, 7.58 (0.76); P, 2.18 (0.19); R, 0.13 (0.03).

Two-dimensional ordination plots show strong grouping of plots from the three habitats both on abundance (Fig. 2a) and biomass (Fig. 2b) data. Assemblages were significantly different using both variables, as tested with ANOSIM (Table 2). Pairwise ANOSIM comparisons using abundance data showed that all habitats were different, and using biomass data showed that habitat R was different from habitats C and P (Table 2).

***Fig 2 and Tables 2 and 3 about here

Mean abundances for the three habitats of total epifauna and of key taxa contributing to differences amongst assemblages are shown in Table 3 along with ANOVA and Tukey results. The total abundance of epifauna was different for all habitats, being highest in habitat C, intermediate in habitat P and lowest in habitat R. The same pattern was found in abundances of amphipods, the most prominent taxon contributing to differences in assemblages, and harpacticoids. There were fewer polychaetes in habitat R

than in habitats C and P. Numbers of gastropods and tanaids were higher in C than in R but although numbers in P were intermediate, they were not significantly different from either C or R. No differences were detected amongst abundances of calanoids or mysids. The overwhelming pattern of abundances is of decreasing abundance from C to P to R.

Mean biomass of total epifauna and of key species are shown in Table 4. Differences in total biomass amongst habitats were significant, with biomass in habitat C higher than in R and biomass in P intermediate but not significantly different from either C or R. Harpacticoid biomass was highest in C, intermediate in P and lowest in R. Amphipod biomass was not significantly different between C and P but was lower in R, whereas polychaete biomass was not different between P and R but was higher in C. Significant differences were not detected in biomasses of other taxa. The general pattern of decreasing abundance from C to P to R was also evident for biomass but was less marked.

***Tables 4 and 5 about here

Total epifaunal production and total crustacean production both differed amongst habitats (Table 5). Total production was higher in habitat C than in R, and was intermediate in P but not significantly different from the other habitats. Crustacean production was lowest in habitat R and not significantly different between C and P.

2. Etang de Diana-Night

Leaf lengths in the three habitats prior to cutting were: C, mean = 281 mm (s.e. = 26.3); P, 281 (28.0); R, 268 (25.7), and at the time of epifauna collection were: C, 273 (22.2); P, 82 (6.9); R, 14 (1.9). Surface areas prior to cutting were: C, mean = 9.35 m^2 leaf area/m^2 sediment surface area (s.e. = 1.90); P, 10.19 (2.08); R, 9.15 (1.87), and after epifauna collection were: C, 9.24 (1.78); P, 2.23 (0.34); R, 0.11 (0.02).

Two-dimensional ordination plots show strong grouping of plots from the three habitats both on abundance (Fig. 3a) and biomass (Fig. 3b), and differences were shown to be significant using ANOSIM (Table 6). On both abundances and biomass, all habitats differed according to pairwise ANOSIM tests.

***Fig 3 and Tables 6 and 7 about here

The total abundance of epifauna was highest in habitat C, intermediate in P and lowest in R (Table 7). The same pattern was found for gastropods, the most prominent taxon contributing to multivariate differences, and tanaids, harpacticoids, calanoids and polychaetes. Isopod and chironomid numbers were greater in habitat C and not significantly different between P and R. Tukey's test failed to detect any pairwise differences in anemone numbers despite a significant ANOVA result. This reflects the infrequent occurrence of anemones in habitats P and R. The total numbers of anemones caught were as follows: C, 11; P, 2; R, 2. As for Embiez data, there was an overwhelming pattern of abundances decreasing from habitat C to P to R.

Total biomass was higher in habitat C than in R, and was intermediate in P but not significantly different from the other habitats (Table 8). The biomasses of gastropods, amphipods, tanaids and harpacticoids were highest in habitat C, intermediate in P and lowest in R. Biomasses of isopods, polychaetes, anemones and chironomids were higher in habitat C and not significantly different between P and R. The general pattern of decreasing biomass from C to P to R is evident but is less marked, judging from total epifaunal biomass, than for abundances.

***Tables 8 and 9 about here

Total epifaunal production and total crustacean production were both higher in habitat C than in R, and were intermediate in P but not significantly different from the other habitats (Table 9).

3. Etang de Diana-Day

Leaf lengths in the three habitats prior to cutting were: C, mean = 269 mm (s.e. = 25.3); P, 270 (17.7); R, 257 (24.0), and at the time of epifauna collection were: C, 262 (27.2); P, 79 (7.1); R, 13 (2.1). Surface areas prior to cutting were: C, mean = 9.44 m^2 leaf area/m^2 sediment surface area (s.e. = 1.80); P, 9.51 (1.24); R, 9.21 (1.23), and after epifauna collection were: C, 8.94 (1.51); P, 1.99 (0.32); R, 0.09 (0.02).

Two-dimensional ordination plots again show strong grouping of plots from the three habitats both on abundance (Fig. 4a) and biomass (Fig. 4b), and differences were shown to be significant using ANOSIM (Table 10). On abundance data, pairwise ANOSIM comparisons were significant only between habitats C and R. Results for the other comparisons had probabilities not much higher than the 0.05 critical level. Although no formal statistical power calculations are possible with this method, the low number of plots (four) from each habitat serve as a reminder that the failure to detect differences does not mean that no difference exists. On biomass data, C was different from P and R, with these two habitats not shown to be different, although the above warning about power is again relevant.

***Fig 4 and Tables 10 and 11 about here

The total abundance of epifauna was greatest in habitat C, intermediate in P and lowest in R (Table 11). The most conspicuous taxon contributing to differences amongst assemblages was harpacticoids, which showed the same pattern as total epifaunal abundance. Amphipod numbers were lower in habitat R and not significantly different in C and P. Tanaid and polychaete numbers were higher in C than in R, and were intermediate in P but not significantly different from the other habitats. Porcellid, gastropod and bivalve abundances were not found to be different amongst habitats. Patterns for epifauna collected during the day were similar to those for epifauna collected at night, with total abundances and those for several important species decreasing from habitat C to P to R.

Total epifaunal biomass was greater in habitat C and not significantly different in P and R (Table 12). The same pattern was found for the biomass of harpacticoids and polychaetes. Amphipod and tanaid biomasses were greater in C than in R, and intermediate in P but not significantly different from the other habitats. Porcellid, gastropod and bivalve biomasses were not significantly different amongst habitats. As for epifauna collected at night, biomasses of epifauna collected during the day showed the same pattern as abundances but the trend from C to P to R was less obvious.

***Tables 12 and 13 about here

Estimates of total epifaunal production from daytime collections were higher in habitat C, and not significantly different between P and R (Table 13). Total crustacean production was highest in habitat C, intermediate in P, and lowest in R.

4. Abundance - Biomass relationships

At all locations the pattern of decreasing abundance from C to P to R was stronger than the pattern for biomass. This implies that the average weight of individual animals increased from C to P to R. Abundance - Biomass comparisons show a consistent trend at all locations of increasing dominance of larger animals from C to P to R (Fig. 5). The plots for Diana-Night and Diana-Day have a similar gap between abundance and biomass curves for each habitat. The curves for abundance and biomass at Embiez are further apart at each habitat than those from Diana.

***Fig 5 about here

5. Location differences

The two-dimensional ordination plots of assemblages based on abundance and biomass from all locations show strong grouping of habitats within locations, as expected from individual ordinations, but there is also an overriding separation of locations (Fig 6). In plots for both abundance and biomass,

habitat groups from Embiez are distinct but close together, and are all entirely separate from those of Diana. Diana-Night and Diana-Day positions overlap, but Diana-Night habitat groups, whilst distinct, are close together, whereas Diana-Day habitat groups are more widely spread. Although the spacing amongst habitat groups is different for Diana-Night and Day, the effect of partly cutting and removing seagrass was the same, with groups C, P and R positioned in that order along a straight line gradient. This gradient is also evident for Embiez habitat groups. Differences amongst habitats and locations were statistically significant (Table 14). ANOSIM comparisons between pairs of habitats were all significant but are not shown in Table 14 because they have been reported for each location separately. Pairwise comparisons amongst locations were all significant on both abundance and biomass data; that is, after taking into account habitat differences, assemblages from all three locations were significantly different from each other.

***Fig 6 and Tables 14 and 15 about here

Results of two-factor ANOVA tests on location and habitat for abundance and production data are presented in Table 15. Significant interaction was detected on abundance data, so that probabilities for main effects should be treated with caution. In this case, main effect probabilities are both highly significant and since interaction tends to diminish main effects, it can be concluded that habitats differed regardless of location and locations differed regardless of habitat. Given the interaction, however, Tukey's tests are best used to compare, for example, pairwise differences amongst locations separately for each habitat. Habitat differences are not shown because these have already been reported under separate location sections. Total abundances were higher at Diana-Night than at Embiez for each habitat. Diana-Day abundances were similar to those at Diana-Night in habitat C, but were similar to those at Embiez in habitat P, and even lower than those at Embiez in habitat R. The interaction term in the ANOVA test on production data was not significant but nearly so. As for abundance data, the main effects were highly significant indicating that habitat differences were obvious regardless of location and location differences were obvious regardless of habitat. Again it is informative to make pairwise Tukey comparisons of locations separately for each habitat. Total production was higher at Diana-Night than at Embiez for each habitat. Production at Diana-Day was similar to that at Diana-Night in habitat C, but was similar to that at Embiez in habitats P and R.

Discussion

The epifauna of the three experimental habitats was different whether measured as abundance, biomass or production. At each location, total epifaunal production within habitats declined in line with decreasing vegetation cover. Epifaunal assemblages differed amongst habitats, and the types of differences, but not their magnitudes, were consistent at the two sites and at night and day. At each location, the same taxa tended to be dominant numerically and by weight, although the importance of these taxa in distinguishing amongst habitats varied with location. These dominant taxa showed a very strong pattern of decreasing abundance and biomass from habitat C to P to R. As a result, the total abundance and biomass, and therefore estimates of production, declined from C to P to R.

The pattern of decline from C to P to R was less obvious on biomass data than on abundance data, and abundance - biomass comparisons highlighted a trend from more numerous, lighter animals in uncut plots to fewer, heavier animals in plots from which seagrass was removed. These dominance curves have been used to detect the effects of disturbance on marine benthic fauna (WARWICK, 1986) by comparing the relative positions of the two curves. According to disturbance and competition theory, the fauna at undisturbed sites should be dominated by fewer, heavier animals relative to the fauna at disturbed sites which should be characterised by a greater diversity of abundant, lighter animals (WARWICK, 1986). Although in the current study a special effort was made to inflict the same disturbance on all plots including those used as controls, it would not have been surprising if the abundance - biomass comparison method had indicated greater disturbance in habitats P and R, given that canopy was actually removed from these habitats. The indications from this method are, however, that at both sites and during both night and day, the fauna in control plots gave the strongest indication of being disturbed. Abundance - biomass comparisons can be influenced by major recruitment events but, in the current experiment, replication and interspersion of treatments and the running of the experiment at two sites make it unlikely that the observed pattern is due to chance recruitment in any

treatment. Heavier animals were not more abundant in habitats from which vegetation had been removed, rather the relative importance of heavier animals was greater. Heavier animals were perhaps less averse to the modified habitats than lighter animals. There are many plausible explanations for the increased importance of heavier animals in habitats from which vegetation had been removed. Predators may have removed animals differentially according to size, or the food resource available in modified habitats may have been more attractive to larger animals. The responses to reduced canopy could be instinctive selection of habitat. An alternative explanation for the increased dominance of larger animals with decreasing canopy cover is that heavier animals, either because of their weight or because they are more powerful swimmers, may have been less likely to be removed along with vegetation at the time of cutting. If smaller animals removed accidentally along with vegetation had not returned by the time epifauna was collected, then the relative importance of heavier animals would increase as the amount of vegetation removed increased. WARWICK (1986) also warns that whilst the theory behind the abundance-biomass comparisons is appropriate for macrofauna, it does not necessarily hold for meiofauna. The present study includes animals from the large end of the meiofauna range as well as macrofauna, and this may have affected the relationship between abundance and biomass.

The differences amongst habitats lay in the abundance or biomass of taxa, not in the presence or absence of taxa. This result may reflect the gross clumping of species and possibly functional groups into single, higher taxa, so that changes in the fauna at these levels would not have been detected. Nevertheless, WARWICK (1988) showed that multivariate analyses at family level reproduced very closely the results obtained at species level, and even analyses at the level of phylum generally agreed surprisingly well with those at lower taxonomic levels. WARWICK suggests that for some purposes analyses based on higher taxa may more closely reflect the information of interest than those based on species. In any case, the taxa used in this study are adequate when discussing differences in prey availability, at least for fish species that become less abundant in response to a reduction in seagrass canopy. Three species of fish increased in abundance in response to reduction in seagrass canopy in experiments in *Zostera capricorni* beds reported by BELL & WESTOBY (1986a). To examine the possibility that food availability is the direct cause of increases in abundances shown by some fish species, epifauna may need to be identified to lower levels, or into taxa representing groups of animals actually able to be caught by those fish species.

The epifauna of Embiez and Diana-Night were consistently different. Multivariate analysis showed no overlap of assemblages from the two sites. Epifaunal abundance, biomass and production were always higher at Diana in all habitats. The fauna from Embiez was more strongly characterised by numerous, lighter animals in all habitats. As explained above, this can signify effects of pollution or disturbance, raising the possibility that the fauna at Embiez is more affected by disturbance than the fauna at Diana. The Lagune du Brusc, a sheltered, shallow waterway between the mainland and the main island of Embiez, is the site of many human activities, such as water sports, boating, the harvesting of *Upogebia* sp., and at times pollution by sewage, and it would be no surprise if the fauna was grossly affected by these activities. As a comparison with Diana, however, the current study is confounded by time. Differences in the fauna could be attributable to changes in any number of environmental variables occurring during the ten day period between experiments.

Although the fauna at the two sites differed, the effects of partly cutting and totally removing the seagrass canopy were the same at both sites. Habitat groups in the ordination plots based on abundance and biomass were related in the same way at the two sites. The differences in abundance, biomass and production of habitats were also consistent at both sites.

The effects of partly cutting and removing seagrass were different on the night fauna and day fauna at Diana. Multivariate and univariate analyses on abundance and biomass show that modifying seagrass canopy had the same type of effect on day and night fauna, but that the magnitude of those effects differed with time of day. Habitat groups on the ordination plots for abundance and biomass of day fauna were more spread out than for night fauna. Abundance and production of day fauna matched that of night fauna in control plots, but were as low as or lower than that at Embiez in modified habitats. Plausible explanations for the greater reduction in fauna in modified habitats during the day include the possibility of an increase in predation during the day, or of instinctive selection of habitat with more canopy cover (behaviour for which the ultimate agent may have been increased risk of predation during the day). Reducing seagrass canopy may increase the number of invertebrates burrowing in sediment

during the day, thereby reducing the number in the epifauna. The effect of reducing seagrass canopy on the amount or quality of food for invertebrates may differ between night and day. Although only 12 hours elapsed between day and night sampling, comparisons between the night and day fauna are confounded by time. The different effects of modifying habitat between the day and night samples may be attributable to changes in environmental factors during that period.

The control and shortened canopy treatments in this study were similar to the control and shortened treatments shown by BELL & WESTOBY (1986a) to affect fish abundances, and the seagrass of their study (*Zostera capricorni*) is similar in height, width, density, and general form to the seagrass (*Cymodocea nodosa*) in the present study. Fish communities of south-eastern Australia are also similar to those of the Mediterranean region at familial level (POLLARD, 1984). Results presented here can therefore be sensibly combined with results from the fish studies of BELL & WESTOBY (1986a) to sharpen explanations of fish distributions.

Summary

The main aim of this study was to determine whether alterations to the seagrass canopy affected epifauna, the major dietary component of fish from shallow, sheltered embayments. At both sites, and at night and day at Diana, the abundance, biomass and production of epifauna were reduced in line with reduction in canopy height. The results of the present study are consistent with, although they do not alone demonstrate, the model in which fish abundances are explained by food availability.

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Table 1. List of taxa into which animals were grouped.

Abbreviations shown are those used in Tables 2,6,10 and 14.

	Crustacea		Non-crustacea
	Caridea	Pol	Polychaeta
Mys	Mysidacea	Gas	Gastropoda
Amp	Amphipoda - Gammaroidea	Biv	Bivalvia
	Amphipoda - Caprellidea		Ophiuroidea
Tan	Tanaidacea		Echinodermata, larvae
Iso	Isopoda	Ane	Actiniaria (Anemones)
	Cumacea		Chaetognatha
Har	Copepoda - Harpacticoida	Chi	Chironomidae, larvae
Por	Copepoda - Harpacticoida - Porcellidiidae		Ascidiacea, larvae
Cyc	Copepoda - Cyclopoida		
Cal	Copepoda - Calanoida		
	Copepoda - nauplii (unidentified)		
	Ostracoda		

Table 2. Results of ANOSIM comparisons amongst epifaunal assemblages from Embiez.

Global test is for any differences amongst habitats, and pairwise tests are for differences between pairs of habitats. Significance level for each comparison is 0.05 (ns = not significant). Contributing taxa are those making a consistently large contribution to differences between samples from the two habitats, listed in order of decreasing importance.

Variable	Global ANOSIM result	Pairwise ANOSIM results	Main contributing taxa
Abundance	< 0.001	C,P 0.017	Amp, Pol, Gas, Har
		C,R 0.004	Amp, Har, Pol
		P,R 0.009	Amp, Cal, Har, Mys
Biomass	0.001	C,P 0.1 ns	Pol, Har, Tan, Amp, Cal
		C,R 0.002	Har, Amp, Pol
		P,R 0.006	Amp, Har, Cal, Mys, Gas

Table 3. Abundances of total epifauna and key taxa in each habitat at Embiez.

Numbers are means with standard errors in parentheses. ANOVA results are probabilities; ns = not significant. Tukey's results show significant differences as letters not grouped by underlining: C = control, P = partly cut, R = removed.

	Control	Partly cut	Removed	ANOVA result	Tukey's results
All species combined	838 (45)	509 (43)	256 (34)	< 0.001	C P R
Amphipods	64 (8)	28 (2)	10 (2)	< 0.001	C P R
Tanaids	23 (6)	112 (3)	3 (1)	0.011	C̲ ̲P̲ ̲R̲
Harpacticoids	548 (35)	319 (34)	153 (20)	< 0.001	C P R
Calanoids	15 (4)	19 (3)	22 (9)	0.689 ns	
Mysids	8 (5)	3 (1)	3 (2)	0.358 ns	
Polychaetes	135 (14)	78 (6)	49 (7)	< 0.001	C̲ ̲P̲ R
Gastropods	15 (4)	4 (1)	3 (2)	0.046	

Table 4. Biomasses (AFDW in mg) of total epifauna and key taxa in each habitat at Embiez.

Numbers are means with standard errors in parentheses. ANOVA results are probabilities; ns = not significant. Tukey's results show significant differences as letters not grouped by underlining: C = control, P = partly cut, R = removed.

	Control	Partly cut	Removed	ANOVA result	Tukey's results
All species combined	13.4 (2.1)	8.9 (1.6)	4.3 (0.9)	0.004	<u>C P R</u>
Amphipods	4.2 (0.8)	2.2 (0.5)	0.3 (0.1)	< 0.001	<u>C P</u> R
Tanaids	1.9 (0.6)	0.8 (0.3)	0.3 (0.1)	0.051 ns	
Harpacticoids	2.1 (0.2)	1.3 (0.3)	0.5 (0.1)	< 0.001	C P R
Calanoids	0.1 (0.0)	0.1 (0.0)	0.2 (0.1)	0.431 ns	
Mysids	0.3 (0.1)	0.5 (0.3)	0.1 (0.1)	0.296 ns	
Polychaetes	2.5 (0.3)	1.0 (0.1)	0.6 (0.1)	< 0.001	C <u>P R</u>
Gastropods	0.4 (0.1)	1.2 (0.5)	0.1 (0.0)	0.059 ns	

Table 5. Total epifaunal production and total crustacean production (μg/day/0.0625m^2) in each habitat at Embiez.

Numbers are means with standard errors in parentheses. ANOVA results are probabilities. Tukey's results show significant differences as letters not grouped by underlining: C = control, P = partly cut, R = removed.

	Control	Partly cut	Removed	ANOVA result	Tukey's results
All species combined	221 (31)	158 (24)	86 (15)	0.004	<u>C P R</u>
All crustaceans combined	171 (24)	106 (15)	37 (6)	< 0.001	<u>C P</u> R

Table 6. Results of ANOSIM comparisons amongst epifaunal assemblages from Diana-Night.

Global test is for any differences amongst habitats, and pairwise tests are for differences between pairs of habitats. Significance level for each comparison is 0.05 (ns = not significant). Contributing taxa are those making a consistently large contribution to differences between samples from the two habitats, listed in order of decreasing importance.

Variable	Global ANOSIM result	Pairwise ANOSIM results	Main contributing taxa
Abundance	< 0.001	C,P 0.002	Har, Tan, Pol, Gas, Amp
		C,R 0.002	Gas, Iso, Cal, Har
		P,R 0.002	Gas, Amp, Tan, Cal, Har
Biomass	< 0.001	C,P 0.002	Har, Chi, Ane
		C,R 0.002	Gas, Har, Iso, Cal
		P,R 0.002	Gas, Cal, Tan, Amp

Table 7. Abundances of total epifauna and key taxa in each habitat at Diana-Night.

Numbers are means with standard errors in parentheses. ANOVA results are probabilities; ns = not significant. Tukey's results show significant differences as letters not grouped by underlining: C = control, P = partly cut, R = removed.

	Control	Partly cut	Removed	ANOVA result	Tukey's results
All species combined	2554 (314)	912 (57)	521 (59)	< 0.001	C P R
Amphipods	187 (22)	86 (10)	30 (4)	< 0.001	C P R
Tanaids	197 (41)	49 (6)	15 (2)	< 0.001	C P R
Isopods	2 (1)	1 (0)	0 (0)	0.003	C <u>P R</u>
Harpacticoids	1395 (117)	537 (28)	313 (30)	< 0.001	C P R
Calanoids	16 (4)	5 (2)	0 (0)	< 0.001	C P R
Polychaetes	569 (135)	140 (13)	78 (9)	< 0.001	C P R
Gastropods	11 (2)	3 (1)	0 (0)	< 0.001	C P R
Anemones	2 (1)	0 (0)	0 (0)	0.045	<u>C P R</u>
Chironomids	29 (14)	1 (0)	1 (1)	< 0.001	C <u>P R</u>

Table 8. Biomasses (AFDW in mg) of total epifauna and key taxa in each habitat at Diana-Night.

Numbers are means with standard errors in parentheses. ANOVA results are probabilities; ns = not significant. Tukey's results show significant differences as letters not grouped by underlining: C = control, P = partly cut, R = removed.

	Control	Partly cut	Removed	ANOVA result	Tukey's results
All species combined	67.9 (16.7)	31.3 (5.4)	18.4 (3.9)	0.002	C P R
Amphipods	16.9 (2.9)	7.8 (1.3)	2.9 (0.5)	< 0.001	C P R
Tanaids	11.4 (4.1)	3.3 (0.2)	1.0 (0.5)	< 0.001	C P R
Isopods	0.8 (0.3)	0.1 (0.1)	0.0 (0.0)	0.003	C P R
Harpacticoids	3.8 (0.4)	1.5 (0.1)	0.8 (0.1)	< 0.001	C P R
Calanoids	0.1 (0.0)	0.0 (0.0)	0.0 (0.0)	0.058 ns	
Polychaetes	12.8 (4.0)	3.7 (0.8)	3.0 (0.6)	0.001	C P R
Gastropods	4.8 (1.1)	1.5 (0.5)	0.0 (0.0)	< 0.001	C P R
Anemones	1.5 (0.3)	0.0 (0.0)	0.1 (0.1)	< 0.001	C P R
Chironomids	0.8 (0.5)	0.0 (0.0)	0.0 (0.0)	0.028	C P R

Table 9. Total epifaunal production and total crustacean production (μg/day/0.0625m^2) in each habitat at Diana-Night.

Numbers are means with standard errors in parentheses. ANOVA results are probabilities. Tukey's results show significant differences as letters not grouped by underlining: C = control, P = partly cut, R = removed.

	Control	Partly cut	Removed	ANOVA result	Tukey's results
All species combined	812 (151)	434 (55)	277 (51)	0.002	C P R
All crustaceans combined	568 (101)	347 (42)	226 (42)	0.006	C P R

Table 10. Results of ANOSIM comparisons amongst epifaunal assemblages from Diana-Day.

Global test is for any differences amongst habitats, and pairwise tests are for differences between pairs of habitats. Significance level for each comparison is 0.05 (ns = not significant). Contributing taxa are those making a consistently large contribution to differences between samples from the two habitats, listed in order of decreasing importance.

Variable	Global ANOSIM result	Pairwise ANOSIM results	Main contributing taxa
Abundance	0.001	C,P 0.086 ns	Har, Tan, Pol
		C,R 0.029	Har, Amp, Pol
		P,R 0.057 ns	Biv, Amp, Pol
Biomass	0.001	C,P 0.029	Tan, Har, Biv
		C,R 0.029	Har, Biv, Pol, Gas
		P,R 0.086 ns	Pol, Por, Har

Table 11. Abundances of total epifauna and key taxa in each habitat at Diana-Day.

Numbers are means with standard errors in parentheses. ANOVA results are probabilities; ns = not significant. Tukey's results show significant differences as letters not grouped by underlining: C = control, P = partly cut, R = removed.

	Control	Partly cut	Removed	ANOVA result	Tukey's results
All species combined	2308 (625)	451 (87)	143 (20)	< 0.001	C P R
Amphipods	141 (65)	49 (16)	3 (2)	0.001	<u>C P</u> R
Tanaids	64 (28)	10 (2)	2 (2)	0.028	<u>C P R</u>
Harpacticoids	1414 (304)	248 (42)	95 (15)	< 0.001	C P R
Porcellids	52 (27)	35 (15)	6 (3)	0.493 ns	
Polychaetes	391 (149)	73 (24)	19 (3)	0.006	<u>C P</u> R
Gastropods	27 (15)	3 (3)	2 (2)	0.056 ns	
Bivalves	34 (30)	11 (5)	1 (1)	0.131 ns	

Table 12. Biomass (AFDW in mg) of total epifauna and key taxa in each habitat at Diana-Day.

Numbers are means with standard errors in parentheses. ANOVA results are probabilities; ns = not significant. Tukey's results show significant differences as letters not grouped by underlining: C = control, P = partly cut, R = removed.

	Control	Partly cut	Removed	ANOVA result	Tukey's results
All species combined	44.9 (11.2)	11.0 (2.4)	3.6 (1.2)	0.001	C P R
Amphipods	15.4 (7.9)	3.1 (1.2)	0.4 (0.2)	0.025	C P R
Tanaids	3.2 (1.1)	0.3 (0.1)	0.2 (0.1)	0.022	C P R
Harpacticoids	5.4 (1.4)	0.8 (0.2)	0.2 (0.1)	< 0.001	C P R
Porcellids	0.2 (0.1)	0.2 (0.1)	0.0 (0.0)	0.194 ns	
Polychaetes	6.4 (2.2)	1.0 (0.2)	0.2 (0.1)	0.007	C P R
Gastropods	2.6 (1.1)	1.7 (1.2)	0.0 (0.0)	0.115 ns	
Bivalves	0.5 (0.1)	0.3 (0.3)	0.0 (0.0)	0.124 ns	

Table 13. Total epifaunal production and total crustacean production (μg/day/0.0625m^2) in each habitat at Diana-Day.

Numbers are means with standard errors in parentheses. ANOVA results are probabilities. Tukey's results show significant differences as letters not grouped by underlining: C = control, P = partly cut, R = removed.

	Control	Partly cut	Removed	ANOVA result	Tukey's results
All species combined	589 (121)	188 (36)	71 (21)	0.001	C P R
All crustaceans combined	478 (109)	136 (38)	44 (24)	0.001	C P R

Table 14. Results of two-way crossed ANOSIM comparisons amongst epifaunal assemblages of locations and habitats.

Global test is for differences amongst habitats or locations, as specified. Pairwise tests for habitats were all significant but are not shown. Pairwise tests amongst locations are shown. Significance level for each comparison is 0.05. Contributing taxa are those making a consistently large contribution to differences between samples from the two habitats, listed in order of decreasing importance.

Variable	Global ANOSIM result	Pairwise ANOSIM results for location	Main contributing taxa
Abundance	Habitat < 0.001 Location < 0.001	EM,DN < 0.001 EM,DD < 0.001 DN,DD < 0.001	Por, Biv, Mys Cal, Cyc, Pol, Har Cyc, Pol, Har
Biomass	Habitat < 0.001 Location < 0.001	EM,DN < 0.001 EM,DD < 0.001 DN,DD < 0.001	Por, Mys, Biv, Gas, Pol Cal, Cyc, Mys, Pol, Har, Gas Cyc, Har, Pol

Table 15. Total epifaunal abundance and production comparisons across locations.

Results shown as probabilities for two factors and interaction term in two-way ANOVA. Tukey's results for Location only, separately for each Habitat, show significant differences as letter not grouped by underlining. EM = Embiez, DN = Diana-Night, DD = Diana-Day.

	Term	ANOVA result	Habitat	Tukey's results for Location comparisons
Abundance	Habitat	< 0.001	C	<u>DN DD</u> EM
	Location	< 0.001	P	DN <u>EM DD</u>
	Interaction	< 0.001	R	DN EM DD
Production	Habitat	< 0.001	C	<u>DN DD</u> EM
	Location	< 0.001	P	DN <u>DD EM</u>
	Interaction	0.049	R	DN <u>EM DD</u>

Figure captions

Fig. 1. Map showing location of sites.

Fig. 2. Two-dimensional MDS ordination plots of epifaunal assemblages from Ile des Embiez based on a) abundance (stress = 0.146) and b) biomass (0.138) data. C = control; P = partly cut; R = removed.

Fig. 3. Two-dimensional MDS ordination plots of epifaunal assemblages from Etang de Diana-Night based on a) abundance (stress = 0.068) and b) biomass (0.073) data. C = control; P = partly cut; R = removed.

Fig. 4. Two-dimensional MDS ordination plots of epifaunal assemblages from Etang de Diana-Day based on a) abundance (stress = 0.073) and b) biomass (0.042) data. C = control; P = partly cut; R = removed.

Fig. 5. Cumulative dominance plots of abundance - biomass from each site and treatment. □ = abundance; ● = biomass.

Fig. 6. Two-dimensional MDS ordination plots of epifaunal assemblages from all locations based on a) abundance (stress = 0.123) and b) biomass (0.124) data. C = control; P = partly cut; R = removed.
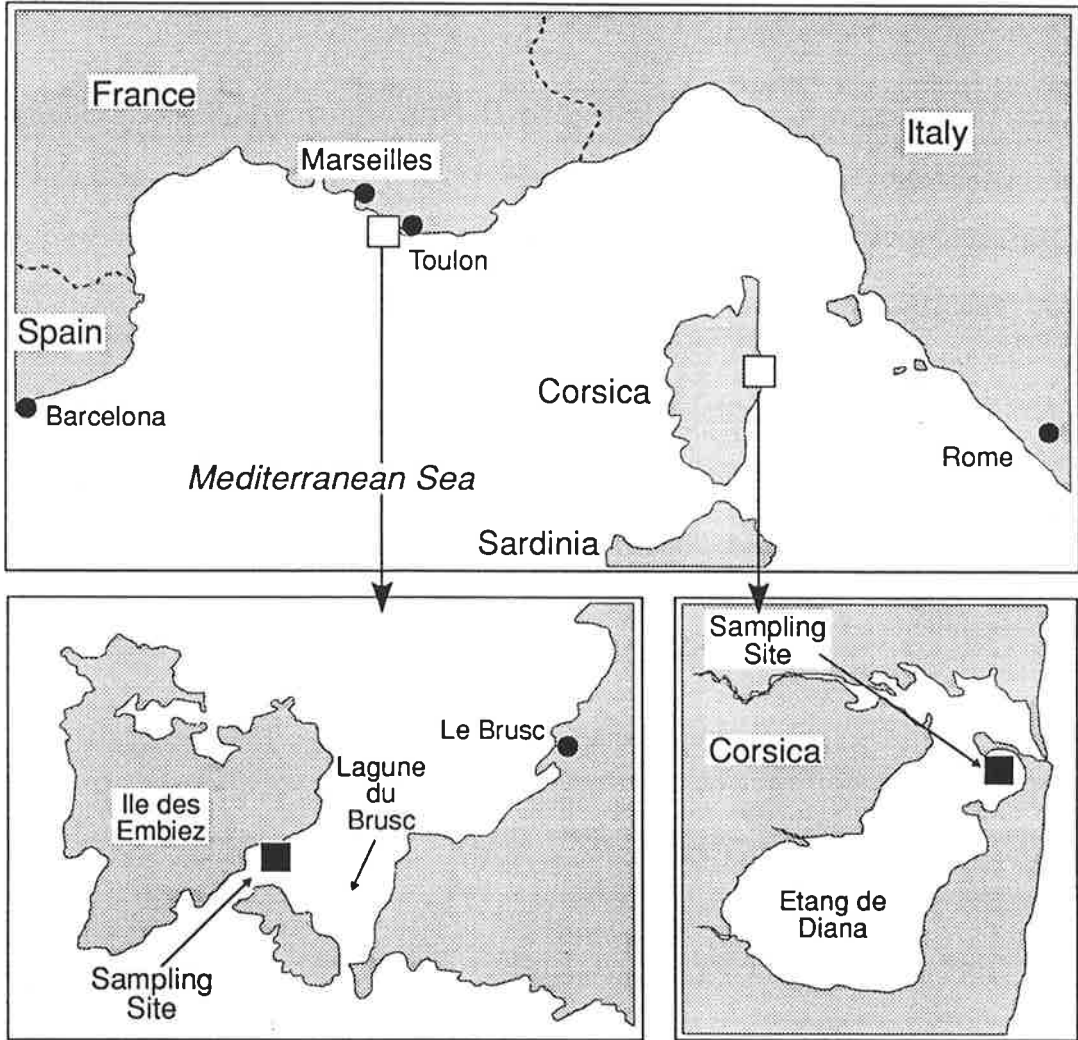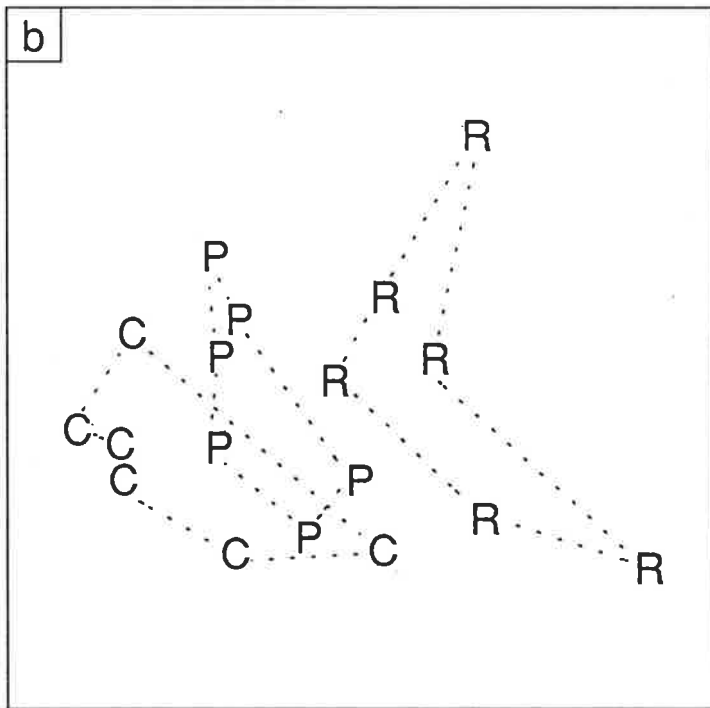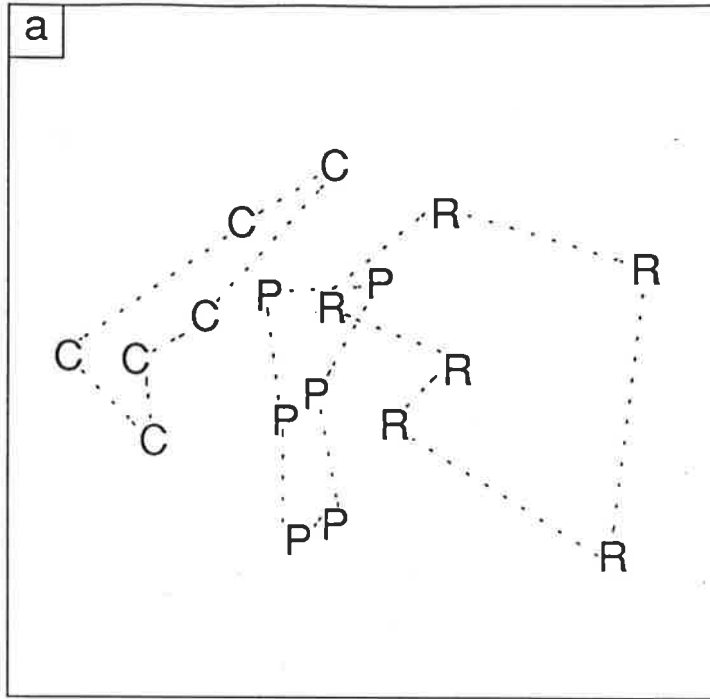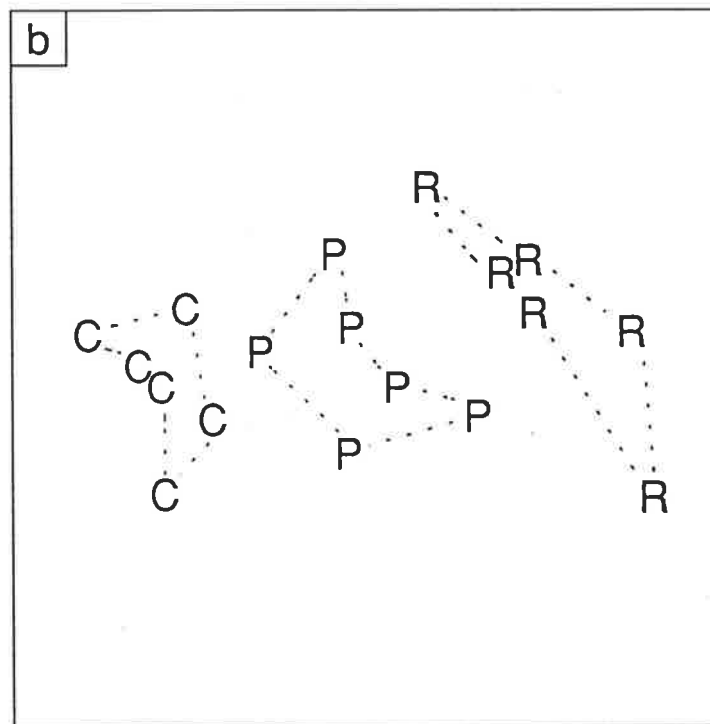
France

Marseilles

Toulon

Spain

Barcelona

Mediterranean Sea

Italy

Corsica

Rome

Sardinia

Le Brusc

Lagune
du
Brusc

Ile des
Embiez

Sampling
Site

Sampling
Site

Corsica

Etang de
Diana

Fig. 1

Fig. 2
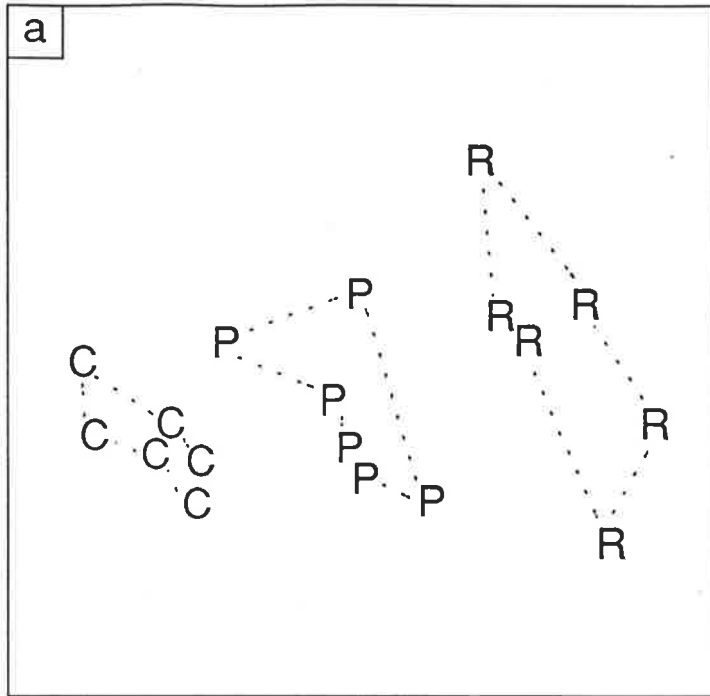
Fig. 3
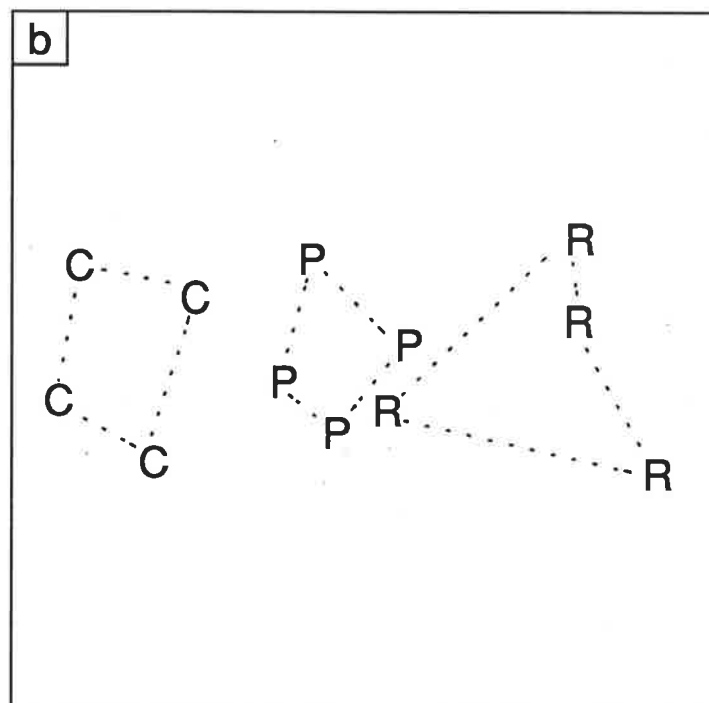
Fig. 4

Fig. 5

Fig. 6

Connolly, R. (1994) The role of seagrass as preferred habitat for juvenile *Sillaginodes punctata* (Cuv. & Val.) (Sillaginidae, Picses): habitat selection or feeding?
Journal of Experimental Marine Biology and Ecology, v. 180(1), pp. 39-47

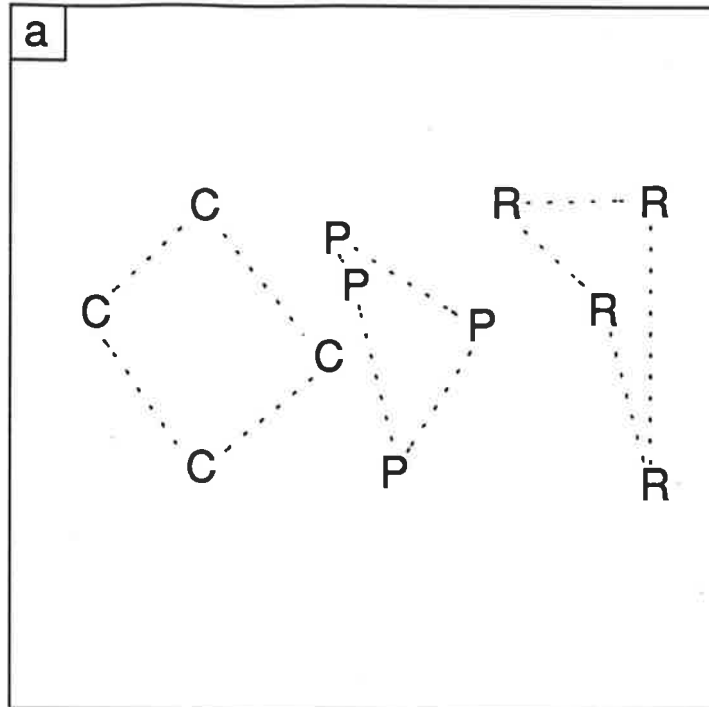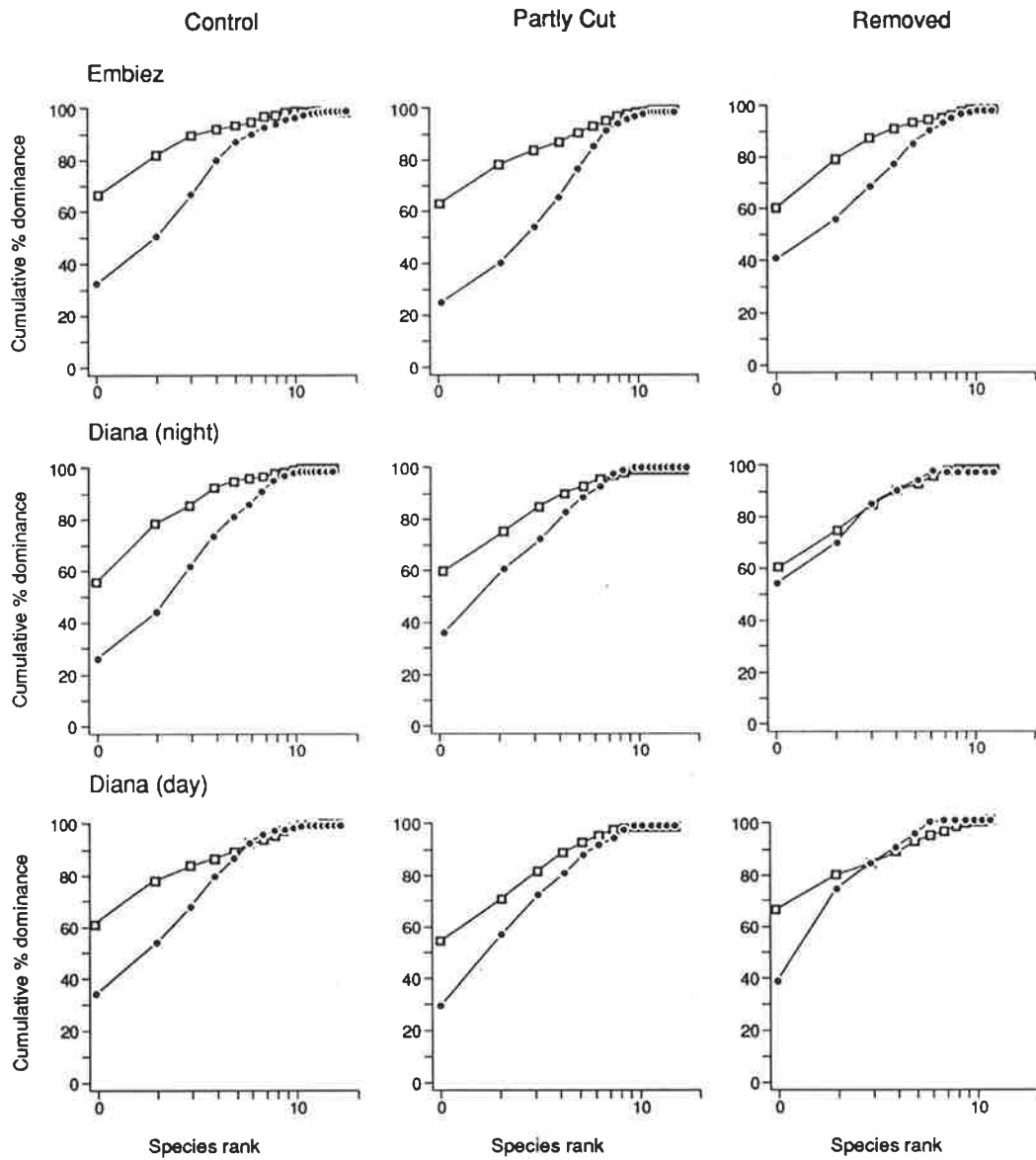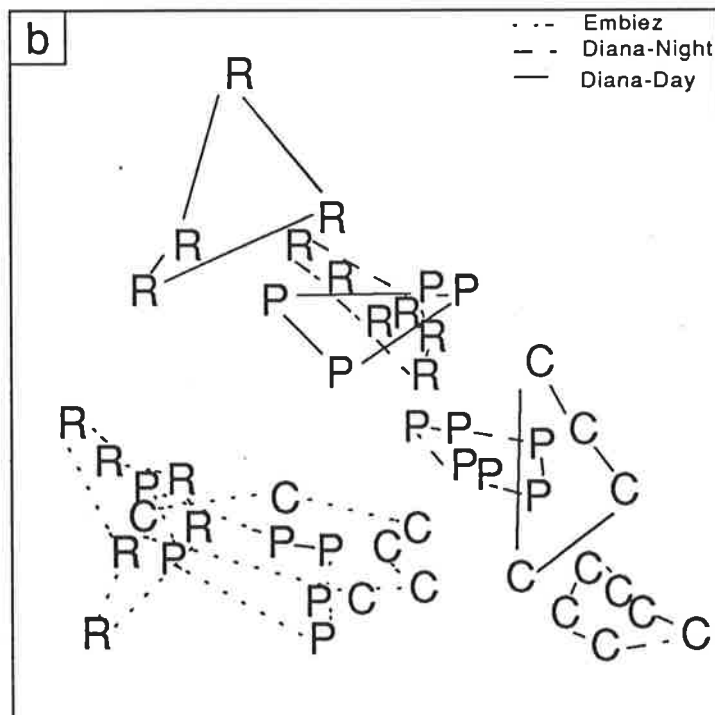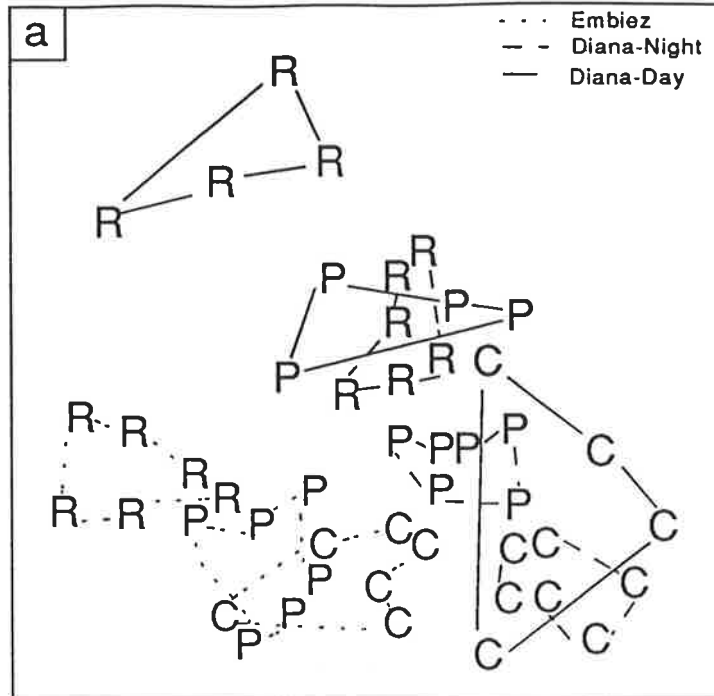# The ratio of the probabilities of Type I and Type II errors: what to set, what to forget

Rod M Connolly

Abstract

Thinking about the consequences of Type I (α) and Type II (β) errors in tests of significance is important and is becoming part of accepted experimental practice in ecology and environmental science. The ratio α:β should reflect the relative seriousness of the consequences of the two error types. The usual statistical procedure of fixing α inviolably prior to an experiment results in an actual α:β ratio different (sometimes very different) from that wanted by the investigator. The α:β ratio, not the α level, should be set prior to an experiment and maintained throughout. The α level for the test of significance is determined after the experiment but before testing. The variability of data from the experiment is the best estimate of population variability. The number of useful replicates in the experiment is known exactly. The relationship between α and β is then solved to determine the α value that results in maintenance of the required α:β ratio. Setting the α:β ratio should be standard practice for scientists, but there are few guides about which factors to consider.

Introduction

Ecological and environmental questions are answered by calculating a statistic from sampling observations and comparing this with a theoretical distribution. A decision is made either to reject the null hypothesis -- with a certain probability α of doing so incorrectly (Type I error) -- or to accept it -- with a probability β of being wrong (Type II error).

Whereas biologists used to be concerned almost solely with α, they are now encouraged to consider β also. Statistical power is the chance of correctly rejecting the null hypothesis and is the complement of β ($= 1 - \beta$). The importance of power in both the planning stage and in reporting results has been convincingly demonstrated (e.g. Toft & Shea 1983; Peterman 1990; Fairweather 1991). Prior to an experiment the sample size needed to give an acceptable power for a specified effect should be calculated. When reporting the results of an experiment in which the null hypothesis was accepted, the power to detect an effect (of a specified size) should be stated so that readers can decide with what confidence they view the result. When a null hypothesis is accepted

(or, more informatively, is not rejected (Peterman 1990)) and power is low, only weak conclusions can be drawn. Surveys and manipulative experiments in ecology and environmental science often *do* have low power because sample sizes are small and variability (both natural and that due to sampling) is high. For this reason, and also because the consequences of making a Type II error can be great relative to the consequences of a Type I error, ß must be considered. Attending to ß (and thus power) results in better decisions in tests of hypotheses and increases the efficiency and usefulness of research.

My aim is to further improve hypothesis testing by proposing a development in experimental procedure; namely that, prior to an experiment, the α:ß ratio -- not the α level -- should be fixed, along with the minimum effect size of interest.

The account of ß and power above merely introduces the context within which the procedural improvements are proposed. Readers wanting a thorough grounding can read Andrew and Mapstone (1987) on power and other design considerations, Peterman (1990; and references therein) on power generally, and volumes such as Cohen (1988) and Winer (1971) for computational details. Fairweather (1991) lists up-to-date references.

To make the proposed improvement clear, the basic procedural steps of current experimental practice are outlined first. The problem is then exposed, and the solution given as amended procedural steps. The procedure appropriate when no pilot study has been done follows. Finally, setting α:ß ratios is discussed.

Current Experimental Practice

The following six steps are intended as a guide to current *statistical procedure* for research well done. There are many critical points in design and execution of experiments not mentioned here; see, for example, Green (1979), Hurlbert (1984), Andrew and Mapstone (1987), Hairston (1989) and Underwood (1990).
In working through the six steps below, I will use as an example my comparison of the capture success of two netting methods on small fish in intertidal seagrass meadows. The two methods, described below, are aimed at catching all small fish (< 10 cm) over seagrass-covered mudflats when the water is between 40 and 100 cm deep.

1) Seine Net. A 5 m long seine net of 1 mm mesh is pulled by two people for 20 m, netting a total of 84 m^2 (s.e. = 1.19). This method is easily learned, quick and cheap, but the seine net probably does not catch all fish. I have used this method in surveys which are part of research into the importance of seagrass to small fish.

2) Pop-up Net. A 5 x 5 m buoyant pop-up net (similar to that in Larson *et al.* (1986) but with no 'floor') is set at low tide and released by remote control one day later, ensnaring all fish over the 25 m^2 area. Fish are collected by passing a 5 x 1 m long rigid-framed net through the area three times. This method is expensive, time-consuming and difficult to operate, and needs four people at each release, but seems to catch all fish.

The experiment was planned as a paired comparison of the two methods using catches standardized to fish/m^2.

Step 1. Pilot Study

An estimate of variability and an approximate guide to scale are needed for later steps; these are obtained from the pilot study, or from another source such as a similar experiment described in the literature.

For the paired analysis appropriate to the comparison of netting methods, I used an estimate of the variability of *differences* between (paired) pop-up and seine catches. Four nettings of each type were done two months prior to the main experiment along the same 1 km stretch of coast. Results are in Table 1.

There are many other important benefits gained from a pilot study, such as information about the accuracy and precision of methods and time and money costs per replicate; see Green (1979), Underwood (1981) and Andrew and Mapstone (1987).

Step 2. Specify Minimum Effect Size

The researcher must specify the minimum effect size of interest so that ß can later be calculated. The lack of guidance for setting effect sizes and the sensitivity of power calculations to differences in effect size have been pointed out by Rotenberry and Wiens (1985). Notwithstanding these difficulties, the minimum effect size should be specified with the individual circumstances of the research and the researcher in mind.
For the paired comparison of netting methods, effect size is expressed as a mean *difference*. The difference of interest can be specified as a proportion of the catch in pop-up nets (which give, I think, the more accurate fish densities). Taking into account 1) the high variability of fish numbers at the scale of the study and 2) the relative crudeness of the questions being asked about fish in seagrass that surveys using seines are intended to answer but also 3) the need to convince colleagues of the usefulness of the seine method, I was interested in detecting a mean difference between seine and pop-up densities of 20% or more of the pop-up mean: 0.2 x 3.80 = 0.76 fish/m^2. The alternative hypothesis was non-directional, requiring a 2-tailed test.

Step 3. Set α and ß

Ideally, the desired probabilities of making Type I and Type II errors should be set so that the ratio α:ß reflects the *relative* seriousness of the consequences of the error types (Toft & Shea 1983; Peterman 1990). In practice, conventional levels of, for example, α = 0.05 and ß = 0.2 are often used, but this is unsatisfactory (see later section "Setting α:ß Ratios"). It is always desirable to keep both α and ß as small as possible but since to do so the sample size must be increased, a compromise will always be needed to keep a practicable sample size. Judging consequences of the two error types is an unfamiliar pastime for biologists and may seem hazardous; there is little guidance in the literature.

I consider that the main purpose of my comparison of the pop-up and seine methods was to gauge the effectiveness of the seine, a quick approximate method which I had

used in several surveys over the previous two years, against the pop-up net, which had been designed to catch fish very accurately but only with great effort. Rejection of the null hypothesis that both methods caught similar numbers of fish would, depending on which method caught fewer fish, reduce my confidence either in results of the surveys using the seine, or in the usefulness of the pop-up net for future experiments. If the null hypothesis was actually true and a Type I error had been made, then the doubt over the usefulness of either the survey results or future use of the pop-up net would be unnecessary.

If the null hypothesis was not rejected, it could be concluded that fish numbers from surveys using the seine are as accurate as numbers from pop-up nets. In drawing this positive conclusion (going ahead and believing the survey results) from a negative result (failure to reject the null hypothesis), it is particularly important to have only a small chance of wrongly retaining the null hypothesis (making a Type II error). Keeping ß low (power high) in this experiment guards against "encouraging" the more palatable result, namely, retention of the null hypothesis.

In trying to judge the relative consequences of the two error types, I consider that the main points are:
1) that falsely rejecting the null hypothesis casts unnecessary doubt on the accuracy of either the seine catches and therefore also on part of a study about the importance of conserving shallow seagrass beds, or the pop-up catches and therefore on the suitability of these for future research, and 2) that falsely retaining the null hypothesis may wrongly encourage the results of surveys using the seine to be considered more reliable than they really are, and may cause inappropriate amendments to models about fish and seagrass.

Both consequences are equally unappealing, and therefore imply an α:ß ratio of 1:1. The actual values of α and ß are a compromise between levels low enough to convince readers that results are meaningful and levels high enough to be practicable in the circumstances. Aiming for the lowest practicable levels of α and ß, taking into account the cost and effort involved in using pop-up nets and the high variability of fish densities, I believe that $\alpha = ß = 0.05$ is appropriate.

Step 4. Calculate Required Sample Size

For the chosen α (Step 3) and effect size (Step 2) and using the estimate of variability (Step 1), the sample size needed to give the required ß is calculated.

In the present example, the values were: $\alpha = 0.05$, ß $= 0.05$, effect size $= 0.76$ fish/m^2 and standard deviation of differences $= 0.80$ fish/m^2.

The required sample size is 17 pairs of nets, calculated using either Dallal's (1987) program or Equation 8.8 from Zar (1984).

Step 5. Do the Experiment

A rather important step. Be vigilant. If possible a larger sample size than that suggested in Step 4 should be used to cover for replicates that may in some way be rendered useless. It was a struggle, however, to use 17 pairs in the comparison of seines with pop-ups and I was unable to increase the sample size.

Step 6. Analyse Results and Draw Conclusions

Interpreting results of ecological experiments nearly always requires statistical tests. In deciding whether to reject the null hypothesis, the α level decided upon in Step 3 is used. It is advocated (Toft & Shea 1983; Rotenberry & Wiens 1985; Peterman 1990; Fairweather 1991), but still not routine practice in published papers, that when the null hypothesis is *not* rejected, then either the power of the test to detect the minimum effect specified in Step 2, or the minimum effect that could have been detected at the β level specified in Step 3 should be calculated.

What is wrong with current practice?

The usual practice described above fixes the α level inviolably prior to the main experiment. This means that in the test of the main experiment, for one or both of the following reasons, the actual β level -- and thus the $\alpha{:}\beta$ ratio -- is *not* that decided upon in Step 3.

1) Variability estimated from the pilot study (Step 1) differs from that actually occurring in the main experiment.

2) The sample size suggested in Step 4 and the number of useful replicates in the main experiment are different.

Differing Variability

The variability of the pilot study used to calculate the sample size required for the main study may differ, sometimes markedly, from the variability actually found in the main study because the studies are separated spatially and/or temporally. This remains true even in the most useful pilot studies, which are relatively close spatially and temporally to the main study. Variability in a pilot study may also differ from that in the main study if the sample size of the pilot study is much smaller (usually the case), giving a less reliable estimate of the population variability (Fairweather 1991).

The pilot study for the comparison of netting methods took place on the same stretch of coast as the main study, on similar tides, but was done two months earlier and had only four replicate pairs. The standard deviation of the differences in the main study was 2.10 fish/m^2, compared with 0.80 fish/m^2 in the pilot study --that is an increase of about 2.6 times, which would have dictated six times as many replicates (N = 102) to achieve the desired power.

Differing Sample Size

The intended sample size may differ from the actual number of useful replicates in the main study because of losses in the field due to, for example, weather damage or vandalism, or losses in the laboratory due to blunders in handling or counting.

It is good practice to use replicates additional to the number suggested in Step 4, but this still does not ensure that the final number of useful replicates will be as required. In comparing the two netting methods, I was only able to use the sample size of 17 suggested in Step 4 (I should have striven for more, perhaps 20) and, in this case, good fortune reigned and the useful sample size was indeed 17.

Example of the Problem

The determination, *post hoc*, of ß for the comparison of netting methods provides a striking example of the problem with maintaining the α level fixed at Step 3. Given $\alpha = 0.05$ (as set in Step 3), sample size = 17, standard deviation of differences = 2.1 and effect size = 1.26 (calculation of this value will be explained in revised Step 6 below), ß for the paired t-test is 0.36 (chance of detecting the effect size (power) = 0.64). That gives an α:ß ratio of 0.05:0.36, meaning that a Type II error was about seven times as likely as a Type I error, even though I had decided in advance that their consequences were equally serious.

A Better Way

It is the desired α:ß ratio, not the α level, that should be preserved against the changing statistical scene. To achieve this, follow steps 1 - 5 above, and then continue with Steps 6 and 7 below. Be sure to slip your friend (better, *not* a friend) the α:ß *ratio* of Step 3 for impartiality.

Step 6. Determine Required α

Prior to doing the statistical test, calculate the variability in the main study. (When using computer programs, the variability is often shown as output along with the observed test statistic. Note the variability but, at this stage, do nothing with the test statistic.) Register also the sample size used. Along with the effect size specified in Step 2, this is all the information needed to calculate ß, given any α(see below).

If the effect size used to calculate the required sample size was originally determined as some proportional effect, that proportion (rather than the specific *magnitude*) can be used again now. This procedure results in an effect size of different magnitude but with the same biological meaning intended in Step 2.

Using these values of variability, effect size and sample size, find the α level that results in a ß level such that the α:ß ratio of Step 3 is maintained. In effect, the inverse mathematical relationship should be solved for the desired α:ß ratio. In practice, it is

easier to seed the equation being used to calculate ß with any αvalue and then, in iterative steps, alter α until the resultant ß value gives the desired α:ß ratio. This process takes just a few minutes whether done by hand calculation or computer program. The same result can be achieved by graphing ß against α. First the line ß = kα is drawn (where k gives the desired α:ß ratio) and then ß is found for enough α values to plot the curvilinear relationship. The intersection shows the desired values of α and ß (Fig. 1). The α level thus determined is now used in the test of significance.

In the comparison of netting methods, the standard deviation of the differences was 2.1 fish/m^2, and the sample size was 17. The effect size chosen for the net comparison was a mean difference of 0.2 of the mean fish density using pop-up nets. In Step 2, that resulted in an effect size of 0.2 x 3.80 (mean from pilot study) = 0.76, but now gives an effect size 0.2 x 6.32 (mean from main study) = 1.26.

The relationship between α and ß in the paired t-test comparing netting methods is shown in Fig. 1. The value giving the desired α:ß ratio of 1:1 (determined iteratively) is α = ß = 0.1605.

Step 7. Analyse Results and Draw Conclusions

Using the α level determined in the previous step, test the data from the main study for significance.

For the paired t-test comparing netting methods (see Table 1), t = 9.28, df = 16, p = 7.7 x 10^{-8}. Since α = 0.1605, the null hypothesis of no difference is rejected; it is concluded that the seine method caught fewer fish/m^2. Use of the paired t-test assumes that differences come from a normally distributed population of differences (Zar 1984). The distribution of differences in the present example (Fig. 2) is almost symmetrical (g_1 = 0.30) but is platykurtic (g_2 = -1.22). With a sample size of 17, however, this degree of departure from normality has no non-trivial effect on either α (Ratcliffe 1968) or ß (Srivastava 1958).

Procedure When No Pilot Study Has Been Done

A researcher without information about variability (from a pilot study or another source) has no reasonable way of selecting a sample size and usually will have an increased chance of making a Type I or II error. This weakened position should be avoided but is frequently encountered when tests are done on what are pilot data themselves. When the information needed to calculate the required sample size is not available prior to an experiment, the usual procedure described above should be used, but with the following differences.

1) It may be more difficult to specify the smallest effect of interest (Step 2) without the approximate guide to scale provided by a pilot study. A good guess is necessary, as it is when choosing a sample size.

2) The α:ß ratio must be set prior to the experiment (Step 3) but there is no need to specify actual values of α and ß, since these would only be used to calculate the

required sample size and this calculation is impossible because an estimate of variability is lacking.

Setting α:ß Ratios

Judging the Consequences

The point of this paper is not to suggest that ecologists should consider their α:ß ratio. *They should already be doing that.* However, since the importance of the α:ß ratio has been emphasised, some discussion is necessary.

Both α and ß are probabilities of making mistakes so we want them both to be small. We achieve that by having large sample sizes (relative to the variability of the data). α and ß are inversely related; like Scylla and Charybdis, the further you sail from one the more likely you are to founder on the other.

The ratio of α:ß should imply the relative seriousness of the consequences of the two error types. In ecology, the call to contemplate the consequences of the errors has become strident over the last decade (Toft & Shea 1983; Peterman 1990) but there are few guides on what factors to consider in judging the consequences. Biometry texts generally give a poor account, and there are no thorough reviews as yet. A brief introduction is offered in an outline of major philosophical positions by Shrader-Frechette and McCoy (1992; and see references therein), and some specific examples are discussed by Simberloff (1990).

There is a notable focus in ecological literature on situations where failure to reject the null hypothesis leads to positive conclusions (Toft & Shea 1983). The case of comparing two netting methods is an example of this; failure to reject the null hypothesis would encourage the belief that the seine method was as accurate as the pop-up method. The consequences of wrongly making these "positive" conclusions are relatively serious and it is important to keep ß low (power high) in these situations (Toft & Shea 1983; Hayes 1987). Fairweather (1991) gives instructive catchphrases for environmental impact work: Type I errors are false alarms and Type II errors give a false sense of security. Although Type I errors tend to burden developers unnecessarily with additional costs or impediments they may not be serious in the longer term. Type II errors probably lead to some (possibly detrimental) impact going unnoticed, so that remedial action is not taken when it should be; this can have long-lasting repercussions for the environment and can be very expensive for the community (Fairweather 1991; Peterman & M'Gonigle 1992). Again, it is important to keep ß low.

There is a nascent convention that, along with α = 0.05, has ß = 0.2 (Cohen 1988; Dallal 1987). An investigator using this ratio implies that the consequences of a Type I error are four times worse than the consequences of a Type II error (Cohen 1988). In ecology and environmental science at least, this ratio has no compelling generality; it should be ignored, and instead the ratio should reflect the individual circumstances of each test.

Practical Problems

Tables for calculating ß (or power) usually have the full range of ß values but a very limited range of α values. This impedes the selection of α:ß ratios, and is unhelpful in the critical step of this paper (Step 6 in "A Better Way") in which an αvalue must be selected such that the desired α:ß ratio is maintained. Cohen's (1988) tables are limited to α values of 0.01, 0.05 and 0.1 for most tests. For analysis of variance, Pearson & Hartley (1976) and Winer (1971) have figures with α values of 0.01 and 0.05, but Odeh & Fox (1975) give a better range, providing values of 0.001, 0.005, 0.01, 0.025, 0.05, 0.1, 0.25 and 0.5. The graphic approach to finding the desired α and ß values (Fig. 1) facilitates interpolation between the limited values in tables, and computer programs (see Goldstein (1989)) are another flexible alternative, permitting calculation of ß for any α.

If the α level determined in Step 6 is outside the range of α in the table consulted for critical values, extrapolation is necessary. α is in any case likely to be an irregular number (such as 0.1605 in the comparison of netting methods) requiring interpolation. Again, computer programs are a better alternative; when doing a statistical test, the output usually includes the probability of making a Type I error --simply compare this with α. Ensure that the computer has used the model and the hypothesis (1- or 2-tailed) that you had in mind.

The ideas in this paper rely on being able to calculate ß (or power) but there are some difficulties in doing these calculations; see Fairweather (1991) for a discussion of these difficulties.

Information for Readers

Siegel (1956) suggested that scientists should state not only whether they accept or reject the null hypothesis but also the probability of the test statistic obtained; this permits readers to judge whether the test result is significant at some different α. In the same way, scientists should include all the information needed by readers wanting to select an α:ß ratio different from that used in the paper.

The information required (from the main experiment, regardless of whether a pilot study was done) is: 1) variability of the data, 2) the minimum effect size of interest, 3) the sample size used, and 4) the test used, in addition to 5) the obvious statistics normally presented (e.g. means, t-value and probability in the comparison of netting methods). It is even more helpful to graph the relationship between α and ß for the above values, highlighting the values of α and ß actually used in the test (e.g. Fig. 1 for the comparison of netting methods).

Conclusions

An increasing awareness of Type II errors in ecology results in more useful research being done. It is not enough, though, to decide upon an α:β ratio prior to an experiment only to have it changed at the time of testing as a result of holding α inviolable. And in this situation, analysing power after the test informs us only that the realised α:β ratio was not the ratio desired. The improved experimental procedure described in this paper will, with only a little extra effort, further increase the efficacy and usefulness of research by allowing better decisions -- decisions that reflect the circumstances of the research. The improved procedure is useful to all ecologists (indeed, to all researchers testing statistical hypotheses) and is particularly helpful in environmental impact detection where the repercussions of statistical errors can be very serious.

Acknowledgements

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Table 1 Results of pilot study and main study: paired comparisons of netting methods (in fish/m^2).

| | Pilot | | Main | |
	Mean	s.e.	Mean	s.e.
Pop-up	3.80	1.50	6.32	2.09
Seine	1.70	1.24	1.62	0.71
Differences	2.10	0.80	4.70	2.10

Captions for Figures.

Fig. 1 Relationship of ß to α for paired t-test on fish catches of pop-up versus seine nets using information from main study (curved line). Straight line represents my desired ratio. Values of intersection determined using iterative technique described in text.

Fig. 2 Frequency distribution of DIFFERENCES between fish catches (fish/m^2) in paired pop-up and seine nets (N = 17 pairs).

Fig. 1
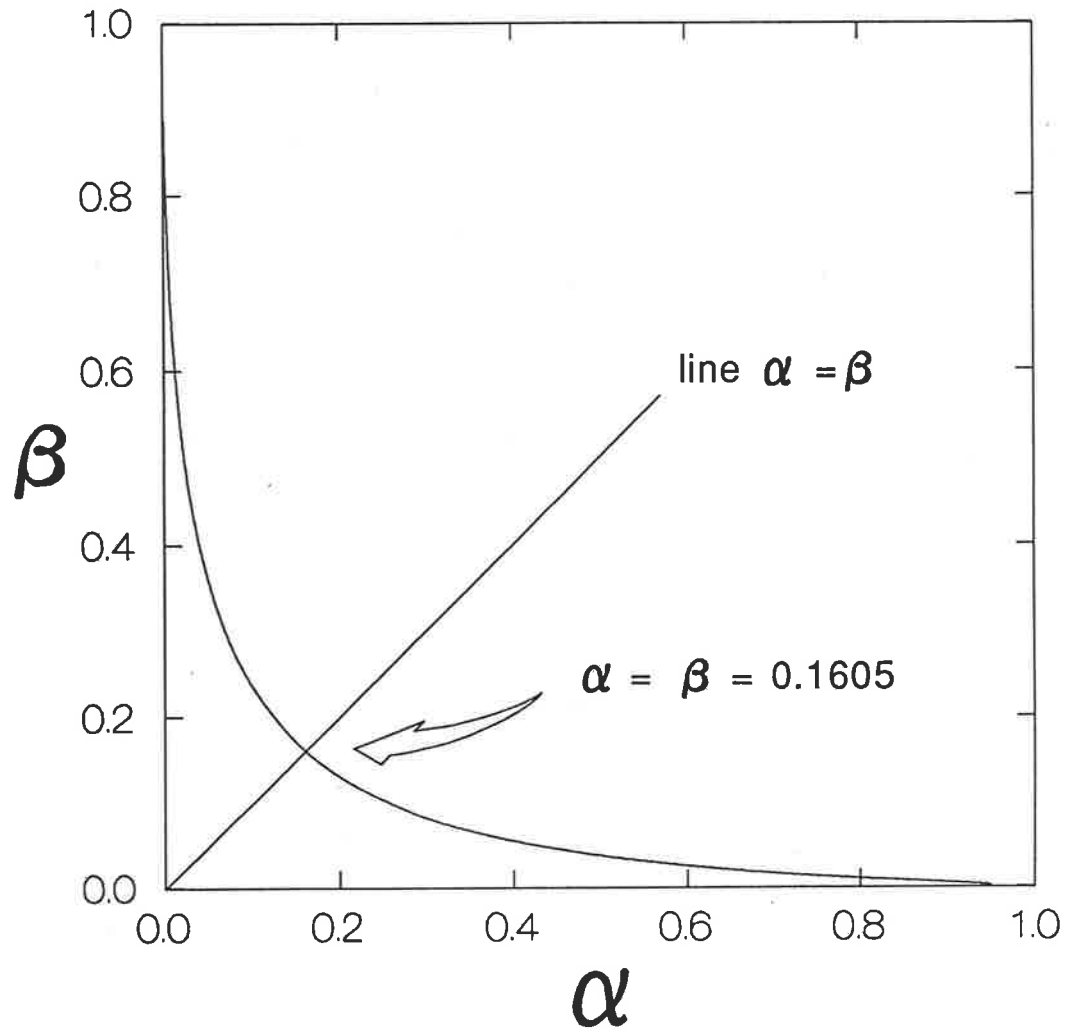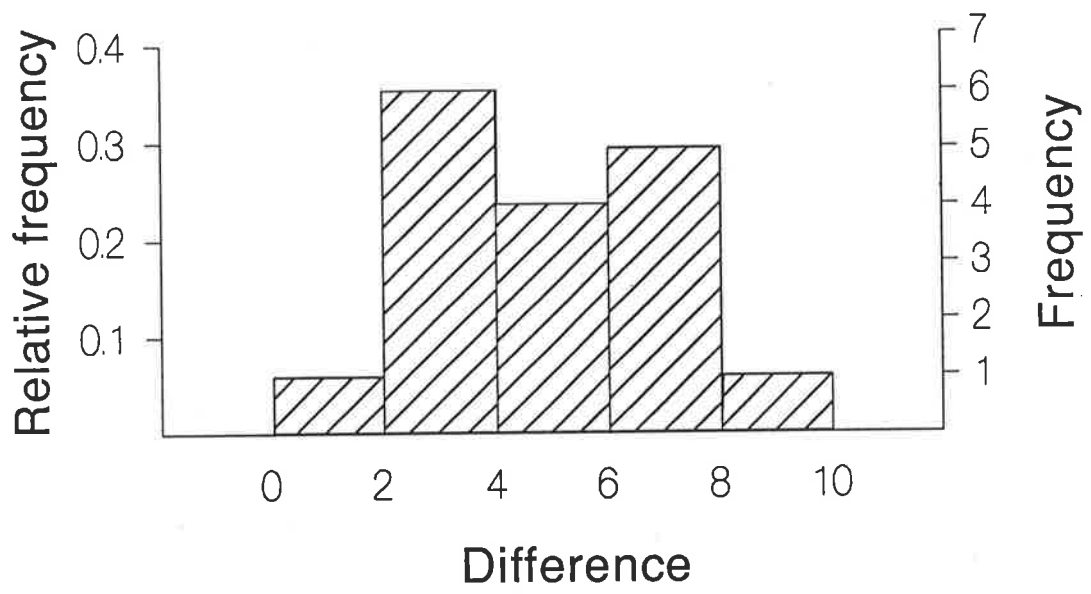
Fig. 2

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