An investigation into the trophic nature of small reef fish from the tribe Salariini, family Blenniidae.

Thesis submitted by

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Abstract

The nutritional value of detritus to small coral reef fishes was examined by assessing the trophic status of salariin blennies on the Great Barrier Reef, Australia. It is often assumed that filamentous algae satisfies the dietary requirements of fish feeding on the epilithc algal matrix (EAM); however, gut content analysis of nine blenny species, from five genera, found they all ingested predominantly detrital aggregates and only small amounts of filamentous algae. For the representative species examined in greater detail, *Salarias patzneri*, detrital aggregates were the dominant item ingested irrespective of season or age. Comparisons of *S. patzneri* gut contents with EAM samples from within their territories showed that *S. patzneri* ingested a significantly higher proportion of particles <125μm and significantly lower proportion of filamentous algae than was present in their territory.

Visual examination of particulates <125μm collected from *Salarias patzneri* territories found that organic matter was almost exclusively in the form of amorphic detrital aggregates. Biochemical comparisons of detrital particles <125μm with filamentous algae determined that protein:energy ratios of particulates <125μm were slightly higher than those of filamentous algae, suggesting detritus is of comparable, if not slightly higher nutritional value than filamentous algae. The mean protein:energy ratio of particulates <125μm in the summer (11.3 ± 0.8 $mg.Kj^{-1}$) and winter (10.3 ± 1.3 $mg.Kj^{-1}$) was also greater than values required to sustain fish growth. The organic content of particulates <125μm was high (15-20%), and, overall, these particles accounted for approximately 41-44% of the total organic matter in the EAM. Detrital particulates <125μm were therefore a nutritionally valuable and abundant component of the EAM in *S. patzneri* territories.

Lipid biomarkers were used to identify the source of amorphic detritus <125μm in *Salarias patzneri* territories. Fatty acid, hydrocarbon and sterol profiles from detrital aggregates and filamentous algae samples were similar, suggesting a large portion of the amorphic detritus is derived from filamentous algae. However, differences in the relative concentration of specific lipid biomarkers in detritus and algal samples indicated additional inputs to the detritus from microalgae, coral mucus and bacteria.

The concentration of fatty acid biomarkers in *Salarias patzneri* tissues were also compared to detritus and filamentous algae samples to assess which of these resources were assimilated by blennies. The ratio of the fatty acid 16:1ω7 to 16:0 and the percentage of 18:2ω7 in detrital samples and *S. patzneri* tissues were similar; however, the ratio/ percentage of these dietary biomarkers were significantly different in filamentous algae and *S. patzneri* tissues. This difference suggested that *S. patzneri* assimilated lipids primarily from detrital aggregates rather than filamentous algae.

Detrital aggregates therefore represent 62 ± 5% of *S. patzneri* gut contents, are of a high nutritional value, represent a major source of organic matter available to *S. patzneri* in their territories and are assimilated by this species. *Salarias patzneri* can, therefore, unequivocally be classified as a detritivorous fish, and, based on the gut contents of 9 salariin species, other blennies are also likely to be detritivores.

As a group, salariin blennies were most abundant, and biomass estimates greatest, on the tops of exposed seaward reefs. At a finer spatial scale, individual species showed a preference for dead coral microhabitats. It is hypothesised that both broad and fine scale habitat associations of blennies are partially related to the availability and quality of detritus. On the exposed reef crests at Lizard Island, where the number and biomass of detritivorous and herbivorous fishes is greatest, blennies

accounted for approximately half of the density and one fifth of the estimated biomass of territorial detritivores. Compared to other functional groups, territorial detritivores accounted for approximately a third of all detritivorous/herbivorous fishes and one quarter of total biomass estimates on exposed reef tops. The substantial contribution of blennies and other detritivores to fish assemblages that feed on the EAM, relatively high nutritional value and abundance of detritus, combined with its undeniable contribution to blenny diets, indicates that detritus and detritivorous fishes are an integral and important component of coral reef trophodynamics.

Preface

A fundamental aspect of ecology is understanding how energy flows between different trophic levels. In almost all ecosystems, energy is initially fixed by primary producers then transferred to different trophic levels via the grazing of herbivores, or less directly, via the detrital pathway (Begon et al. 1990). On coral reefs it is often assumed that grazing by herbivorous fishes and invertebrates forms the main trophic link between primary production by algae and secondary consumers (Hatcher 1983a). However, studies that have compared levels of primary production and grazing by herbivorous fauna on coral reefs have found algal production always exceeds herbivore consumption (Hatcher 1981, Klumpp and Polunin 1990, Polunin and Klumpp 1992). Algae that are not ingested by grazers may enter the detrital food web when dislodged, or via the secretion of dissolved organic matter and subsequent formation of particulate organic matter. In addition, a large percentage of the organic matter that is ingested by herbivores is not assimilated (Montgomery and Gerking 1980, Klumpp and Polunin 1989, Galetto and Bellwood 1994), and fecal matter from these grazers will also contribute to the detrital pool. Organic matter from coastal outwellings (Alongi 1990), dead organisms and exudates from other reef fauna, particularly mucus from corals (Ducklow and Mitchell 1979), may also add to detritus on coral reefs. Consequently, given the large range of potential sources, it seems likely that a substantial proportion of the energy on coral reefs is in the form of detritus.

A trophodynamic model of a coral reef predicts that 59-69% of the net primary production enters detrital food webs (Arias-Gonzalez et al. 1997). This high estimate is supported by observations recording large inputs of organic carbon to a coral reef lagoon from detritus relative to primary productivity (Hansen et al. 1992),

and a higher standing biomass of detritus relative to filamentous algae in the territories of three species of pomacentrids (Wilson and Bellwood 1997).

The detritus produced on reefs may contribute to further algal production, become incorporated into sediments and buried, transported off reefs, or consumed by microorganisms and detritivores (Hatcher 1983b). Microorganisms, in particular bacteria, are believed to be responsible for recycling large amounts of detritus and making it available for metazoan consumers (Alongi 1988). Similarly, benthic and pelagic invertebrates have been identified as consumers of detritus (Alongi 1988), and, in conjunction with microbial organisms, may provide an important link between detritus and coral reef fishes. However, the possibility that coral reef fish ingest, digest and assimilate detritus directly has been largely overlooked, especially in hard substartum environments. This omission is despite findings that detritivorous fishes are a major part of fish communities in fresh water ecosystems (Gerking 1994) and that some of these fishes are capable of assimilating detritus without bacterial or invertebrate intermediates (Bowen 1981).

On coral reefs most of the fish that are classified as herbivores feed on the epilithic algae that grow on the hard reef substratum (Hatcher 1983a). Amongst these algal filaments there is detritus, invertebrates, microbes and sediment (Hatcher 1983b, Choat 1991), a conglomeration of items, that together with the filamentous algae has been described as the epilithic algal matrix (EAM) (Wilson and Bellwood 1997). Fish that feed on the EAM are likely to ingest not only filamentous algae, but also other items within the EAM (Choat 1991). Recent findings have shown that three species of territorial pomacentrids ingest more detritus than filamentous algae (Wilson and Bellwood 1997) and that one of the most prominent acanthurids on coral reefs, *Ctenochaetus striatus*, is a specialised detritivore (Purcell and Bellwood 1993). In

fact, gut content analyses of fish from the Scaridae, Acanthuridae and Pomacentridae reveal that many species, often regarded as herbivores, ingest substantial amounts of detritus (Sano et al. 1984). The detritus ingested by pomacentrids was of irregular shape, lacked cellular structures and was composed of particles <100 μm (Wilson and Bellwood 1997), characteristics typical of amorphic detritus (Bowen 1984). Compared to morphic detritus, which contains cellular remnants of its organic origin and is typically composed of particles >100 μm (Bowen 1984), amorphic detritus is of a relatively high nutritional value (Alber and Valiela 1994). The contribution of detritus to secondary production by these fish is unclear, however, these fish do ingest large quantities of a nutritious form of detritus, which raises questions regarding their true trophic status and the relative importance of grazing and detritivory in coral reef trophodynamics.

Of the fish that feed on the EAM, members of the family Blenniidae, tribe Salariini, represent some of the smallest known detritivorous/herbivorous fishes. Based on data from Myers (1989) members of the salariin blennies have maximum standard lengths of between 27 and 137mm, with a mean of 74mm, making them considerably smaller than any other group of coral reef fish that feed on the EAM (Choat 1991). Fish with small body mass have a higher resting metabolic rate than larger fish and therefore require relatively more energy per gram of body mass than larger conspecifics (Clarke and Johnston 1999). In warm, tropical waters metabolic demands are even greater than in cooler aquatic habitats, as oxygen consumption increases in a curvilinear fashion with environmental temperature (Clarke and Johnston 1999). Being of small body size and living in tropical waters, the salariin blennies must, therefore, by necessity, feed on a dietary resource that is readily available and of high nutritional value.

The salariin blennies feed on the EAM, where the two most likely sources of organic matter and energy are filamentous algae and detritus. The nutritional value of these two resources is often considered to be low; however, with selective feeding and high consumption levels, it is feasible that blennies could live on a detrital and/or algal based diet (Bowen et al. 1995). Indeed, although they are small and cryptic, the salariin blennies are often highly abundant on coral reefs (Hatcher 1981, Labelle and Nursall 1992, Townsend and Tibbetts 2000) and in some locations they have been identified as major consumers of the EAM (Hatcher 1981). Salariin blennies also have been identified as important prey items of common coral reef fish from the families Serranidae and Lutjanidae (Kingsford 1992, Connell 1998, St John 1999), as well as primary prey of specialised predatory fish from the families Fistularidae and Aulostomidae (Hiatt and Strasburg 1960, Randall 1967). The salraiin blennies are a group of fish that provide an important trophic link between resources within the EAM and secondary consumers and are therefore of both ecological and physiological interest.

The primary aim of this dissertation was to examine the trophic status of salariin blennies on coral reefs. This study initially involved examination of ingested material from a number of different blenny species collected from different regions of the Great Barrier Reef, Australia (Chapter 1). Selection of dietary items and the nutritional value of dietary resources were then examined for a representative species, *Salarias patzneri* (Chapters 1 and 2). Comparisons of dietary selection and quality concentrated on differences between detritus and filamentous algae collected from the EAM within *S. patzneri* territories. The origin of detritus in territories also was investigated using lipid biomarkers (Chapter 3). The composition of lipid biomarkers in detritus and algae were then compared to tissue samples of *S. patzneri* to assess the

source of dietary lipids (Chapter 4). Finally, the distribution patterns of salariin blennies were examined with respect to their primary dietary resource, and their contribution to fish assemblages that feed on the EAM were assessed (Chapter 5).

Thus, by examining the feeding selectivity, ingestion and assimilation by blennies, this thesis will provide information on their trophic status, whilst distribution studies will give an indication of their ecological significance. Given the metabolic constraints that are applicable to blennies, information on their diet, its nutritional value and origin may also provide insights into the trophic nature of other fishes that feed on the EAM, laying the foundation for a broader understanding of coral reef trophodynamics.

Chapter 1. Diet and feeding selectivity of coral reef blennies (Blenniidae: Salariini)

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1.1 Introduction

The use of the term herbivore to describe the trophic status of reef fishes that feed on epilithic algae often can be misleading. Although many of these fish can be seen feeding on epilithic algae, the algae are not necessarily the main item they ingest. This is because on coral reefs epilithic algae are a conglomeration of algae, sediment, invertebrates, microbes and detritus (Hatcher 1983a, Choat 1991), a combination of potential dietary resources that has previously been referred to as the epilithic algal matrix (EAM) (Wilson and Bellwood 1997). Consequently, fish feeding on the EAM potentially can ingest nutritional resources other than algae, and the value of these alternative resources to a fish's diet often is unclear (Choat 1991). To assess the trophic nature of fish that feed on the EAM, it is therefore essential to determine which of the EAM resources they ingest and ultimately use.

Members of the family Blenniidae, tribe Salariini, have been identified as major grazers of epilithic algae (Hatcher 1981) and previously described as herbivores (Graham et al. 1985, Randall et al. 1990, Stepien 1990, Williams 1990) or detritivores (Williams 1990). Studies that have examined their gut contents have found they ingest a combination of detritus, sediment and algae (e.g. Hiatt and Strasburg 1960, Randall 1967, Sano et al. 1984). However, these studies have tended to group algae and detritus or sediment and detritus as single categories, making it difficult to assess the independent contribution of these items to the gut contents. The coherent nature of the EAM also makes it difficult to physically separate detritus, sediment and algae. A

visual method of analysis however has been developed to allow the relative contribution of different items to be measured quantitatively (Wilson and Bellwood 1997). This technique is used herein to assess the relative contribution of detritus, sediment, filamentous algae and other ingested items in the gut contents of coral reef blennies from the tribe Salariini.

In temperate waters, gut content analyses of blennies from the tribe Blenniini, which are closely related to salariin blennies (Springer 1968), have detected interspecific (Goldschmid et al. 1984), seasonal (Nieder 1997) and ontogenetic (Milton 1983) changes in diet. Consequently, a comprehensive analysis of diet in the salariin blennies should investigate potential dietary differences between species and the possible effect of season and age on blenny diet. Furthermore, if blennies predominantly ingest a particular item within the EAM, it is possible that they preferentially feed on this EAM component, and such feeding behaviour may help to identify trophic status. The size of particles ingested also may be important, particularly if detritus is a major component of the gut contents. Smaller detrital particles are more likely to be amorphic (Bowen 1984), which is more nutritious and better assimilated than larger-sized morphous detritus (Alber and Valiela 1994, D'Avanzo et al. 1991). If blennies feed selectively on a particular size range of particles, it may therefore give an indication of their preferred detrital type.

The morphology of the oral and pharyngeal jaws of fish is also often examined in conjunction with dietary studies (e.g. Purcell and Bellwood 1993). Examining jaw morphology can help identify the manner in which fish procure and process dietary items, as well as the type of dietary items they are capable of ingesting. By examining the jaw structure of the blenny, *Salarias patzneri*, I hope to gain an insight into how this species, and other salariin blennies, obtain dietary items from the EAM.

Finally, it has been demonstrated that feeding intensity of blennies in temperate waters can be correlated to diurnal changes in the nutritional quality of algae (Horn et al. 1990, Zoufal and Taborsky 1991). Monitoring the feeding patterns of salariin blennies on coral reefs may therefore give an indication of whether blennies have the potential for similar temporal feeding selectivity in this environment.

In this chapter I provide an initial assessment of the trophic status of salariin blennies on coral reefs by assessing their gut contents and feeding patterns. Gut content analyses were carried out on nine species of salariin blenny, collected from three regions of the Great Barrier Reef, Australia. The possibility of temporal and ontogenetic diet shifts will be investigated by examining the gut contents of a representative species, *Salarias patzneri.* Preliminary investigations found *S. patzneri* had dentition (e.g. Kotrschal 1989, Ebner 1993), and diet (Ebner 1993) similar to other salariin blennies and was therefore a suitable representative species. *Salarias patzneri* were collected during the summer and winter to examine potential temporal variability in diet, whilst a full size range of *S. patzneri* were collected to examine potential ontogenetic shifts in diet. Selection of dietary categories, different sized particles and temporal feeding patterns of *S. patzneri* also will be examined to determine the dietary and temporal feeding preferences of this blenny.

1.2 Materials and Methods

1.2.1 Collection of samples

Inter-specific differences in diet were evaluated by examining the gut contents of nine species of salariin blennies. Fish were collected from the reefs surrounding: Lizard (14° 42'S, 145° 30'E), Orpheus (18° 40'S, 145° 30'E), and One Tree Islands (23°

30'S, 152° 06'E) (Table 1.1). Species collected from each of these islands represented the dominant salariin taxa from each island. Blennies used for inter-specific comparisons and other analyses were collected between August 1997 and February 1999, using either hand spears or the anaesthetic clove oil (see Munday and Wilson 1997). All fish were collected during the afternoon, as this is when blennies typically have full guts (Klumpp and Polunin 1989,1990, Ebner 1993). Fish were killed immediately after capture to prevent regurgitation, placed on ice, returned to the laboratory and frozen.

Temporal variation in diet was investigated by examining the gut contents of *Salarias patzneri* collected from Lizard Island. Sixteen *S. patzneri* were collected during August 1998 (Austral winter) and a further sixteen in February 1999 (Austral summer). Blennies were sexed by examining genital papillae and gonad morphology. The gut contents of 60 *S. patzneri*, all collected from Lizard Island, and with standard lengths between 17.9 and 58.8 mm (measured with calipers) were examined for possible ontogenetic changes in diet.

Table 1.1 Collection sites of blenny species used for gut-content analysis. Sample sizes for *Salarias patzneri,* a = inter-specific, b = seasonal, c = ontogenetic comparisons.

Species	Location	n
Ecsenius bicolor	Lizard	10
Ecsenius stictus	Lizard	12
Glyptoparus delicatulus	Lizard	10
Salarias patzneri	Lizard	$11^{a}\,32^{b}\,60^{c}$
Cirripectes chelomatus	Orpheus	4
Ecsenius mandibularis	Orpheus	14
Salarias guttatus	Orpheus	11
Atrosalarias fuscus	One Tree	10
Salarias fasciatus	One Tree	7

To assess feeding selectivity by blennies, a sample of total EAM and detritus were collected from eight *Salarias patzneri* territories and compared to the gut contents of fish collected from the same territory. Fish and EAM samples were collected from the Lizard Island lagoon, between the 1st and 10th of March, 1998. Prior to sampling, *S. patzneri* were observed for a period of 10 minutes (recorded with a stopwatch) and their feeding behaviour and bite locations recorded. A square plastic template, outlining an area of 200cm^2, was then carefully pinned to the substratum, in an area where fish had been feeding. Loose sediment and associated detrital aggregates were removed from this area using an underwater sediment sampler fitted with a soft brush (Purcell 1996). This sample herein is referred to as the detrital or detritus sample. A second square template, covering an area of 100cm^2, was pinned to a different area of the territory where fish had also been observed feeding. This area was sampled by scraping the substratum with a metal pipe (12mm internal diameter) fitted to the intake pipe of the underwater sediment sampler. Samples collected in this manner contained filamentous algae that had been scraped from the substratum, sediment and detrital aggregates and will be referred to as the complete EAM. After collection, detrital and complete EAM samples were returned to the laboratory within 30 minutes, placed in settling containers and left for three hours in a cool location. This allowed particles >10 μm to settle (see Dyer 1986). Excess water was then drained through a tap at the base of the settling container and the remaining concentrate placed in a plastic bag and frozen. Fish were collected from territories immediately after removal of detrital and complete EAM samples.

To assess if blennies feed at particular times of the day, the feeding behaviour of *Salarias patzneri* was monitored in the early morning (0600-0859), late morning (0900-1159), early afternoon (1200-1459) and late afternoon (1500-1800). Feeding

observations were made in the Lizard Island lagoon over a two-week period in winter (August 1998) and a further two weeks in summer (February 1999). A minimum of 20 feeding observations was conducted within each sampling period during both seasons. Feeding observations were conducted on snorkel and involved recording the number of bites taken by an individual fish (approximately 35mm or greater total length, estimated visually) over 10 minutes. Before each feeding observation was conducted, blennies were watched for a 5-10 minute period to allow time for fish to become accustomed to the observer's presence.

Data were analysed statistically using ANOVA, with season entered as a fixed factor and time category entered as a random factor. Bite rates were square-root transformed prior to analysis to meet the assumptions of ANOVA (Zar 1999). These assumptions were tested using Levene's test and Q-Q plots.

1.2.2 Sample analyses

Gut contents from blenny guts and complete samples of the EAM from the territories of *Salarias patzneri* were examined visually under an Olympus dissecting microscope. Blenny guts were stretched out and the contents from the anterior half dissected from the gut. Gut content and EAM samples were quantified using the method of Wilson and Bellwood (1997). Each sample was spread evenly over a glass Petrie dish and a 25x25 grid affixed to its base, from which 50 randomly marked grid quadrats were examined. In each of the 50 quadrats the dominant item was recorded and categorised into either: detrital aggregates, inorganic sediment, filamentous algae or invertebrates. Detrital aggregates were defined as dead organic matter with associated autotrophic and heterotrophic microbes (Bowen 1979). Other items present

in the quadrat also were recorded. Values for each category were expressed as a percentage of quadrats in which the category was dominant and present.

To evaluate selectivity of dietary categories by *Salarias patzneri*, the dominant data from visual analyses of gut contents and the corresponding sample of complete EAM were compared. Selectivity ratios, with 95% confidence intervals, were used to assess patterns of food intake (Manly et al. 1993). Ratios, that were less than 1.0 indicate fish had ingested a lower proportion of that dietary category than was present in the complete EAM sample. Ratios that were greater than 1.0 suggested fish ingested a greater proportion of that dietary category than was present in the complete EAM. The 95% confidence limits were used to assess if selectivity ratios varied significantly from a value of 1.

Equation for calculation of selectivity ratios. From Manly et al. (1993) Design type III, sampling protocol A.

$$W_i = U_{i+} / \sum_{j=1}^{n} \pi_{ij}.U_{+j}$$

W_i = Selectivity ratio for category i and n fish.

U_{i+} = Sum of category i from n fish.

π_{ij} = amount of category i (expressed proportionally) available to fish j.

U_{+j} = Sum of all categories used by fish j

A comparison of the size distribution of inorganic particles in the anterior half of *Salarias patzneri* guts and detrital samples of the EAM were carried out to assess if fish selected a particular size range of particles. Inorganic particles from the gut contents of *S. patzneri* and detrital samples were obtained by bleaching samples to

remove organic matter (following Wilson and Bellwood 1997). Particles from these samples then were sifted successively through 500, 250, 125 and 63μm sieves. Each of the resulting five size categories were weighed using a Mettler AE240 digital balance to an accuracy of ± 5μg. Weights for each category were expressed as a percentage of the total inorganic sediment weight. Selectivity ratios with 95% confidence intervals (Manly et al. 1993) then were used to compare the distribution of inorganic particles in the gut and detritus samples.

The oral jaws of a *Salarias patzneri* were photographed using a Cannon P450 camera fitted with a macro lens. This allowed a rudimentary examination of jaw structure in this blenny species. The pharyngeal jaws were dissected from the fish and place in a proteinase solution for 1 week to remove excess solution. The jaws were stained with alizarin red and examined under an Olympus stereo dissecting microscope.

1.3 Results

1.3.1 Comparative gut analysis of nine species of blennies

Gut contents of all nine blenny species were characterised by large amounts of detrital aggregates, moderate amounts of sediment and generally small amounts of filamentous algae. Detrital aggregates were clearly the dominant organic category (Figure 1.1) and were present in almost 100% of quadrats (Table 1.2). In contrast, algae seldom dominated and were often absent from quadrats. Algae present in the guts of all fish were mainly filamentous, from the classes Rhodophyta and Chlorophyta. Invertebrates were a small component in the gut contents of all individuals. Foraminifera and copepods were the most commonly encountered

invertebrates and were present in specimens from all species. Other invertebrates included bivalves, gastropods and nematodes.

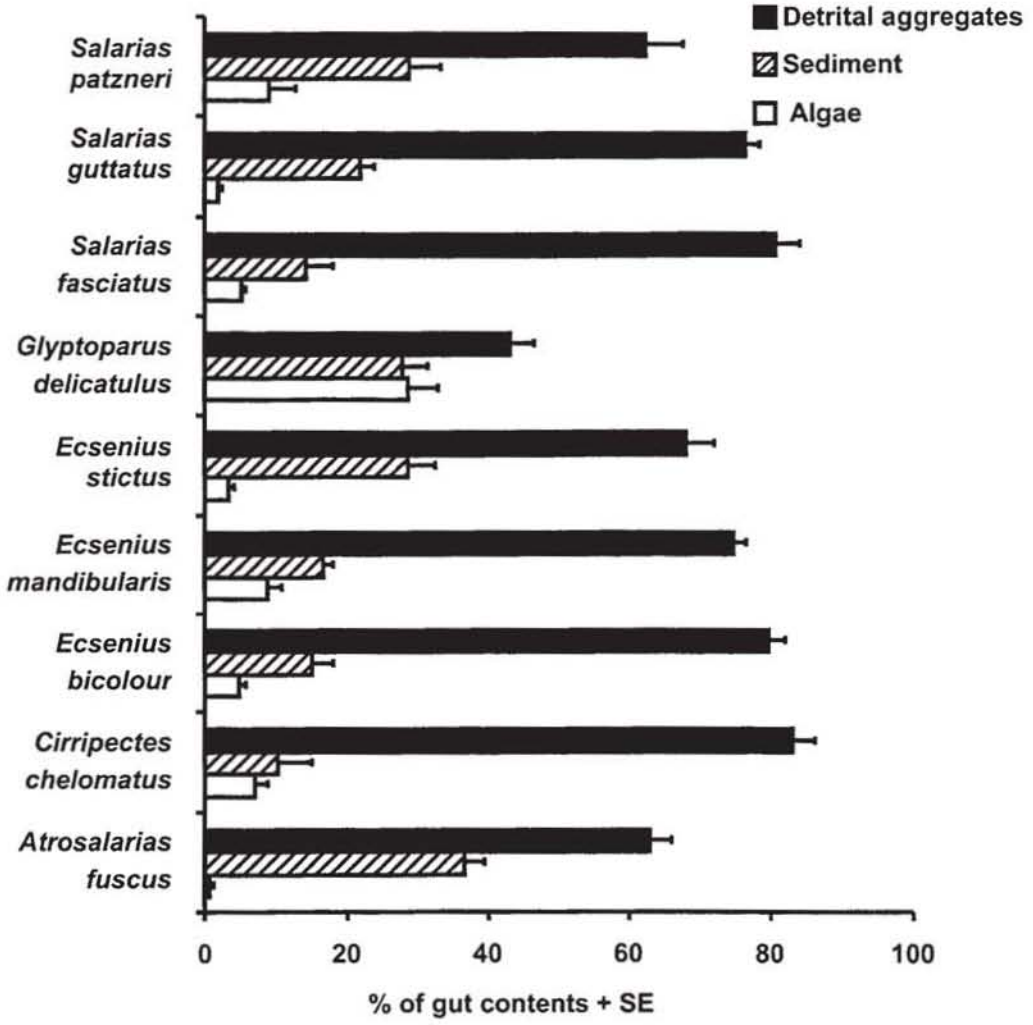

Figure 1.1 Mean percentage dominance + standard error of three prey classes (invertebrates never dominant) from gut-content analysis of nine species of salariin blennies. Values calculated from mean percentage of quadrats in which each dietary category was dominant (50 quadrats per sample).

Table 1.2. Presence of dietary categories in guts of salariin blennies (Mean % + SE, n as in Table 1.1 and 50 quadrats per sample) (*detritus* detrital aggregates; *algae* filamentous algae; *invert* invertebrates)

Species	Mean % of quadrats where dietary category present			
	Detritus	sediment	algae	invert
Salarias patzneri	99.3 (0.3)	97.2 (0.9)	71.0 (6.2)	8.8 (1.2)
Salarias guttatus	100	99.6 (0.2)	60.2 (4.7)	11.5 (1.5)
Salarias fasciatus	100	97.0 (1.3)	73.4 (4.4)	11.0 (1.6)
Glyptoparus delicatulus	98.6 (0.5)	97.4 (0.9)	87.6 (3.1)	8.3 (1.6)
Ecsenius stictus	99.5 (0.3)	96.3 (1.6)	61.4 (5.0)	6.3 (1.2)
Ecsenius mandibularis	99.3 (0.4)	97.9 (0.5)	70.8 (4.6)	8.3 (1.3)
Ecsenius bicolor	100	74.2 (4.5)	51.5 (2.9)	9.8 (1.8)
Cirripectes chelomatus	100	95.6 (2.4)	69.9 (12.5)	13.2 (2.2)
Atrosalarias fuscus	100	98.5 (0.8)	72.9 (4.6)	3.3 (1.0)

The percentage dominance of detrital aggregates in the guts of *Salarias patzneri* were consistently four to five times greater than the percentage of filamentous algae in the gut, irrespective of season or sex (Figure 1.2). Male fish collected during the summer did, however, have a higher percentage of detritus and filamentous algae relative to sediment in their guts than other *S. patzneri* (Figure 1.2). Apart from detrital aggregates, sediment and filamentous algae, *S. patzneri* also ingested cyanobacteria, diatoms and invertebrates (principally foraminifera and copepods); however, these categories were a minor component of gut contents in both male and female fish collected during both seasons.

The relative percentages of detrital aggregates, sediment and filamentous algae in *Salarias patzneri* guts were consistent among fish of different standard lengths (Figure 1.3). Bivariate correlations show no significant relationship between any of the dietary resources and fish length, although their variation in the gut contents of larger fish is high compared to smaller fish.

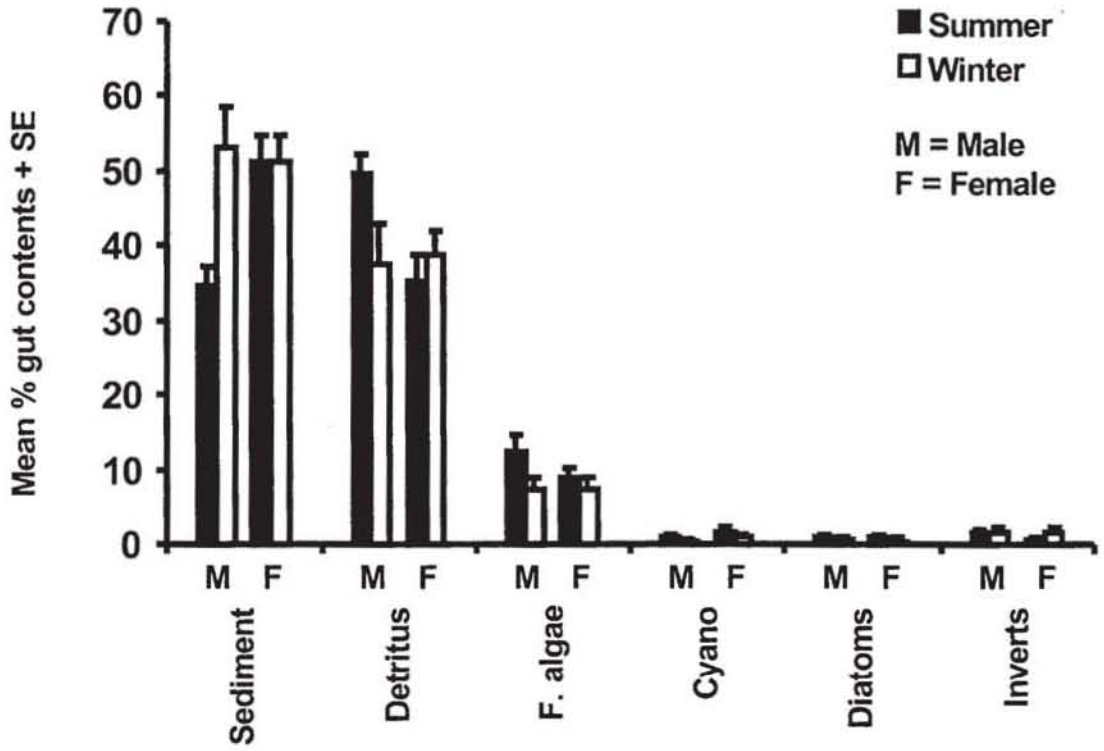

Figure 1.2 Variation in the diet of male and female *Salarias patzneri* collected during the summer and winter. Graph shows mean % dominance of gut contents + standard error. Detritus = Detrital aggregates, F. algae = filamentous algae, Cyano = Filamentous Cyanobacteria, Inverts = Invertebrates. Means and standard errors are calculated from 10 males and 6 females collected during the summer, and 11 males and 5 females collected during the winter. Values calculated from mean percentage of quadrats in which each dietary category was dominant (50 quadrats per sample).

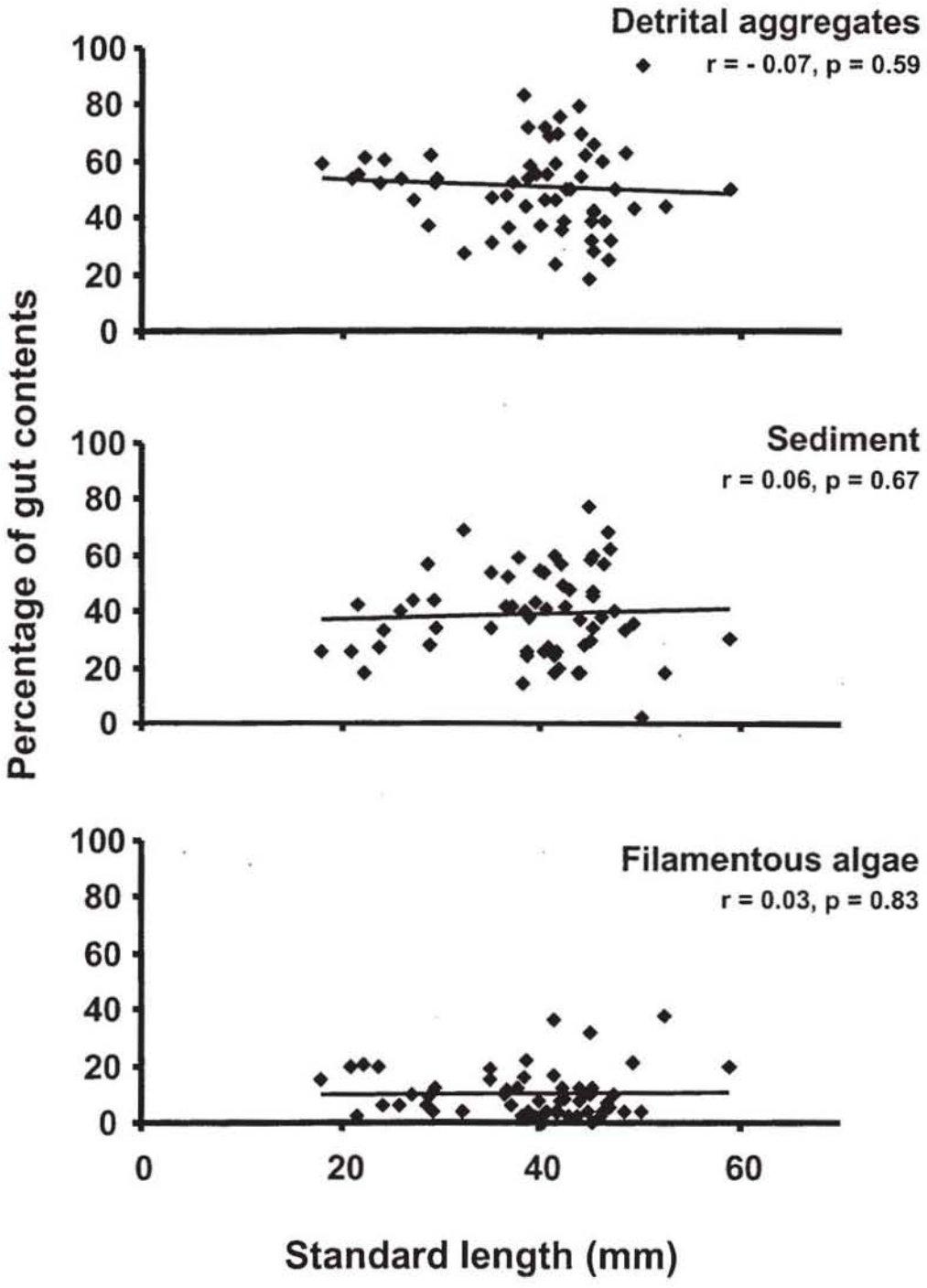

Figure 1.3 Ontogenetic variation in the diet of post-settlement *Salarias patzneri.* Based on the dominant gut content data from 60 fish collected from Lizard Island. Fish were collected throughout the year. Lines on each graph are the "line of best fit".

1.3.2 Feeding selectivity

Visual examination of *Salarias patzneri* gut contents and the complete EAM from their territory found both sample types contained large amounts of detrital aggregates and relatively small amounts of filamentous algae (Figure 1.4). However, the percentage of detrital aggregates in *S. patzneri* guts was marginally greater than that present in the EAM, whilst the percentage of filamentous algae in *S. patzneri* guts was less than that present in the EAM. Selectivity ratios, with 95% confidence limits, found fish ingested a significantly lower proportion of filamentous algae than was available in the EAM (Figure 1.5). In contrast, although the relative amount of detrital aggregates ingested by *S. patzneri* was greater than is present in the EAM, selectivity ratios suggested differences in the proportion of detritus in blenny guts and the EAM were non-significant. Similarly, although the mean percentage of sediment in the guts of *S. patzneri* was lower than that in the EAM, this difference was not significant. Selectivity indices could not be calculated for the invertebrate category, as invertebrates were rarely dominant in either gut or EAM samples.

The distribution of different-sized inorganic particles in *Salarias patzneri* guts and detrital samples collected from their territories were compared to assess if fish select a particular size range of particles. Both sample types contained predominantly smaller-sized particles (<250μm); however, *S. patzneri* guts contained a higher percentage of small particles and lower percentage of large particles than detrital samples (Figure 1.6). Selectivity ratios, with 95% confidence intervals, suggested *S. patzneri* ingested a significantly higher proportion of particles <125μm and significantly lower proportion of particles >250μm than were present in detritus samples (Figure 1.7).

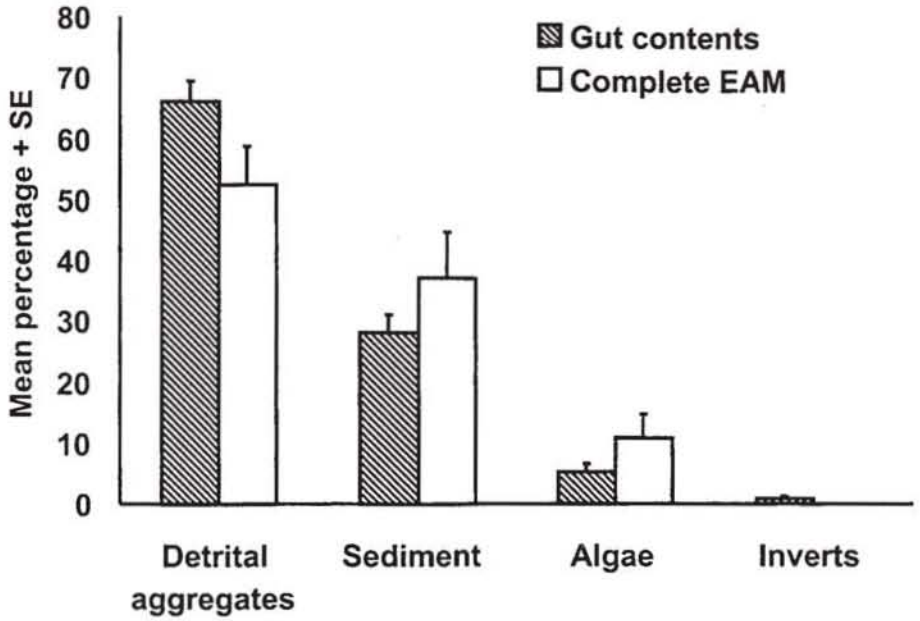

Figure 1.4 Comparison of *Salarias patzneri* gut contents with the complete epilithic algal matrix (EAM), showing mean percentage of quadrats in which each dietary category was dominant (50 quadrats per sample; n = 8 samples) (*inverts,* invertebrates).

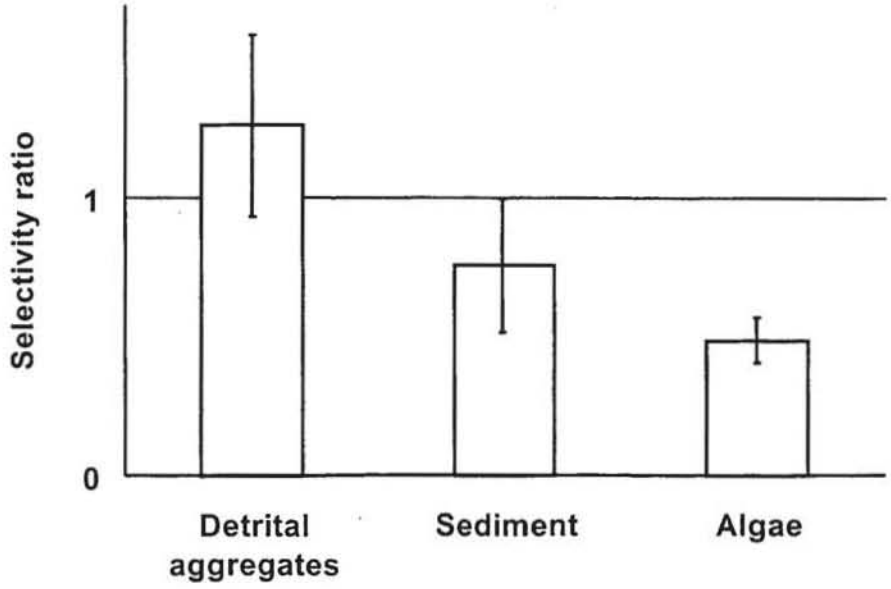

Figure 1.5 Selection of dietary categories by *Salarias patzneri*. Ratios >1 infer selection for a category, ratios <1 infer avoidance (Error bars represent 95% confidence limits; n=8). Based on Selection ratios in Manly et al. (1993) for Design III, sampling protocol A.

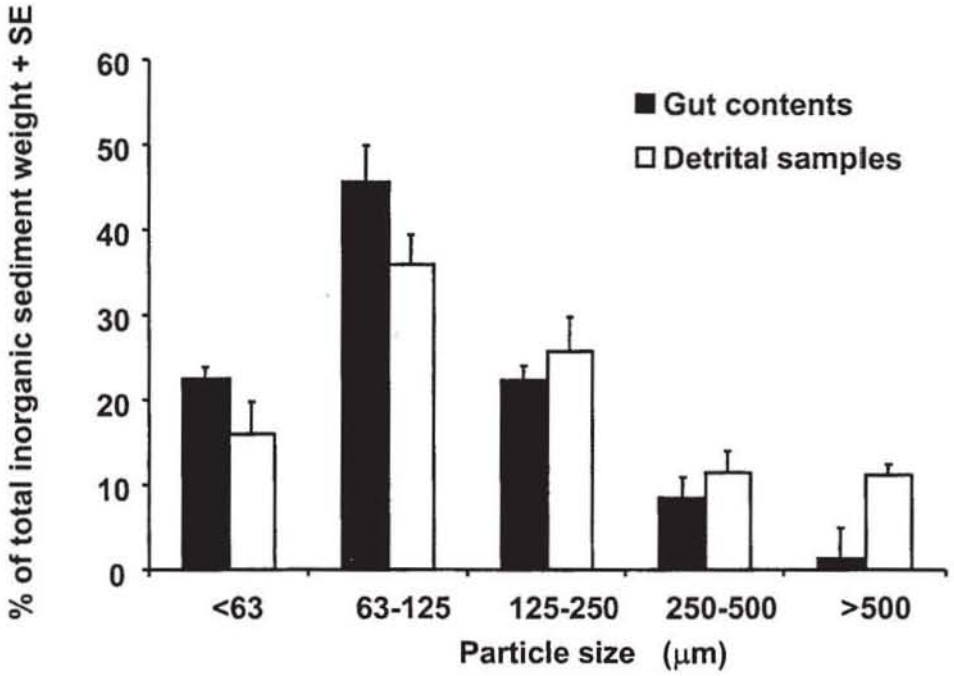

Figure 1.6 Size distribution of inorganic particles in *Salarias patzneri* guts and the detrital samples (n = 8). Graph shows mean % of total inorganic weight + standard error.

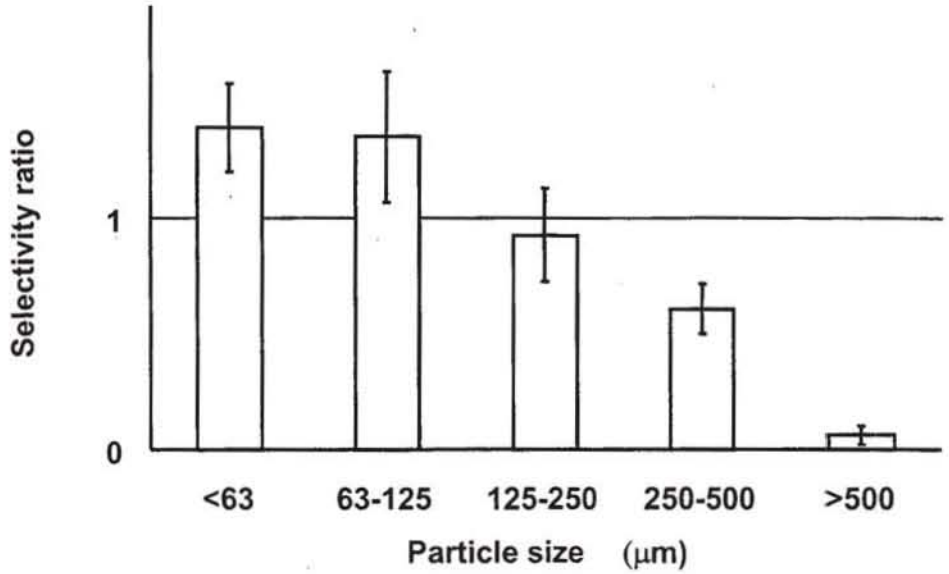

Figure 1.7 Selection of inorganic particles by *Salarias patzneri* from detrital samples. Ratios >1 infer selection for a size category, ratios <1 infer avoidance (Error bars represent 95% confidence limits; n=8). Selectivity ratios from Manly et al. (1993).

The feeding intensity of *Salarias patzneri* was found to vary significantly throughout the day ($F_{3,231}$= 144, p=0.001). In the early morning, feeding rates were lowest, increasing in the late morning and peaking in the early afternoon, before slightly decreasing in the late afternoon (Figure 1.8). Feeding rates were also significantly greater in the summer at all times of the day ($F_{1,231}$= 60, p=0.002).

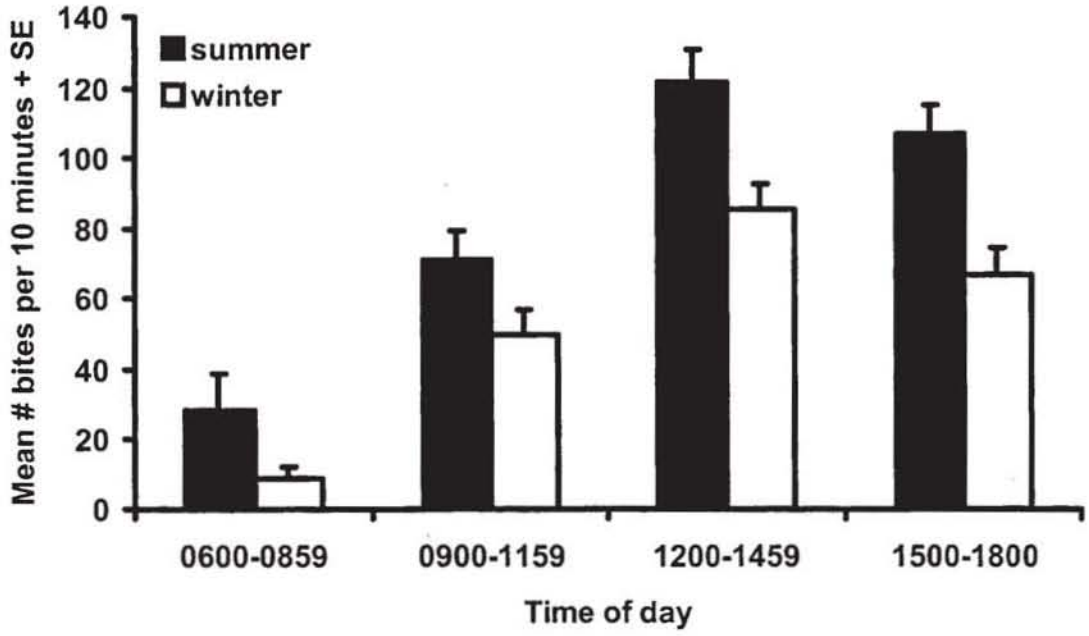

Figure 1.8 Diurnal and seasonal feeding intensity of *Salarias patzneri*. Mean and standard error for each time category calculated from 20-37 ten-minute feeding observations.

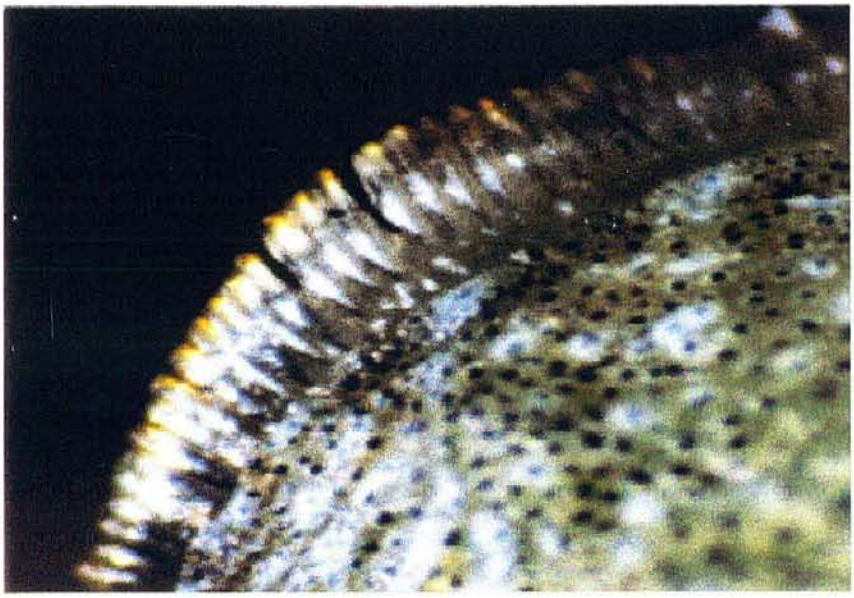

Plate 1.1 Lower jaw of *Salarias patzneri*, showing elongate flexible teeth.

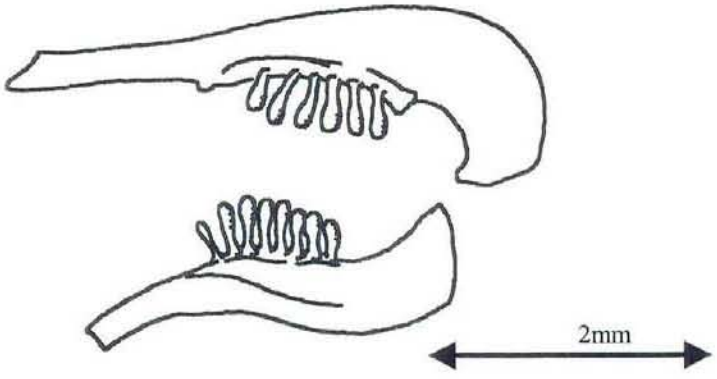

Figure 1.9 Pharyngeal jaws of *Salarias patzneri.*

The upper and lower oral jaws of *Salarias patzneri* contain numerous elongate flexible teeth (Plate 1.1). The teeth are held in position with connective tissue. On the upper and lower pharyngeal jaws there are six to eight teeth (Figure 1.9). The teeth have are club-shaped, with rounded tips. They are attached directly to the pharyngeal jaw.

1.4 Discussion

Gut content analyses of salariin blennies collected from the Great Barrier Reef reveals that these fish predominantly ingest detrital aggregates, irrespective of location or species. Detrital aggregates in the guts of salariin blennies are composed of small, indistinct particles with no identifiable structures, which suggests these particles are amorphous detritus, a potentially valuable dietary resource (Alber and Valiela 1994). Detrital aggregates in blenny guts are unlikely to have originated from mechanical trituration by oral or pharyngeal teeth, as the form of these structures suggest they are incapable of extensive mechanical trituration (Plate 1.1, Figure 1.9). Transverse sections of the alimentary canal of the blenny *Istiblennius meleagris*, also show that specialized musculature, typically associated with a gizzard is lacking (Ebner 1993), suggesting that little trituration occurs within the gut of blennies. Furthermore, detrital aggregates found in all blenny guts appear to be similar to aggregates in the EAM of *Salarias patzneri* territories, which strongly suggests that detritus in the gut was obtained from detritus in the EAM, rather than an artifact of blenny digestion.

Detrital aggregates in the diet of blennies may contain microscopic organisms, which are a potentially important source of nutrition for detritivores (Newell 1965). Choat and Clements (1998) have provided evidence that some detrital and sediment

feeding fish digest bacteria as a means of obtaining nutrition. Bacteria are ubiquitous in coral reef sediments (Sorokin 1981, Moriarty et al. 1985) and may therefore contribute to blenny diets. Similarly, sediments in tropical neritic ecosystems can contain high levels of productive microalgae (Cahoon 1999), and there is some evidence that microalgae are an important part of the diet of some tropical acanthurids (Montgomery et al. 1999). Chapter 3 further explores the composition of detrital aggregates and potential sources of detritus in the feeding territories of *S. patzneri*.

Seasonal changes in the quality and availability of dietary resources can result in dietary shifts by detritivorous fishes (Ahlgren 1990, Little et al. 1998). On coral reefs, seasonal variation in the productivity and biomass of bacteria (Moriarty et al 1985) and microalgae (Uthicke and Klumpp 1998) in sediments may alter the nutritional quality of detrital aggregates. Similarly, seasonal changes in the amount of organic detritus deposited (Hansen et al 1992) and productivity of epilithic algae (Atkinson and Grigg 1984, Klumpp and McKinnon 1989) may alter the availability and quality of these resources. However, despite possible changes to the quality and availability of detritus, *Salarias patzneri* continues to ingest predominantly detrital aggregates in the summer and winter, and the ratio of detrital aggregates to filamentous algae in the guts remained constant between seasons.

The comparison of male and female *Salarias patzneri* gut contents in this chapter found there was no disparity in the ratio of detritus to filamentous algae ingested. However, the percentage of detrital aggregates relative to inorganic sediments in the guts of male *S. patzneri* during the summer was greater than in males during the winter and females during both seasons. A high ratio of detritus to inorganic sediment in the guts of males during the summer suggests that males may be ingesting food of a higher quality than females during this season, as a high ratio of

detritus to sediment implies food resources of a higher organic content, which can be an indication of greater nutritional value (Bowen 1987). Summer also is likely to be a period of concentrated reproductive activity by blennies, when males spend much of their time in their holes guarding eggs (Thresher 1984). It is therefore surprising that males, which are likely to spend less time foraging, have higher quality detritus in their guts than other blennies. Males may feed selectively on sediments of high organic content during this season and defend this area from conspecifics. However, this study has been limited to sampling over a single year and replication is required at the season level. Consequently, detailed field observations, in conjunction with further gut content analyses on fish collected from several summer and winter periods, are required to evaluate these hypotheses.

The mean percentage of detrital aggregates, filamentous algae and sediment in the guts of *Salarias patzneri* remained constant throughout post settlement ontogeny. Because of metabolic constraints imposed on smaller individuals, smaller fish may require relatively more energy than larger conspecifics (Clarke and Johnston 1999). A high percentage of detritus in the guts of *S. patzneri* therefore implies that detritus is a suitable source of energy throughout post-settlement life, including the small recruits and juveniles.

Salarias patzneri grow rapidly, juveniles reaching adult size at approximately 6 months of age (Wilson unpublished data). This rapid growth suggests detrital aggregates ingested by juvenile blennies are of high nutritional value and capable of sustaining high growth rates. In freshwater ecosystems juvenile *Tilapia mossambica* feed on detrital aggregates, grow rapidly and maintain good body conditions (Bowen 1979). *Salarias patzneri* and other blennies are, however, considerably smaller than juvenile *T. mossambica.* The ability of small blennies to grow quickly on a detritus

diet infers detrital aggregates may be a viable and potentially important dietary resource for other, larger bodied coral reef fishes.

A combination of selective feeding and high ingestion rates are required for fish to live on a detrital based diet (Bowen et al. 1995). Comparisons of *Salarias patzneri* gut contents with the EAM collected from their territory suggest they avoid filamentous algae and select fine detrital particles <125μm. Blenniid fish of the tribe Salariini are commonly known as the combtooth blennies, because of the large number of soft, bristle-like teeth present in the upper and lower oral jaws. Similar tooth morphology can be seen in the acanthurid, *Ctenochaetus striatus*, which uses elongate, flexible teeth to brush particulate material from epilithic algae (Choat 1991, Purcell and Bellwood 1993). Blennies may use a feeding strategy similar to that of *C. striatus,* selectively removing the fine, loose particulate matter from within the EAM and leaving filamentous algae attached to the substratum. In this manner, *S. patzneri* and other salariin blennies may effectively avoid ingesting filamentous algae and ingest proportionally more detrital aggregates than are present in the complete EAM.

Salarias patzneri displayed a preference for a certain size range of particles. A comparison of the size distribution of inorganic particles in detrital samples of the feeding zone with *S. patzneri* gut contents found that fish selected particles <125μm and avoided those >250μm. Within the EAM particles <125μm are predominantly detrital aggregates, whilst those >250μm are mainly filamentous algae (Wilson 2000). Consequently, by selectively ingesting fine particles, *S. patzneri* are targeting detrital aggregates and avoiding filamentous algae. This is advantageous, as particles <125μm are likely to be amorphous detritus, rather than morphous detritus (Bowen 1984) and therefore more nutritious (Alber and Valiela 1994). Small particles within the EAM

also are more likely to have a higher organic content (Wilson and Bellwood 1997), which can also be indicative of a higher nutritional vale (Bowen 1987).

Variations in the diurnal and seasonal feeding intensity of *Salraias patzneri* also may be a form of selective feeding by blennies. The diurnal feeding periodicity of the blenny *Parablennius sanguinolentus* (tribe Blenniini) in temperate waters has been correlated with diurnal changes in the carbohydrate and energy content of the algae on which it feeds (Horn et al. 1990, Zoufal and Taborsky 1991). Changes in the energy content of filamentous algae are unlikely to influence the feeding behaviour of *S. patzneri*, as filamentous algae are a small component of their gut contents. However, increased productivity of micro-organisms, particularly microalgae and bacteria within detrital aggregates during the summer, and accumulation of their products in the afternoon, may alter the nutritional quality of detritus, seasonally and diurnally. Temporal changes in the nutritional quality of detritus may therefore influence the feeding behaviour of *S. patzneri* and is further investigated in Chapter 2.

Data presented in this chapter, in conjunction with evidence presented by Wilson and Bellwood (1997) on territorial pomacentrids, have shown that two of the most common groups of supposedly herbivorous fishes on the GBR selectively ingest large amounts of detrital aggregates. Furthermore, the most abundant acanthurid on many coral reefs, *Ctenochaetus* spp (Russ 1984a, Choat and Bellwood 1985), ingests detritus and sediment (Robertson and Gaines 1986).

Detrital aggregates are a dietary resource that is abundant in the feeding areas of salariin blennies and territorial pomacentrids and is likely to be of high nutritional quality. These two groups of fish, functionally classified as algal croppers (Hatcher 1981), are believed to have a major impact on reef ecology through the removal of turf algae. The results of this chapter however, have, shown that blennies clearly

ingest large amounts of detrital aggregates from the EAM and remove only small amounts of filamentous algae. The ecological significance of these fish feeding on detritus is unclear although it would seem that detritus and the fish that feed on detritus have a more important role in coral reef trophodynamics than previously thought.

Chapter 2. Nutritional comparison of detritus and algae collected from blenny feeding territories.

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2.1 Introduction

Many of the small fish that feed on the epilithic algal community ingest a combination of potential dietary items including filamentous algae, detrital aggregates, invertebrates and bacteria (Choat 1991). Combined, these resources represent a conglomeration of living and dead material, which may be referred to as the epilithic algal matrix (EAM) (Wilson and Bellwood 1997). Two of the most prominent families of fishes that feed on the EAM are pomacentrids and blennies (Hatcher 1981). Gut content analysis from species of both these families has determined that detrital aggregates are the dominant item in their guts, whilst filamentous algae is often a minor component of gut contents (Chapter 1, Wilson and Bellwood 1997). Comparisons of gut contents with dietary items in the EAM also have demonstrated that fish from both families tend to ingest proportionally more detritus than is available, particularly particles <125μm, and both groups ingest proportionally less filamentous algae than is available. However, selective ingestion of large quantities of detritus relative to filamentous algae does not necessarily mean that detritus is a superior source of nutrition for these fish. To assess the relative nutritional value of detritus and filamentous algae qualitative evaluation of these dietary resources is required.

Bowen (1987) described three parameters that could be used to assess the nutritional value of detritus: microbial biomass, organic or energetic content and

amino acid or protein content. Energetic and protein content are probably the two main biochemical components of diet that influence growth (Bowen 1987), and as these components are likely to affect fish growth dependently they are often combined to produce protein/energy ratios. These ratios have previously been used to determine optimal growth diets in the aquaculture industry (e.g. Hossain et al. 1998) and in ecological studies to assess the value of natural diets (e.g. Bowen 1979, Horn et al. 1986). Protein can be measured readily in detrital and algae samples, whilst estimates of useable energy can be calculated from protein, carbohydrate and lipid content (Henken et al. 1986).

Microbial biomass also can be estimated using direct counts, or indirectly by measuring compounds such as chlorophyll a. However, this measure of nutritional value is applicable only to detritus and therefore inappropriate for comparisons of detritus and algae. Micronutrients, fatty acids and vitamin content of dietary sources also may influence the nutritional value of detritus and algae, although none of these compounds are expected to have the same affect on fish growth as the protein and energy content of diet (Bowen 1987).

Previous biochemical assessments of detritus on coral reefs have concentrated on particulate organic matter collected from sediment traps (e.g. Koop and Larkum 1987, Hansen et al. 1992) or the detritus within soft sediments between reefs (e.g. Johnstone et al. 1990), rather than the detritus within the EAM, where most grazing fish feed (Hatcher 1983a). The protein and energy content of filamentous algae on coral reefs also have been evaluated (e.g. Bruggeman et al. 1994a, b), although these values have not been compared to those from detritus collected in the same environment. Recently, studies have examined the biochemical content of detritus and filamentous algae collected from the EAM in different reef zones (Purcell and

Bellwood 2001), across mid and outer shelf reefs (Crossman et al. In press) and within fish territories (Wilson and Bellwood 1997, Wilson 2000, Crossman et al. In press). However, these studies have not used protein to energy ratios to assess nutritional value of detritus and filamentous algae and have not examined temporal variation in the quality of detritus and algae. This is despite hypotheses that diurnal variation in the quality of dietary resources can influence the feeding patterns of fish that feed on the EAM (Polunin and Klumpp 1989, Horn et al. 1990, Zoufal and Taborsky 1991, Bruggemann et al. 1994b) and that seasonal variation in productivity by microbes and filamentous algae may influence the composition and nutritional value of detritus.

In this chapter I assess the nutritional value of detritus and filamentous algae in blenny territories by comparing their organic and biochemical compositions. Samples were collected from the EAM within the territories of the salariin blenny, *Salarias patzneri*. This blenny ingests predominantly detritus and relatively small amounts of filamentous algae, which is typical of most salariin blennies (Chapter 1). Detritus and filamentous algae from *S. patzneri* territories were assayed for total organic, protein, carbohydrate and lipid content, which were used to calculate protein/energy ratios. Temporal variation in the nutritional value of detritus and filamentous algae also was assessed by collecting samples diurnally, during the summer and winter.

2.2 Methods

2.2.1 Sample collection

Detritus and algal samples were collected from the territories of *Salarias patzneri* in the Lizard Island lagoon (14° 42'S, 145° 30'E). Twenty-four territories

were sampled during August 1998 (Austral winter) and a further twenty-four during February 1999 (Austral summer). To assess diurnal changes in the nutritional quality of detritus and algae, sample collection was divided evenly between early morning (0600-0900), late morning (0900-1200), early afternoon (1200-1500) and late afternoon (1500-1800).

Loose particulate material was collected from the EAM within *Salarias patzneri* territories using an underwater vacuum fitted with a soft brush (Purcell 1996). Loose sediment and detritus particles were gently brushed off a 200cm^2 section of the territory, filtered through a 125μm sieve and allowed to settle for one hour before excess water was decanted. Sieving removed large algal filaments that may have been collected incidentally and produced samples of a particle size representative of those on which *S. patzneri* preferentially feeds (Chapter 1). The sieved samples were collected on pre-weighed and pre-combusted GF/F filters, briefly washed with fresh water of neutral pH to remove sea salt, then frozen. Particulate matter >125μm was also washed with fresh water, frozen and later used to calculate total weight and organic content of the EAM.

After loose particles had been removed from the 200cm^2 section of a *Salarias patzneri* territory, the filamentous algae that were still attached to the substratum were scraped off with a metal pipe and collected using the underwater vacuum. These samples were washed to remove any adherent detritus and sea salt, then frozen. These samples will be referred to as algae or algal samples.

2.2.2 Sample analyses

2.2.2a Visual analysis

Reliable biochemical comparisons of detritus and filamentous algae are dependent on obtaining distinct samples of detritus and algae from the EAM. Therefore, all particulate (<125μm and >125μm) and algal samples were examined under a microscope following the technique of Wilson and Bellwood (1997) and as described in Chapter 1. For each sample the prominent item from 50 quadrats was identified and placed in one of the following categories: detrital aggregates, inorganic sediment, filamentous algae, cyanobacteria, diatoms, foraminifera, copepods or other invertebrates. Detrital aggregates were defined as non-living organic matter with associated autotrophic and heterotrophic microbes (Bowen 1979). In addition, algal samples were categorised according to the functional groups described by Steneck and Watling (1982). In each quadrat all functional groups of algae were recorded and results expressed as the percentage of quadrats where each group was present. After examination, all samples were refrozen and freeze-dried.

2.2.2b Organic content

Particulate and algal components of the EAM collected from *Salarias patzneri* territories (including those portions used in visual analyses) were freeze-dried to determine the weight of each EAM component. A sub-sample of each dried sample was weighed to 0.00001g on a Mettler AE240 balance, then treated with bleach (>70% sodium hypochlorite) to remove organic matter following the procedure of Wilson and Bellwood (1997). After bleach had dissolved the organic matter, samples were rinsed with tap water to remove excess bleach, freeze dried and reweighed. The difference in dry weights before and after bleaching represented the estimated mass of

organic matter in the samples. The total organic content of particulate and algal samples was calculated by multiplying the total weight of each EAM portion by their respective organic contents.

2.2.2c Biochemical analyses

For each biochemical analysis a freeze dried sample of particulate matter <125μm or algae collected from each *Salarias patzneri* territory was ground into a powder with a glass mortar pestle and weighed in a glass vial. Glassware used in all biochemical analyses was acid-washed and rinsed with distilled water prior to use, and all reagents were Analar grade. Spectrophotometric work used for protein and carbohydrate analyses were carried out using a Beckman DU-64 spectrophotometer.

The protein content of samples was determined using a Sigma^{TM} micro BCA testing kit, following the procedures of Ngyuen and Harvey (1994) for the extraction and analysis of total proteins. Extracted and prepared samples were placed in the spectrophotometer, which was set at 562 nm, and protein concentration calculated by comparing absorbance readings to a standard curve, constructed from different concentrations of bovine serum albumin.

To assess carbohydrate content, freeze dried samples were analysed following the procedure described by Gerchakov and Hatcher (1972) for colorimetric determination of sugars. Prepared samples were placed in the spectrophotometer and absorbance read at 485 nm. Carbohydrate concentration of each sample was then calculated from comparisons of sample readings to a standard curve, constructed from different concentrations of anhydrous D-glucose.

The lipids of freeze dried particulate matter <125 μm and algal samples were extracted following the technique of Bligh and Dyer (1959). Extracted lipids were

filtered through combusted GF/C filter paper to remove particulate matter, concentrated by rotary evaporation and stored in 2 ml vials. A 10 µl aliquot was taken from the lipid extract, the solvent evaporated on a hotplate set at 30°C and the remaining lipid weighed on a Perkin Elmer microbalance. Three to four 10 µl aliquots of lipid extract were weighed for each sample and total lipid content calculated from an average lipid content of these aliquots.

The concentration of protein, carbohydrates and lipids were standardised relative to the organic content, as algal samples contained a high percentage of inorganic sediment that was an artifact of sampling. The energetic value of samples was estimated from protein, carbohydrate and lipid content of samples, using published conversion factors (Henken et al. 1986). This method of estimating energy content was used in preference to combustion in a bomb calorimeter, as it does not include energy from refractory material and therefore provides a realistic estimate of energy that is available for assimilation by blennies.

2.2.3 Statistical analysis

Seasonal differences in the organic and biochemical composition of particulates <125µm and algal samples were examined using ANOVA. Sample type (particulates <125µm or algae) and seasons (summer or winter) were fixed factors, and time of day was entered as a co-variate. The percentage of EAM organic matter was arcsine transformed (Zar 1999) and the other biochemical variables ($\log_{10}$ x+1) transformed before analysis. Transformations ensured data meet the assumptions of ANOVA, which were tested using Levene's test and Q-Q plots. Sequential Bonferonni corrections (Rice 1989) were used to reduce the increased probability of type 1 error that is associated with multiple comparisons.

2.3 Results

2.3.1 Visual analysis

Visual examination of particulates <125μm found that the main organic component of samples collected in the summer and winter was detrital aggregates (Table 2.1). The detritus was amorphous in appearance, lacking any definitive structure that could be used to identify its source (Plate 2.1). Filamentous algae were present in these samples although this category was dominant in less than 5% of the quadrats observed in the summer and winter samples. Diatoms, predominantly from the order Pennales, were also seen in samples from both seasons, as were filaments of cyanobacteria; however, these items were a minor component and usually accounted for less than 1 % of samples. Foraminiferans and copepods were the main invertebrates seen, although they were also a minor component of samples. Other invertebrates included coral spat, bryozoans and nematodes. The high percentage of detrital aggregates and low percentage of filamentous algae in particulates <125μm meant they were a good representation of detritus in the EAM.

Particulate matter >125μm contained a higher percentage of detrital aggregates than particles <125μm, particularly in samples collected during the summer (Table 2.1). However, it was estimated that filamentous algae accounted for up to 46% of particulates >125μm and mean filamentous algae content in these samples was 4-5 times higher than in particulates <125μm. Consequently, particulates >125μm independently do not represent detritus or filamentous algae collected from the EAM and were excluded from biochemical analyses.

Table 2.1 Visual analysis of particulates collected from *Salarias patzneri* territories during the summer and winter. Values are the mean percentage of quadrats where each category is dominant (50 quadrats per sample). Figures in parentheses are standard errors based on a sample size of 24.

	< 125 µm		>125µm	
	Summer	**Winter**	**Summer**	**Winter**
Detrital Aggregates	39.1 (2.7)	31.3 (1.6)	48.2 (3.8)	34.0 (4.9)
Sediment	52.9 (3.1)	59.8 (2.2)	36.9 (4.5)	44.5 (6.4)
Filamentous Algae	3.6 (0.5)	4.2 (0.8)	14.5 (3.0)	20.9 (3.4)
Cyanobacteria	0.9 (0.3)	1.3 (0.3)	0	0
Diatom	0.9 (0.2)	0.6 (0.2)	0	0
Foraminifera	0.7 (0.3)	0.7 (0.2)	0	0.2 (0.1)
Copepod	0.2 (0.1)	0.3 (0.2)	0.2 (0.1)	0.5 (0.3)
Other Invertebrates	0.1 (0.1)	0.1 (0.1)	0.2 (0.1)	0

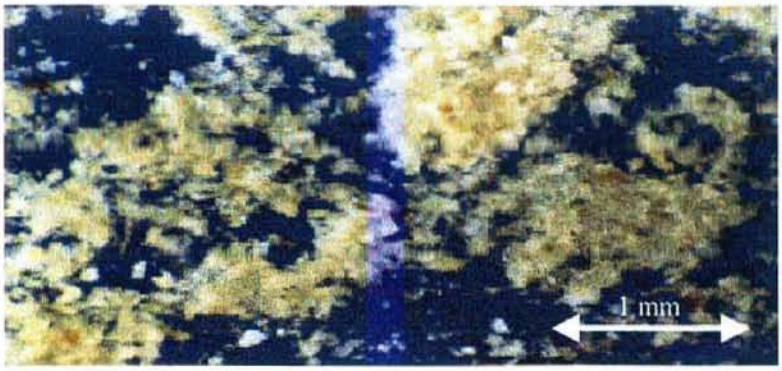

Plate 2.1 Particulates <125µm; sample taken from the territory of a *Salarias patzneri.*

Filamentous algae were the major items in algal samples (Table 2.2) and, although detrital aggregates were present (Plate 2.2), they rarely dominated more than 15% of the quadrats in a sample. Filamentous algae were present in almost all of the quadrats from all samples (Table 2.3), emphasising the prevalence of this functional group. Closer examination of several samples identified genera from the Rhodophyta (*Polysiphonia* sp, *Laurencia* sp, *Champia* sp, *Gelidiella* sp, *Ceramium* sp), Phaeophyta (*Sphacelaria* sp *Sargassum* sp, *Dictyota* sp) and Chlorophyta (*Derbesia* sp). Encrusting and corticated algae were also frequently seen in algal samples, whilst macro-, foliose and calcareous algae were rarely seen in samples.

Table 2.2 Visual analysis of algal samples collected from *Salarias patzneri* territories during the summer and winter. Values are the mean percentage of quadrats where each category is dominant (50 quadrats per sample). Figures in parentheses are standard errors based on a sample size of 24.

	Summer	**Winter**
Detrital Aggregates	13.8 (1.5)	6.6 (0.9)
Sediment	37.8 (4.3)	37.6 (5.0)
Algae	48.4 (4.3)	55.8 (5.1)

Table 2.3 Presence of algal functional groups in algal samples collected from *Salarias patzneri* territories during the summer and winter. Values are the mean percentage of quadrats where each functional group is present (50 quadrats per sample). Figures in parenthesis are standard errors based on a sample size of 24.

	Summer	Winter
Filamentous	96.4 (1.0)	96.7 (1.5)
Foliose	0.7 (0.5)	5.1 (1.7)
Corticated	4.7 (1.3)	15.0 (3.6)
Macro	1.0 (0.6)	0.9 (0.4)
Calcareous	0.8 (0.3)	2.1 (0.9)
Encrusting	17.9 (4.1)	24.3 (3.2)

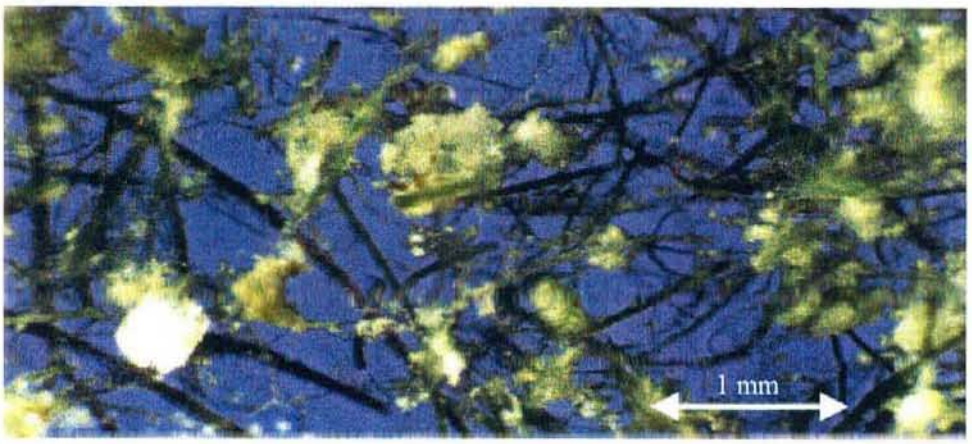

Plate 2.2 Algal sample taken from the territory of a *Salarias patzneri.*

2.3.2 Organic Content

Although the organic content of particulates <125μm was lower than algal samples, the particulates <125μm represented a much larger proportion of the EAM weight than algae (Table 2.4). Consequently, particulates <125μm accounted for 41 ± 2 % of the organic matter present in the EAM during the summer and 44 ± 3 % of the total organic matter in the winter (Figure 2.1). Algal samples collected during the summer accounted for 37 ± 3% of the total organic matter and 41 ± 3% of the organic matter in the winter. The slightly higher contribution to the total organic matter in the EAM by particulates <125μm was non-significant. There was also no significant effect due to time of day when samples were collected, season, or the interaction between season and sample type.

Table 2.4 Mean organic content and contribution to total EAM weight (g) of particulates <125μm (detritus) and algal samples. Organic content is expressed as a percentage of the sample weight. Figures in parenthesis are standard errors, calculated from 24 samples.

	Summer		**Winter**	
	Detritus	**Algae**	**Detritus**	**Algae**
% Organic matter	15.4 (2.4)	22.9 (2.7)	20.6 (3.2)	31.5 (3.0)
% EAM weight	49.7 (3.1)	26.8 (2.7)	44.8 (3.1)	29.6 (4.1)

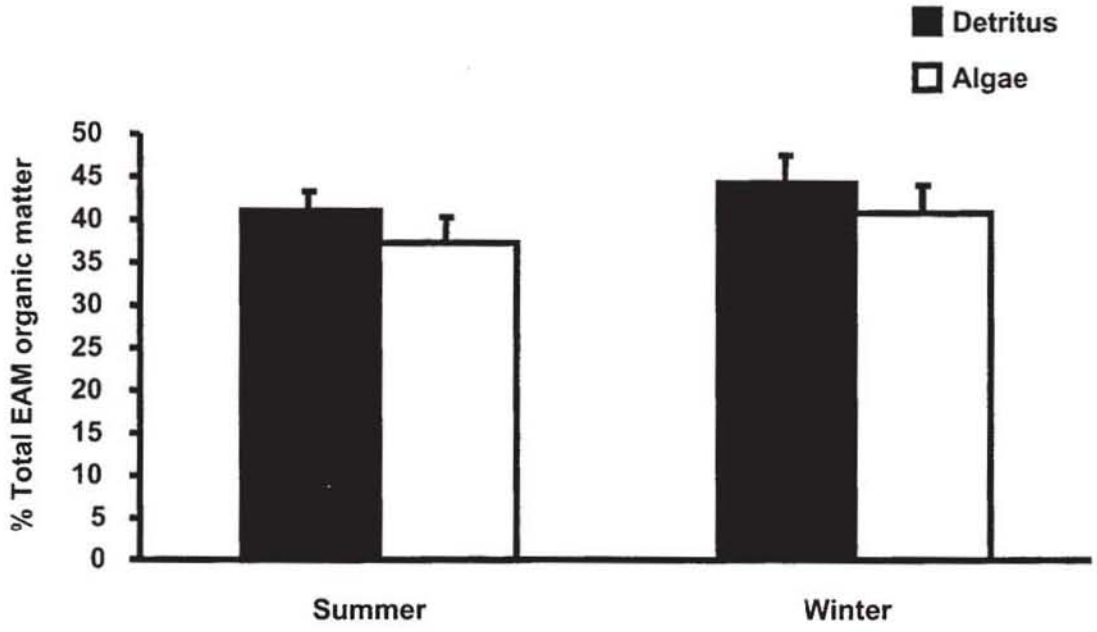

Figure 2.1 Distribution of organic matter in the EAM of *Salarias patzneri* territories during the summer and winter. Detritus = particulates <125μm. Values are means ± SE, n =24.

2.3.3 Biochemical analyses

The concentration of the three principal biochemical components, protein, carbohydrate and lipid, was higher in algae than particulates <125μm (Figure 2.2). At an alpha level of 0.05, this difference is significant for all three variables; however, when sequential Bonferroni adjustments were made to account for multiple comparisons, carbohydrate and lipid concentrations were the only variables for which significant differences were detected (Table 2.5). Seasonally, the concentrations of protein, carbohydrate and lipid were higher in summer samples (Figure 2.2) although only the protein concentrations were found to be significantly different (Table 2.5). For all three of these biochemical variables there was no significant interaction detected between sample type and season, nor was there any significant effect detected resulting from the time of day at which samples were collected.

Table 2.5 Summary of ANOVA statistics where type (particulates <125μm or algae) and season (summer or winter) were fixed factors, and time of day was entered as a co-variate. (*Carbo*, carbohydrate).

	Type		**Season**		**Type x Season**		**Time of Day**	
	$F_{1,91}$	**P**	**$F_{1,91}$**	**P**	**$F_{1,91}$**	**P**	**$F_{1,91}$**	**P**
Protein	4.885	0.030 *	12.038	0.001**	0.431	0.513	2.954	0.089
Carbo	19.572	<0.001**	5.875	0.017*	0.923	.0339	3.852	0.053
Lipid	7.285	0.008**	5.622	0.020*	0.395	0.513	0.012	0.914
P/E	5.009	0.028*	3.225	0.076	0.006	0.938	0.112	0.738

* significant at α=0.05, ** significant at the table-wide significance level.

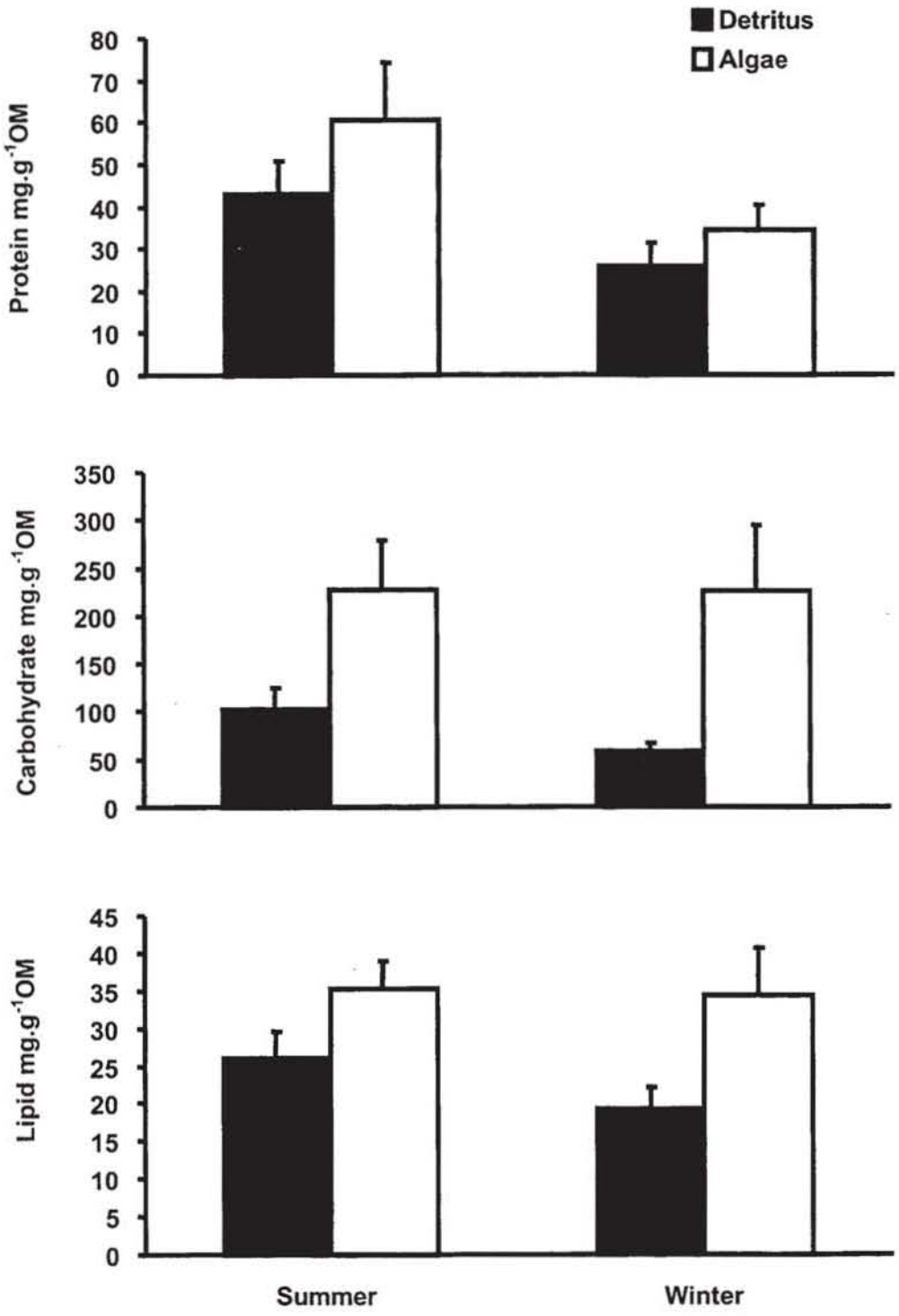

Figure 2.2 Concentration of the principal biochemical components in particulates <125μm (detritus) and algal samples collected from the territories of *Salarias patzneri* during the summer and winter. (Mean ± SE, n=24).

Protein:energy ratios in particulates <125μm were slightly higher than in algal samples collected from both seasons (Figure 2.3). However, this difference was non-significant when α levels were adjusted using sequential Bonferroni corrections (Table 2.5). In the summer, protein:energy ratios were 11.3 ± 0.8 mg.Kj^{-1} and 9.1 ± 0.8 mg.Kj^{-1} for particulates <125μm and algae samples, respectively. Compared to the summer, winter protein:energy ratios were slightly lower in both particulates <125μm (10.3 ± 1.3 mg.Kj^{-1}) and algae (7.9 ± 1.0 mg.Kj^{-1}); however, this difference was non-significant (Table 2.5). Similarly, there was no significant effect of time of day or the interaction between season and sample type.

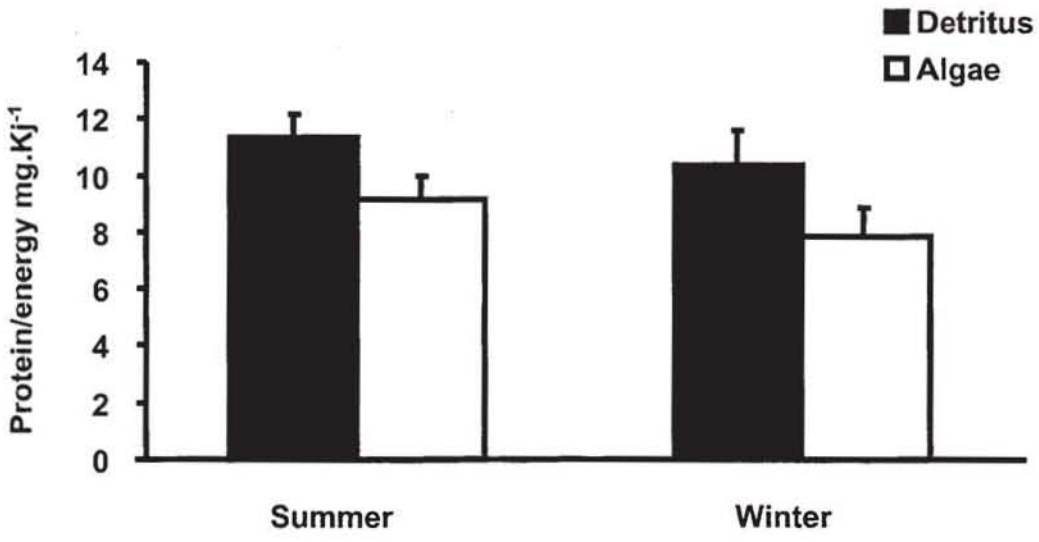

Figure 2.3 Nutritional quality of particulates <125μm (detritus) and algae samples collected from the territories of *Salarias patzneri* during the summer and winter, as measured by protein/energy ratios. (Mean ± SE, n=24).

2.4 Discussion

Detrital aggregates of amorphic appearance were the dominant organic items in particulate matter <125μm collected from *Salarias patzneri* territories. The prevalence of detrital aggregates and low percentage of filamentous algae in particulates <125μm meant that these samples provided a good representation of detritus in the EAM. Amorphic detritus, which is derived from dissolved organic matter, is typically of a higher nutritional quality than morphic detritus, which originates from decomposing organic matter. Comparisons of C:N ratios and protein content in these two detrital forms have found that amorphic detritus has lower C:N ratios and a higher protein content (Alber and Valiela 1994). Furthermore, shrimp, *Palaemonetes pugio,* and minnow, *Cyprinodon variegatus,* fed both forms of detritus were found to assimilate 10-40 times more nitrogen from the amorphic detritus (D'Avanzo et al. 1991). The amorphic appearance of the detritus collected from *Salarias patzneri* territories therefore suggests that these detrital aggregates may be of a high nutritional quality and easily assimilated. Consequently, it is not surprising that these aggregates constitute the bulk of material ingested by salariin blennies and that they are preferentially ingested (Chapter 1).

Filamentous algae dominated the algal samples collected from the territories of *Salarias patzneri*. According to Steneck and Watling (1982), filamentous algae are a functional group with low thallus toughness that are easily grazed compared to most of the other functional algal groups, the exception being microalgae. However, filamentous algae may represent a variety of different algal species (Russ 1987, Klumpp and Polunin 1989, Price and Scott 1992), and many algal species, including members of the genera *Laurencia* and *Dictyota,* which were identified in algal samples collected from *S. patzneri* territories, are capable of producing secondary

metabolites that inhibit grazing or digestion if consumed (Hay 1991). The concentration of secondary metabolites in algae is likely to vary taxonomically, spatially and temporally (Hay 1991). It is however, possible that younger portions of some algae have higher concentrations of secondary metabolites suitable for deterring grazing (Hay 1991). This may mean that young "filamentous" stages of algae found in the EAM have relatively high concentrations of metabolites that deter grazing.

In contrast, detrital particles that originate from algae may lose secondary metabolites that are associated with algae (Duggins and Eckman 1997) making detritus more suitable for microbial colonisation and consumption by fish. Amorphic detritus, which was prevalent in detritus samples, may also contain less refractory material than filamentous algae, because it is believed to originate largely from dissolved organic matter. Furthermore, the small size of detrital particles (<125μm) means that they have a high surface area to volume ratio, which increases the area available for microbial colonization and abundance (Yamamoto and Lopez 1985), and this may improve the nutritional value of detritus (Bowen 1987). Relatively greater surface area also may aid the processing and digestion of small detrital particles, possibly improving their assimilation by blennies. Thus, small particle size, reduced concentrations of secondary metabolites and lower refractory content may improve the effective nutritional value of detritus relative to filamentous algae.

The consistently high contribution of detritus to the total organic content in *Salarias patzneri* territories, coupled with a lack of temporal variation in the organic contribution to the EAM by detritus, suggests that detritus is a prominent and temporally stable component of the EAM within blenny territories. When compared to algae, detritus samples from *Salarias patzneri* territories represented a slightly higher percentage of the total EAM organic matter. This difference was non-

significant, however, a high organic contribution from fine detrital aggregates (<125μm) also has been documented in EAM samples collected from pomacentrid territories in the Lizard Island lagoon (Wilson and Bellwood 1997) and within EAM samples collected from an exposed reef at Lizard Island (Purcell and Bellwood 2001). Interestingly, filamentous algae represented a higher percentage of the total organic matter in EAMs collected from exposed reef crests and this difference may be partially related to higher wave energy on exposed locations reducing the overall detrital biomass (Crossman et al. In press). Alternatively, as the composition of algae inside and outside fish territories may vary (Lassuy 1980, Montgomery 1980), it is also possible that the type of algae inside blenny and pomacentrid territories may favour the accumulation of detritus, resulting in a relatively higher detrital biomass within fish territories.

Particulates <125μm collected from *Salarias patzneri* territories had a high percentage of organic matter when compared to soft sediment samples collected from coral reef lagoons (see Koop and Larkum 1987). The organic content of detritus samples in this study was, however, similar to sediments collected from the windward reef crest at Lizard Island, the reef zone where the organic content of sediments was greatest (Purcell and Bellwood 2001). The organic content of detritus is one way of assessing nutritional quality (Bowen 1987) and relatively high organic content in detrital samples collected from *S. patzneri* territories and on the reef crest suggests that detritus in both these areas may be of high nutritional quality.

As detritivores are expected to have high consumption rates (Bowen et al. 1995), a consistently high standing biomass of detritus in areas of intense detritivore feeding also suggests high rates of detrital accumulation. It has been estimated that a medium sized blenny (2.98g wet weight) ingests 0.024g.C.day^{-1}(Klumpp and Polunin

1989), whilst estimates of detrital deposition within coral reef lagoons vary from 1.5g.C.m^{-2}.day^{-1} at One Tree Island (Koop and Larkum 1987) down to 0.009 g.C.m^{-2}.day^{-1} at Davies reef (Hansen et al. 1992). Such high levels of variation may mean it is necessary for blennies to locate their territories in areas with hydrodynamic or biotic features that favour the accumulation of detritus. The distribution of blennies relative to detritus on coral reefs will be further investigated in Chapter 5.

Algal production also is likely to vary spatially (Klumpp and Mckinnon 1989) and has been estimated at 1.2 to 3.7g.C.m^{-2}.day^{-1}in pomacentrid territories (Russ 1987, Klumpp and Polunin 1989). Accumulation rates of detritus therefore appear low in comparison to algal production. However, such comparisons may be inappropriate, as it is likely that only a proportion of the detritus in the EAM is accounted for when sediment traps are used to estimate rates of detritus accumulation. Some of the detritus within the EAM may be produced within the boundary layer surrounding filamentous algae. Within this layer, dissolved organic matter (DOM), which is released by algae (Aluwihare and Repeta 1999), is likely to be at a higher concentration than the surrounding waters. Relatively high concentrations of DOM in the boundary layer may be converted rapidly to particulate matter by physical processes (Chin et al. 1998) or bacteria (Azzam et al. 1983). A similar mechanism of detrital production has been proposed to explain the origin of particulate matter from phytoplankton in the oligotrophic waters of open oceans (see Mann 1988). It is therefore reasonable to hypothesise that a similar mechanism of detrital production may occur within the EAM. Detritus formed in this manner is, however, unlikely to be included in estimates of detrital deposition calculated from settlement trap data, as traps only sample large particulate organic matter that settles from the water column. Furthermore, settlement traps may not collect very fine organic particles that remain

suspended in the water column but may adhere to mucus excreted by algae. Consequently, current estimates of detrital deposition may underestimate a large and nutritionally important portion of the organic matter within the EAM.

Particulate matter <125μm collected from *Salarias patzneri* territories had a slightly higher protein to energy ratio than algal samples and was greater than those proposed by Bowen (1979) as the minimal requirements for fish growth. This finding supports data from previous comparisons of detritus and algae that have shown detritus to have C:N ratios lower than algae collected from the same EAM and that C:N ratios of detritus are theoretically capable of supporting fish growth (Wilson and Bellwood 1997, Purcell and Bellwood 2001).

Bowen et al. (1995) predicted that protein is the most important nutritional constraint to growth of detritivorous fishes. The protein content of particulates <125μm and filamentous algae collected from *Salarias patzneri* territories was very similar although algae tended to have a slightly higher protein concentration. The protein content of detritus from *S. patzneri* territories however, may, underestimate the nutritional value of this resource, as organic matter unaccounted for by protein, carbohydrates or lipids may contain non-protein amino acids, which are readily assimilated by detritivorous fish (Bowen 1980). In a comparison of detritus and algae collected from the Great Barrier Reef, Crossman et al. (In press) found the concentration of total amino acids in detritus was, on average, twice that of algae collected from the same EAM. Consequently, although protein levels in detritus are slightly lower than algae collected from *S. patzneri* territories, detritus may represent a better source of dietary amino acids than algae.

This study has only examined seasonal change over a period of one year and further replication is required to identify seasonal patterns in protein concentrations of

algae and detritus. Nonetheless, temporal variation in the protein content of detrital samples may be associated with seasonal blooms and increased productivity of microalgae (Faganeli et al. 1995) and bacteria (Moriarty et al. 1985, Hansen et al. 1992), which are both potentially rich sources of protein (Simon and Azzam 1983, Lourenco et al. 1998). A higher protein content in algal samples collected in the summer also may contribute to protein levels in the particulates <125μm, although it also is possible that higher algal protein levels are a consequence of increased nutrient levels in the detritus. Irrespective of causative effects, relatively higher protein concentrations in the summer have important ecological implications, as it is during this period that many fishes increase reproductive output (Thresher 1984) and subsequent recruitment to coral reefs is likely to be high (Doherty 1991, Caley 1995). The feeding intensity of detritivorous/herbivorous fish may also increase in the summer (Polunin and Klumpp 1992, Bellwood 1995, Chapter 1), and therefore maximum use of dietary resources in the EAM coincides with the season when protein content is high.

In summary, a biochemical comparison of the particulates <125 μm and filamentous algae collected from the territories of the blenny, *Salarias patzneri,* found that detritus is at least equivalent and probably superior to filamentous algae as a dietary resource. Protein to energy ratios in detritus samples are slightly higher than algae and indicate that detritus is capable of supporting growth in fish. As detrital aggregates resemble amorphic detritus biochemically and visually, they are also less likely to contain secondary metabolites or refractory material that may be present in algae and can impede digestion. Detritus in *S. patzneri* territories represents a larger proportion of the total organic matter than filamentous algae, and a high organic content of detritus advocates that detritus is of a high nutritional value. These results

support those of recent comparisons between detritus and algae that have found amorphic detritus to be a biochemically viable and abundant dietary resource. As many of the fish species that feed on the EAM ingest large quantities of detritus, these results suggest that detritus may play an important role in satisfying their dietary requirements and that detritus contributes significantly to secondary production by these fishes.

Chapter 3. Identifying sources of organic matter in sediments from the territory of a detritivorous coral reef fish.

Published in Organic Geochemistry 32: 1257-1269.

3.1. Introduction

Detritivorous fishes are an integral part of coral reef foodwebs and are partially responsible for the transfer of organic matter within sediments to secondary consumers. In order to satisfy basic dietary requirements, detritivorous fish ingest large volumes of organic matter, selectively feeding in areas of high quality detritus (Bowen et al. 1995). Detritus is the major source of nitrogen, protein and organic matter in the territories of several species of prominent coral reef fishes (Wilson and Bellwood 1997, Chapter 2). However, the amorphous nature of this detritus means that visual analysis alone is insufficient to identify its sources. Consequently, a large portion of the food web on coral reefs is poorly defined and often overlooked.

An alternative to visual analysis of organic matter is the extraction and identification of lipid biomarkers from detritus in the sediments. Lipids in marine sediments are a combination of compounds that reflect the composition and origin of the organic detritus. Many of these lipid compounds are characteristic of marine organisms and some compounds are unique to particular organisms. Consequently, these lipid biomarkers, can be used to trace the source of organic matter in sediments and in some cases quantify the contribution of different organisms to the detrital load.

Many lipid biomarkers originate from more than one source, making identification and quantification of lipid sources difficult, particularly when based on only one compound. Consequently, identification and quantification of lipid sources are best made on the presence of several characteristic biomarkers. Combinations of

lipid biomarkers have been used extensively to identify material in sediments from deep waters, estuaries and lakes (e.g. Saliot et al. 1991). However, the analysis of lipids in detrital material collected from coral reefs is limited to a few studies (Shaw and Johns 1985, Volkman et al., 1986 and 1992, Currie and Johns 1988, Johns et al. 1994) and there are no studies of sediments collected from the epilithic algal community, where most detritivorous/herbivorous reef fishes feed (Hatcher 1983b, Choat 1991).

For the purposes of this study, lipids were extracted and analysed from sediment or detrital samples within the territory of the salariin blenny, *Salarias patzneri.* Salariin blennies are often abundant on coral reefs (Townsend and Tibbetts 2000), and *S. patzneri* is typical of most species, as it predominantly ingests detrital aggregates (Chapter 1). *Salarias patzneri* also restricts its feeding to relatively small territories (pers. ob.), which is similar to other blennies (Nursall 1977, Gonclaves and Almada 1998). Detritus therefore can be sampled easily from feeding areas within *S. patzneri* territories.

The primary aim of this chapter was to use lipid biomarkers to identify organic inputs to the detritus on which *S. patzneri* feeds. Based on the high productivity of filamentous algae in the EAM, relatively small standing crop (Larkum 1983), and poor assimilation of algae (Horn 1989), it is often proposed that algae are a major source of detritus on coral reefs (Hansen et al. 1992). As detritus is associated closely with filamentous algae and these algae are believed to be a major source of detritus on coral reefs samples of filamentous algae also were collected from *S. patzneri* territories and analysed for lipid biomarkers. This allowed comparison of detritus and algal lipids and assessment of filamentous algae input to detrital organic matter.

3.2 Methods

3.2.1 Sample collection

Detritus and algal samples were collected from areas in *Salarias patzneri* territories where the resident blenny had been observed feeding. The territories were located on the top of contiguous reef within the Lizard Island lagoon (14° 42'S, 145° 30'E) at a depth of 1-2m. Samples were collected from seven different territories during August 1997 (Austral winter) and a further seven territories in February 1998 (Austral summer). Detritus and algal samples were collected using an underwater sampling device (Purcell 1996) and followed the procedures outlined in Chapter 2.

Sub-samples of the detritus and algae in *Salarias patzneri* territories were used to determine total organic content. The freeze-dried samples were soaked in bleach (>70% sodium hypochlorite) to remove organic matter, and the difference in dry weights before and after bleaching was used to estimate the total organic content of the samples after Wilson and Bellwood (1997).

3.2.2 Extraction

Freeze-dried samples of detritus and algae were ground into a powder using a glass mortar and pestle. The ground samples were weighed, then extracted using the single-phase $CHCl_3$- CH_3OH technique of Bligh and Dyer (1959). Samples were extracted overnight then separated into two phases with the addition of $CHCl_3$ and H_2O. The lower phase was retained and poured through a glass funnel lined with a pre-combusted GF/C filter, which removed particulate matter. This filtrate, or total solvent extractable lipids (TSE) was concentrated by rotary evaporation and made up to 2 ml. To estimate the total lipid content, a 10 µl aliquot of TSE was placed on a tared aluminium pan and the solvent evaporated at approximately 30°C. The

remaining lipid was weighed using a Perkin Elmer microbalance. This procedure was repeated 3-4 times for each sample and total lipid content calculated from the average of these values.

The remaining TSE was reduced to dryness under nitrogen and saponified with 5% w/v KOH in 80% CH_3OH (2 ml, 3 hrs, 60° C). After cooling and addition of Milli-Q water (1 ml), the saponified samples were extracted with hexane/$CHCl_3$ (4:1 v/v, 2 ml, 3 times). The upper organic layer contained the total saponified neutrals (TSN) and the lower aqueous layer contained the total fatty acids (TFA). The TSN were separated into sterol and hydrocarbon fractions by column chromatography (3 g fully active alumina). The hydrocarbon fraction was eluted with 35 ml of hexane and 40 ml of 20% v/v CH_2Cl_2 in hexane. Sterols were eluted with 20 ml of $CHCl_3$ and converted to their trimethylsilyl ethers with BSTFA (50 µl, 2 h, 60° C).

The aqueous layer containing the total fatty acids was acidified with concentrated HCl, and the fatty acids were extracted with hexane/$CHCl_3$ (4:1 v/v, 2 ml, 3 times). Solvent was removed under a stream of nitrogen and fatty acids methylated with a solution of $CH_3OH/CHCl_3$/HCl (10:1:1 3 ml) for 1 hour at 100°C. The solution was allowed to cool and 1 ml of Milli-Q water added before the fatty acid methyl esters (FAMEs) were extracted with hexane/$CHCl_3$ (4:1 v/v, 2 ml, 3 times).

The FAME and hydrocarbon fractions were analysed on a Carlo Erba 8080 gas chromatograph with a 30 m x 0.32 mm I.D. J&W Scientific DB5-MS capillary column and a flame ionisation detector. FAMEs and hydrocarbons were identified primarily by comparing retention times to those of known standards. The sterols and several samples of the FAMEs and hydrocarbon fractions were analysed on a Hewlett Packard 5972 gas chromatograph –mass spectrometer with a similar capillary column.

Sterols and phytol within this fraction were identified by comparison of spectra with previously published mass spectral data (e.g. Jones et al. 1994). Mass spectra of FAMEs and hydrocarbon fractions were used to confirm peak identifications and to identify any unknown components in the GC/FID chromatograms. The C_{25} highly branched isoprenoid (HBI) alkenes were identified using mass spectra and relative retention times from Barrick et al. (1980), Barrick and Hedges (1981), and Volkman et al. (1994).

Complete procedural blanks, surrogate (*ortho*-terphenyl and $C_{23:0}$ FA) and internal standards (*n*-eicos-1-ene) were used to assess blank contamination and compound recoveries.

3.2.3 Statistical analyses

The composition of FAMEs in samples were compared using a 2-way MANOVA, with sample type (detritus or algae) and season as fixed factors. Individual FAMEs were expressed as a percentage of the FAMEs fraction and arcsine transformed before analysis (Zar 1999). FAMEs characteristics of detritus and algal samples collected from different seasons were displayed using canonical discriminant analysis (CDA). The same statistical procedure was carried out on the hydrocarbon fraction, but not the sterol fraction, as uneven sample sizes made MANOVA analysis inappropriate due to increased probability of type 1 error (Zar 1999).

3.3 Results

The total organic content of detritus and algae samples were similar; however, the percentage of organic matter in both detritus and algae samples were much higher during the winter. When expressed in terms of organic content the concentrations of

total lipids and lipid fractions were greater in the algal samples than in detritus collected from the same season (Table 3.1). The concentrations of total lipids, free fatty acids, hydrocarbons, sterols and phytol were highest in samples collected during the summer.

Total fatty acids were the major lipid class in all samples, accounting for 6-26% of the total lipid content. Total fatty acids were particularly abundant in the summer detritus, constituting 23 ± 2 (SE)% of the total lipids in these samples. In contrast, the hydrocarbon and sterol fractions were a relatively minor component of all samples, generally accounting for less than 1% of the total lipids.

Table 3.1 Mean organic content and concentration of lipid fractions extracted from samples collected from *Salarias patzneri* territories. Values in parentheses are standard errors, based on 6 or 7 samples for the organic content, total lipids, fatty acids and hydrocarbons and 3 to 6 samples for sterols and phytol.

	Detritus (Summer)	Detritus (Winter)	Algae (Summer)	Algae (Winter)
% Organic matter	14 (1)	29 (8)	18 (3)	28 (5)
Total lipids ($mg.g^{-1}$ OM dry wt)	27 (5)	21 (5)	49 (8)	30 (8)
Total Fatty Acids ($mg.g^{-1}$ OM dry wt)	6.3 (1.0)	2.3 (0.5)	9.7 (2.2)	4.2 (0.8)
Hydrocarbons ($\mu g.g^{-1}$ OM dry wt)	214 (42)	162 (112)	657 (170)	200 (46)
Sterols ($\mu g.g^{-1}$ OM dry wt)	199 (100)	140 (23)	721 (220)	140 (23)
Phytol ($\mu g.g^{-1}$ OM dry wt)	75 (29)	66 (13)	392 (98)	166 (39)

3.3.1 Fatty Acids

Detritus samples collected during the summer and winter were dominated by the even-chain fatty acids 16:0, 16:1ω7,14:0 and 18:1ω9, in descending order of abundance (Figure 3.1). These fatty acids accounted for approximately 77% and 80% of the fatty acid composition of the detritus samples from summer and winter, respectively. The major fatty acids in algal samples collected during the winter were the same as those in the detritus although the relative abundances were different, with 16:0, 18:1ω9,14:0 and 16:1ω7 dominating in descending order. In summer, the major fatty acids of algal samples were 16:0, 14:0, 18:1ω9 and 18:1ω7, in descending order of abundance. Despite the apparent visual similarity of the major fatty acids in samples, MANOVA revealed significant differences in the profiles of fatty acids between detritus and algal samples (Pillai's trace, $p = 0.001$) and seasons (Pillai's trace, $p = 0.017$). There was no interaction between these variables.

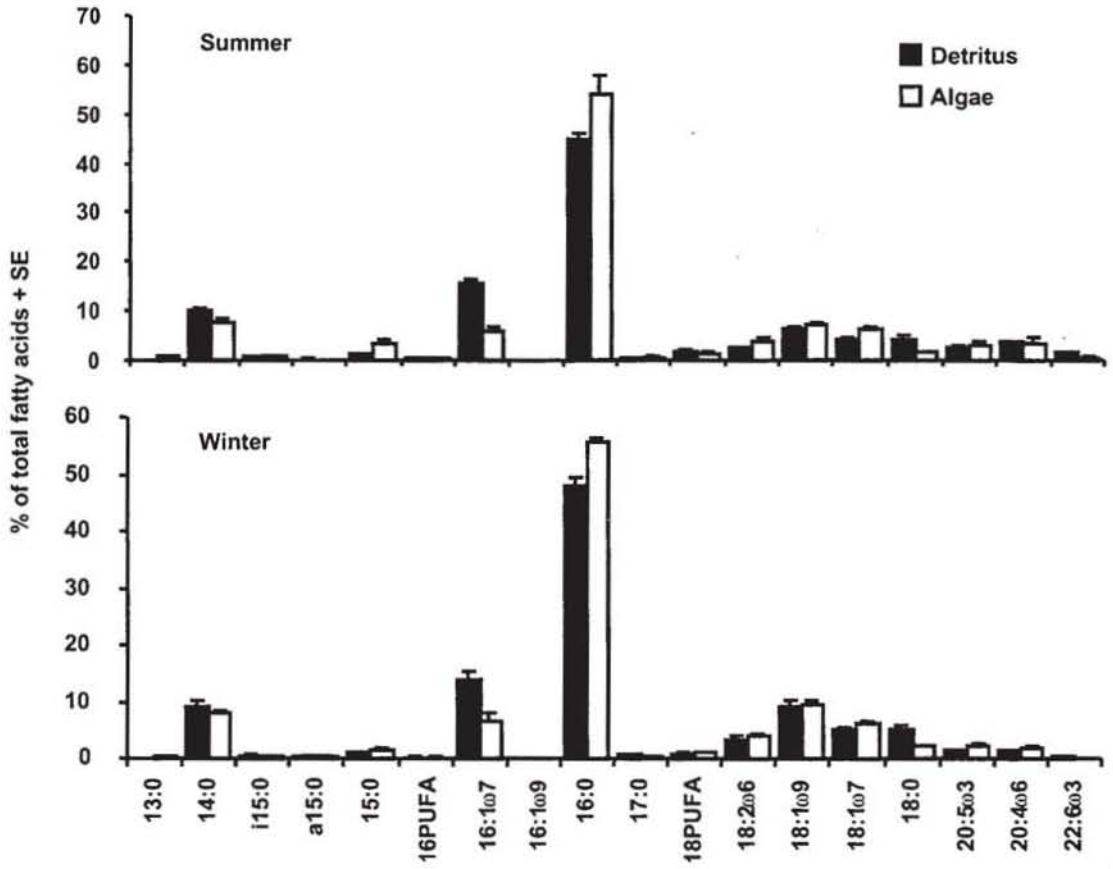

Figure 3.1 Mean percentage of fatty acids in detritus and algal samples collected from *Salarias patzneri* territories during the summer and winter. (Mean ± SE, n = 6-7).

Table 3.2 Mean percentage and ratio of fatty acids in samples collected from *Salarias patzneri* territories. Values in parentheses are standard errors, based on 6 or 7 samples.

	Detritus (Summer)	Detritus (Winter)	Algae (Summer)	Algae (Winter)
Σ Saturates	60.9 (0.9)	64.0 (0.6)	67.8 (3.8)	67.6 (1.0)
Σ Branched	0.9 (0.1)	0.7 (0.1)	0.8 (0.1)	0.6 (0.1)
Σ Monounsaturated	26.0 (0.5)	28.0 (0.4)	19.2 (0.4)	22.4 (0.7)
Σ Polyunsaturated	12.3 (1.0)	7.2 (0.8)	12.2 (3.6)	9.4 (0.7)
18:1ω7/18:1ω9	0.67 (0.02)	0.55 (0.04)	0.93 (0.13)	0.63 (0.05)
15 branched/15:0	0.70 (0.07)	0.80 (0.17)	0.24 (0.03)	0.49 (0.09)
16:1ω7/16:0	0.34 (0.02)	0.29 (0.03)	0.11 (0.03)	0.12 (0.02)
Even/Odd	43.8 (6.5)	44.5 (5.8)	21.2 (4.1)	40.4 (6.6)

Canonical discriminant analysis (CDA) was used to help assess differences in fatty acid distributions between samples (Figure 3.2). Together, the first two axes explained 99% of the variation, with the first canonical discriminant describing variation between detritus and algal samples and the second canonical discriminant describing seasonal variation. The algal samples were characterised by 16:0, 18:2ω3, 13:0 and 15:0 fatty acids, whilst detritus samples had higher amounts of 16:1ω7, 14:0, 18:0 and 22:6ω3. The higher concentration of 16:1ω7 in sediment samples meant that monosaturated fatty acids represented a greater proportion of the total fatty acids in detritus samples and that the ratio of 16:1ω7 to 16:0 was two to three times greater than algal samples collected during the same season (Table 3.2).

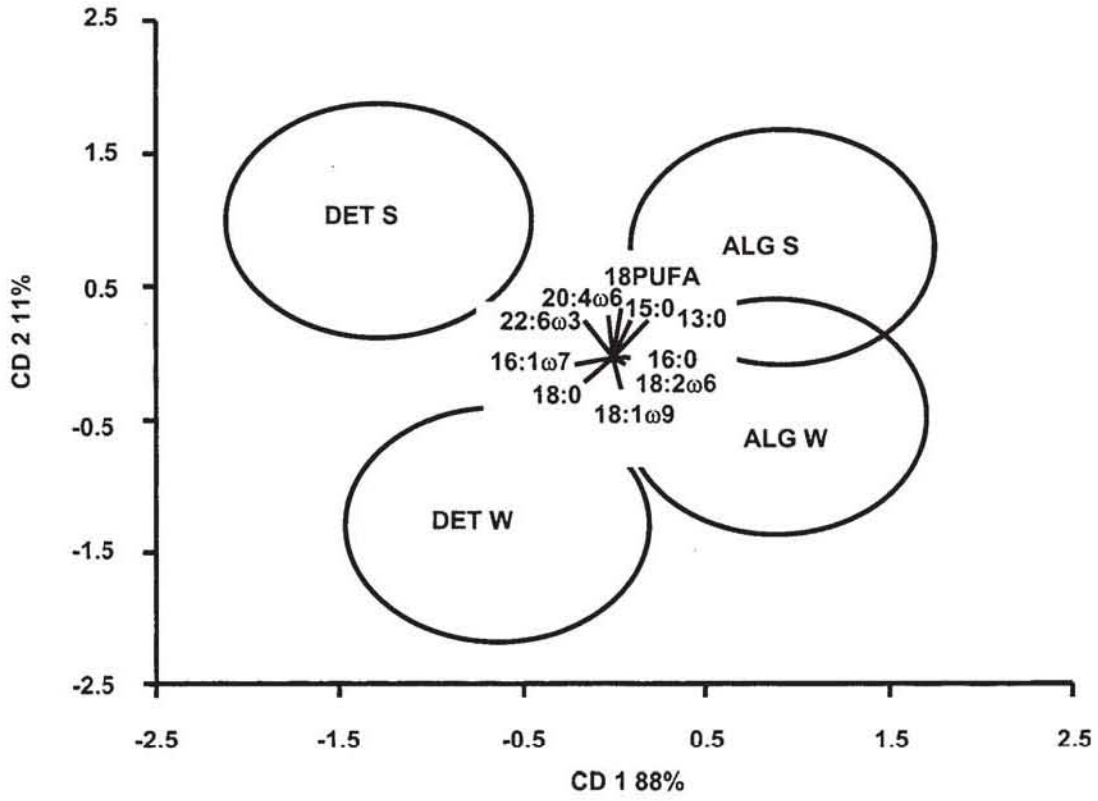

Figure 3.2 Canonical discriminant analysis of fatty acids. Ellipses are 95% confidence limits, based on a sample size of 6 to 7. ALG S = algae summer, ALG W = algae winter, DET S = detritus summer, DET W = detritus winter.

On a seasonal basis, summer samples were characterised by the polyunsaturated fatty acids (PUFA): 20:4, 20:5ω3 and 22:6ω3, as well as 15:0 and 13:0, whilst samples collected during the winter had higher concentrations of 18:1ω9 (Figure 3.2). This meant that PUFA were more abundant in samples collected during the summer, accounting for approximately 12% of the total fatty acids in sediment and algae samples (Table 3.2).

Branched fatty acids were a minor component of all samples, representing less than 1% of the total fatty acids (Table 3.2). Vaccenic acid, (18:1ω7) was also present in all samples and was most abundant in the algae although the ratio of 18:1ω7 to 18:1ω9 was less than 1 for algal and detrital samples collected in both seasons.

3.3.2 Hydrocarbons

The predominant hydrocarbons in detritus samples collected during the summer were *n*-C_{17}, *n*-$C_{18:1}$, *n*-$C_{16:1}$, and *n*-C_{15} in decreasing order of concentration (Figure 3.3). Algal samples collected during the summer also were dominated by n-C_{17} although the percentage of this n-alkane was greater than in detritus samples. Other abundant hydrocarbons in summer algal samples were *n*-C_{15}, *n*-$C_{18:1}$ and *n*-$C_{17:1}$ in decreasing order of abundance (Figure 3.3). In the winter *n*-C_{17}, *n*-$C_{18:1}$, *n*-C_{15} and *n*-C_{18} were the major hydrocarbons in the detritus and *n*-C_{17}, *n*-$C_{17:1}$, *n*-C_{15}, and *n*-C_{30} the main hydrocarbons in algal samples (Figure 3.3). There also was a high concentration of squalene in winter detritus, which can be attributed to a single sample that contained an exceptionally high concentration of this compound.

A MANOVA comparing hydrocarbon content of detritus and algae samples collected during the summer and winter detected a significant interaction between sample type and season (Pillai's trace, $p = 0.035$). CDA conducted on the relative percentage of hydrocarbons in samples explained 97% of the variation between samples with the first two axes (Figure 3.4). The algal samples were characterised by higher percentages of *n*-C_{17} and *n*-$C_{17:1}$, whilst winter detritus samples had higher percentages of *n*-C_{27}. Summer samples of both detritus and algae contained large amounts of *n*-$C_{16:1}$ and *n*-$C_{18:1}$, which can be attributed to samples taken from two of the *S. patzneri* territories.

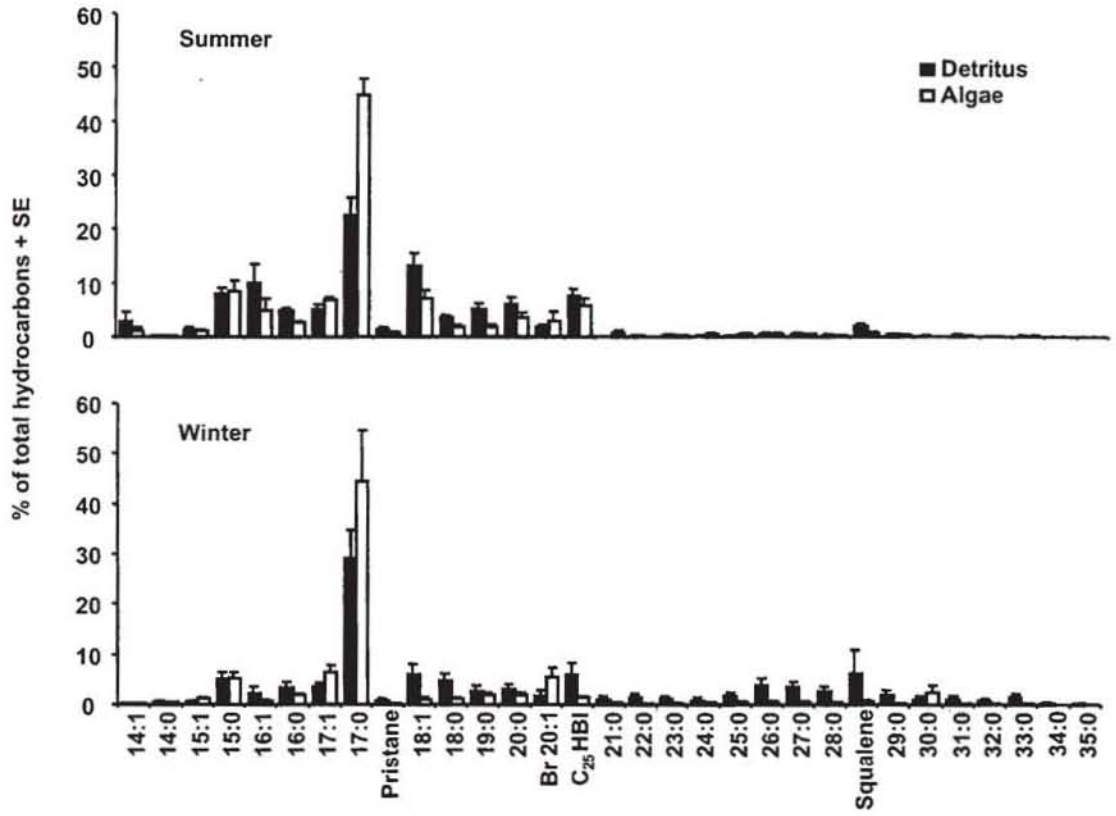

Figure 3.3 Mean percentage of hydrocarbons in detritus and algal samples collected from *Salarias patzneri* territories during the summer and winter, expressed as percentage of total hydrocarbon content. Standard errors are based on sample size of 6 to 7.

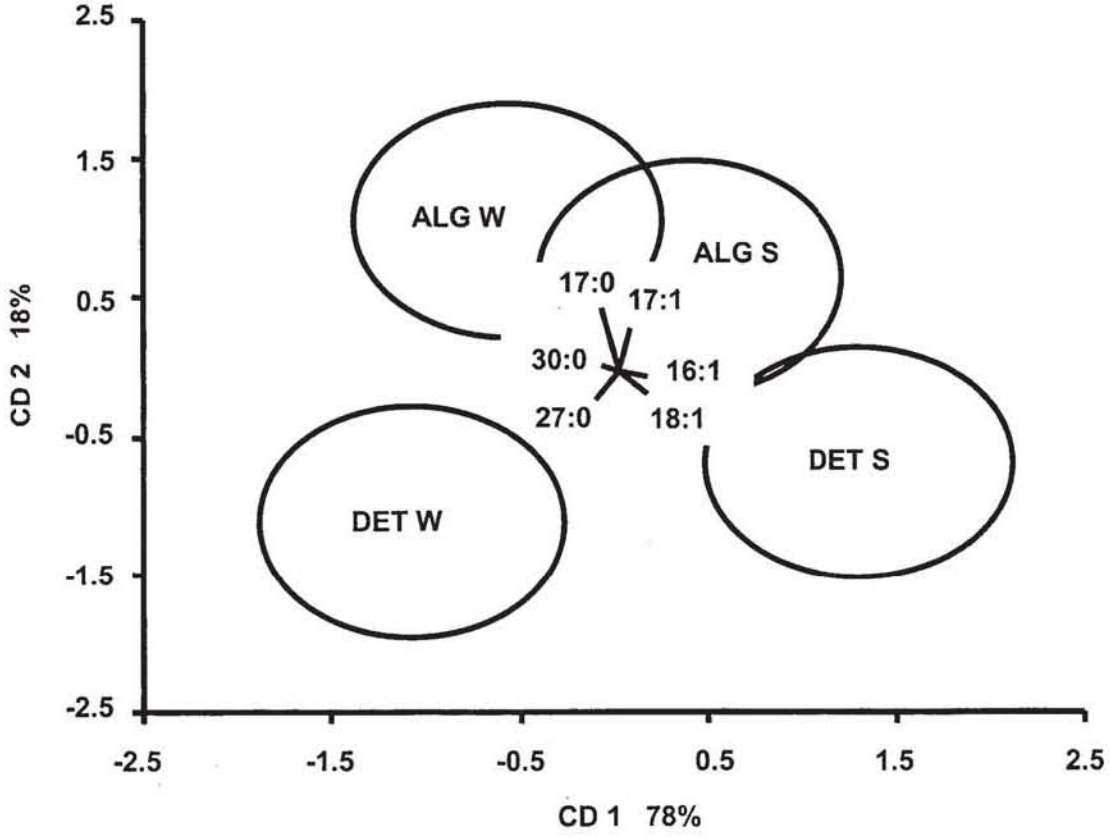

Figure 3.4 Canonical discriminant analysis of hydrocarbons. Ellipses are 95% confidence limits, based on a sample size of 6 to 7. ALG S = algae summer, ALG W = algae winter, DET S = detritus summer, DET W = detritus winter.

C_{25} highly-branched isoprenoid (HBI) alkenes were identified using mass spectra in detritus and algal samples from both seasons. The C_{25} HBIs included $C_{25:3}$, $C_{25:4}$, $C_{25:3}$, $C_{25:4}$, and $C_{25:5}$ and when combined, were most abundant in detritus samples although there was also a high percent of these isoprenoids in algal samples collected during the summer (Table 3.3). The isoprenoids pristane and squalene were also identified and were higher in the detritus samples. (Table 3.3). Examination of the carbon preference index (CPI) over the range *n*-C_{20-35} for alkanes found that detritus collected from both seasons and algae samples collected during the summer had a slight predominance of odd alkanes. Algae collected during the winter had a higher ratio of even numbered alkanes (Table 3.3).

Table 3.3 Mean percentage of hydrocarbons in samples collected from *Salarias patzneri* territories. Values in parentheses are standard errors, based on 6 or 7 samples.

	Detritus (Summer)	Detritus (Winter)	Algae (Summer)	Algae (Winter)
Σ n-Alkanes	57.5 (6.4)	75.2 (5.6)	73.8 (4.3)	82.5 (2.4)
Σ n-Alkenes	36.9 (7.3)	14.1 (4.3)	23.8 (4.5)	14.0 (2.3)
Pristane	1.5 (0.4)	0.8 (0.4)	0.8 (0.2)	0.5 (0.3)
Σ C_{25} HBI Alkenes	7.6 (1.2)	5.9 (2.5)	5.8 (1.1)	1.4 (0.2)
Squalene	2.2 (0.5)	6.1 (4.8)	0.7 (0.3)	1.4 (0.7)
CPI_{20-35}	1.9 (0.8)	1.8 (0.3)	1.7 (0.7)	0.7 (0.1)

$CPI_{20-35} = 0.5*((\sum_{n=35}^{n=21} HC_{odd} / \sum_{n=34}^{n=22} HC_{even}) + (\sum_{n=35}^{n=21} HC_{odd} / \sum_{n=32}^{n=20} HC_{even}))$ from Clark and Blumer (1967)

3.3.3 Sterols

The major sterol in detritus samples collected during the summer was 4,23,24-trimethyl-5α-cholest-22E-en-3β-ol (dinosterol), and this compound accounted for 14

± 1 (SE)% of the sterol fraction. The other major sterols in summer detritus were 24-methylcholesta-5,22E-dien-3β-ol, 23,24-dimethylcholesta-5,22E-dien-3β-ol, 5α-cholest-22E-en-3β-ol (cholesterol) and 24-methylcholest-5-en-3β-ol (Figure 3.5). In winter, dinosterol was less abundant in detritus samples, accounting for 7 ± 1 (SE)% of the sterol fraction. The major sterols in winter detritus were 24-ethylcholest-5-en-3β-ol (sitosterol), cholesterol, 24-methylcholesta-5, 22E-dien-3β-ol and 23,24 dimethylcholesta-5,22E-dien3β-ol (Figure 3.5). The dominant sterols in algal samples collected in the summer and winter were cholesterol and 24-ethylcholest-5-en-3β-ol (Figure 3.5). Summer algal samples also had high percentages of 24-methylcholest-5-en-3β-ol and 24-methylcholesta-5,22E-dien-3β-ol, whereas winter algae contained high percentages of 24-methylcholesta-5,22E-dien-3β-ol and 24-ethylcholest-5-en-3β-ol.

In general, the percentage of cholesterol and 24-ethylcholest-5-en-3β-ol was higher in the algal samples, especially during the summer. In contrast, detritus samples had higher percentages of dinosterol, 22,23-methylene-23,24-dimethyl-cholest-5-en-3β-ol (gorgosterol) and 23,24 dimethylcholesta-5,22E-dien-3β-ol than algal samples. A comparison of the ratio of stanols to sterols also shows a relatively higher percentage of stanols in the detritus than the algae and a slightly higher percentage of stanols in summer samples (Table 3.4).

Table 3.4 Mean ratio of stanols to sterols. Values in parentheses are standard errors, based on 3 to 6 samples.

	Detritus (Summer)	Detritus (Winter)	Algae (Summer)	Algae (Winter)
Σ Stanols/Σ Sterols	0.16 (0.02)	0.13 (0.02)	0.08 (0.02)	0.05 (0.01)

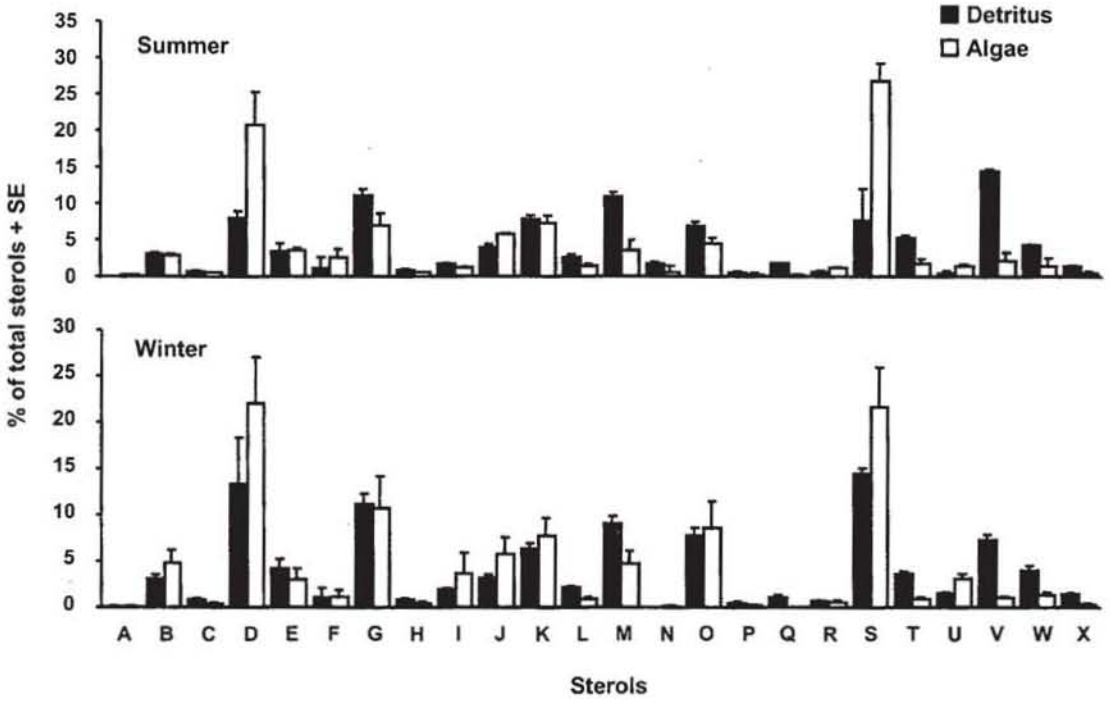

Figure 3.5 Mean percentage of sterols in detritus and algal samples collected from *Salarias patzneri* territories during the summer and winter. Standard errors are based on sample size of 3 to 6. A= 27-nor-24-methylcholest-5,22E-dien-3β-ol, B=cholesta-5,22E-dien-3β-ol, C=5α-cholest-22E-en-3β-ol, D=cholest-5-en-3β-ol (cholesterol), E=5α-cholestan-3β-ol, F=cholesta-5,24-dien-3β-ol, G=24-methylcholesta-5,22E-dien-3β-ol, H=24-methyl-5α-cholest-22E-en-3β-ol, I=5α-cholest-7-en-3β-ol, J=24-methylcholesta-5,24(28)-dien-3β-ol, K=24-methylcholest-5-en-3β-ol, L=4-methyl-5α-cholestan-2β-ol, M=23,24-dimethylcholesta-5,22E-dien-3β-ol, N=23,24-dimethyl-5α-cholest-22E-en-3β-ol, O=24-ethylcholesta-5,22E-dien-3β-ol, P=24-ethyl-5α-cholest-22E-en-3β-ol, Q=4,24-dimethyl-5α-cholest-22E-en-3β-ol, R=23,24-dimethylcholest-5-en-3β-ol, S=24-ethylcholest-5-en-3β-ol, T=24-ethyl-5α-cholestan-3β-ol, U=24-ethylcholesta-5,24(28)Z-dien-3β-ol, V=4,23,24-trimethyl-5α-cholest-22E-en-3β-ol (dinosterol), W=22,23-methylene-23,24-dimethyl-cholest-5-en-3β-ol, X=22,23-methylene-23,24-dimethyl-5α-cholestan-3β-ol.

3.4 Discussion

The detritus samples collected from *Salarias patzneri* territories contained a high percentage of total fatty acids and large amounts of polyunsaturated fatty acids (PUFA). High concentrations of PUFA in detrital material have been used previously as an indicator of high food quality (Ahlgren et al. 1997), particularly when essential fatty acids, which must be obtained from the diet are present (Gurr and Harwood, 1991). The essential fatty acids: 18:2ω6, 20:4ω6, 20:5ω3 and 22:6ω3 all were detected in sediment samples, which, in conjunction with a high total fatty acid and PUFA content, suggests that detritus is a valuable source of nutrition for *S. patzneri*.

3.4.1 Sources of detritus

High diversity in the faunal, floral and microbial communities of coral reefs suggests there are a wide variety of potential detrital sources in *Salarias patzneri* territories. Ratios of, vaccenic acid to oleic acid, branched fatty acids to 15:0 and 16:1ω7 to 16:0 in detrital samples are all typical of detritus that is predominantly derived from eukaryotic organisms (White et al. 1980). In particular, the presence of phytol, dominance of C_{14} to C_{18} fatty acids with even carbon numbers and substantial amounts of PUFA in detritus samples are all characteristic of material derived from marine algae (Mayzaud et al. 1989, Smith et al. 1983). The high abundance of alkanes *n*-C_{17} and *n*-C_{15} in detritus samples also supports the notion that algae are the major source of detritus, as these alkanes are prominent in macroalgae, various microalgae and cyanobacteria (Clark and Blumer 1967, Han and Calvin 1969, Gelpi et al. 1970, Youngblood et al. 1971).

On coral reefs it is the turf or filamentous algae that are responsible for the majority of primary production (Larkum 1983), and a large percentage of the carbon fixed by these algae may be converted into detritus (Hatcher 1983b, Hansen et al. 1992, Arias-Gonzalez et al. 1997). The similarity of fatty acid, hydrocarbon and sterol profiles in detritus and algae samples, combined with low quantities of pristane relative to n-C_{17} (Clark and Blumer 1967) suggests that a large percentage of the detritus in *S. patzneri* territories has originated from filamentous algae.

It has also been suggested that benthic marine algae, such as the filamentous algae collected from *Salarias patzneri* territories, has a higher percentage of fatty acids with double bonds in the ω6 position than many types of phytoplankton or microalgae (Dunstan et al. 1988). Therefore, to obtain an estimate of filamentous algae contribution to detritus in each of the *S. patzneri* territories, the ratios of 18:2ω6 percentages in algal and detrital samples were calculated. Expressing these ratios as a mean percentage, estimated that filamentous algae account for 72 ± 9% and 79 ± 15% of the organic detritus in the summer and winter, respectively. These estimates support lipid biomarker evidence that suggest filamentous algae are the major source of detritus in *S. patzneri* territories. However, these estimates also imply that a substantial portion of the detritus does not come from filamentous algae. Furthermore, although detrital and filamentous algal samples collected from *S. patzneri* territories contained the same lipids, differences in the relative concentration and ratio of some lipids indicate there are sources of detritus other than filamentous algae. Possible inputs to detritus from other sources are discussed separately below.

3.4.1a Microalgae

All algae are characterised by a high percentage of PUFA; however, once the algae die, or become detritus highly unsaturated fatty acids undergo rapid diagenesis (Haddad et al. 1991, Canuel and Martens 1996). Consequently, the presence of PUFA in detrital samples collected from *S. patzneri* territories indicates the material has been recently deposited or indicates the presence of living microalgae.

Further support for the presence of microalgae was the high percentage of 16:1ω7 in detrital samples relative to algal samples. High concentrations of 16:1ω7 are characteristic of diatoms (Volkman et al. 1989, Viso and Marty 1993), and sediments with large diatom inputs have high 16:1ω7 to 16:0 ratios (e.g. Boon et al. 1975, Smith et al. 1983). The presence of C_{25} HBIs in detritus samples also is a strong indication of diatom presence, as these biomarkers are only known to occur in diatoms (Volkman et al. 1994, Sinninghe Damste et al. 1999). Furthermore, the sterol 24-methylcholesta-5,22E-dien-3β-ol, commonly known as brassicasterol, was one of the most prevalent sterols in detrital samples, and, although this compound can be found in other algae, it is often the dominant sterol in diatoms (Volkman 1986). Therefore, data from fatty acid, hydrocarbon and sterol data all support the view that diatoms are an important component of detritus collected from *S. patzneri* territories.

Diatoms also have high amounts of 20:5ω3 (Volkman et al. 1989, Viso and Marty 1993), and, based on a fatty acid content of 10%, of which 10% was 20:5ω3, Currie and Johns (1988) estimated the contribution of diatoms in suspended particulate matter at Lizard Island to be less than 1%. Using the same calculations, it was estimated that diatoms accounted for 14 ± 3% of the organic matter in summer detritus and 3 ± 1% of the winter detritus in this study. These estimates are considerably higher than those of diatom contributions to suspended particulate matter

and may reflect accumulation of diatoms in the sediment, increased input from benthic diatoms and variations due to periodic blooms.

Calculations of diatom input, however, may be imprecise, as 20:5ω3 content varies between diatom species and can be synthesised by other microalgae (Volkman et al. 1989, Viso and Marty 1993). It is also possible that 20:5ω3 in detrital samples has been degraded due to photo-oxidation in the environment (Kieber et al. 1997), or lost during extraction and storage of lipids. Consequently, estimates of diatom input should be considered only as crude approximations.

Dinoflagellates are another group of microalgae commonly found in aquatic sediments. The high percentage of the sterol dinosterol in detritus samples and the presence of the fatty acid 22:6ω3 are indications that dinoflagellates are another source of organic matter in *Salarias patzneri* territories. Dinosterol is considered to be a strong biomarker of dinoflagellate presence (Loeblich 1984, Nichols et al. 1990) and is found in marine sediments where dinoflagellates are abundant (Lee et al. 1980). If it is assumed dinosterol accounts for 0.8% of dinoflagellate dry cell weight (based on data from Nichols et al. 1984), it is estimated that dinoflagellates alone account for approximately 0.4 % of the organic matter in detritus collected during summer and 0.1% of the detritus samples collected during winter. The presence of gorgosterol in the detritus samples also suggests that the dinoflagellates under consideration may be zooxanthellae and provides evidence of organic input to the detritus from their symbiotic hosts (Kokke et al. 1981, Ciereszko 1989, Kerr and Southgate 1993). Both gorgosterol and dinosterol have been identified previously in particulate matter collected from the water column at Lizard Island, and their presence has been used to infer the input of coral mucus to detritus (Gregory 1983 in Currie and Johns 1988). Gorgosterol has been found in a number of other coelenterates and coelenterate

predators (Ciereszko 1989). However, in a coral reef lagoon, the most likely source of dinosterol and gorgosterol is zooxanthellae and associated corals. Coral mucus may therefore be a small, but important source of organics and nutrition in *Salarias patzneri* territories.

Cyanobacteria or blue green algae also are prevalent components of tropical sediments (Wilkinson 1987), and small amounts of filamentous cyanobacteria were evident in detrital samples (Chapter 2). Fatty acid (Parker and Leo 1965, Viso and Marty 1993) and sterol (Volkman 1986) profiles of cyanobacteria are similar to those of many other microalgae, making it difficult to identify positively cyanobacteria as a source of detritus. As cyanobacteria were seen in detrital samples, it is assumed they are a source of lipids in detrital samples; however, it is unclear how important they are as a source of detritus.

3.4.1b Bacteria

Bacteria are a common component of coral reef sediments (Moriarty et al. 1985), and lipid biomarkers suggest they are present in detritus collected from *Salarias patzneri* territories. Detritus samples from both seasons contained branched fatty acids, odd numbered fatty acids and vaccenic acid (18:1ω7), which are all indicative of bacterial presence (Goldfine 1972, Jantzen and Bryn 1985). Furthermore, the presence of stanols in detritus samples provides evidence of bacterial activity in sediments, as stanols are often the product of bacterial reduction of sterols (Nishimura and Koyama 1977, Saliot et al. 1988, Canuel and Martens 1993).

The bacterial biomarker, vaccenic acid, has been used previously to estimate bacterial contributions to the fatty acid (Volkman et al. 1980) and organic content (Currie and Johns 1988) of sediments. Perry et al. (1979) found that vaccenic acid

accounted for 10.5% of the fatty acids in bacteria cultured from tropical sediments. Based on this value and a fatty acid content of 2-8% dry weight for a range of bacteria (Gillan and Johns 1986), it is estimated that bacteria account for 2-10% of the organic matter in summer and 1-4% of the organic matter in winter. However, like estimates of microalgae inputs, these calculations are imprecise, as vaccenic acid has been found in several species of marine macrophytes (Johns et al. 1979, Vaskovsky et al. 1996) and was identified in algal samples. Lipid biomarkers, however, do indicate bacteria are present, and, although a maximum organic input of 10% from bacteria is slightly higher than the 2-4% of organic matter estimated using direct counts (Sorokin 1974), or 3-8% estimated from muramic acid concentrations (Moriarty 1982), these three different methods of estimating the bacterial contribution to detritus are all of a similar magnitude and strongly support the notion that bacteria are a significant component of detritus on coral reefs. Furthermore, as bacteria are rich in nitrogen and phosphorous (Pomeroy 1980) and have a high protein content (Simon and Azam 1989), they also may be an important source of nutrients for *Salarias patzneri*.

3.4.1c Invertebrates

Although invertebrates were present in detrital samples (Chapter 2) and are a potentially important dietary resource, there was little evidence from lipids to suggest that they are major contributors to the detritus in *Salarias patzneri* territories. The presence of the fatty acid 22:6ω3 can be an indication of zooplankton in detritus (Lee et al. 1971, Fraser et al.1989, Cripps et al. 1999), as can cholesterol (Volkman 1986); however, neither of these lipids are definitive markers for zooplankton. Furthermore, the fatty acids 20:1 and 22:1, which are proven biomarkers for copepods (Gatten et al. 1983, Clarke et al. 1987), were not detected in any of the detritus samples. Copepods

are often the dominant mobile invertebrate fauna found in coral reef epilithic algae communities (Zeller 1988, Wilson and Bellwood 1997). The absence of specific invertebrate biomarkers therefore suggests copepods and other invertebrates are an insignificant component of detrital aggregates.

3.4.1d Terrestrial

On some coral reefs there is evidence of input from terrestrial systems (Currie and Johns 1989) and coastal plants such as mangroves (Shaw and Johns 1985). Lizard Island covers an area of approximately 1000 hectares, with over 300 species of terrestrial plants (Byrnes et al. 1977). There is no permanent water on the island to facilitate the continual flow of terrestrial organic matter onto surrounding reefs; however, seasonal rain and the close proximity of the island to sample sites in the lagoon suggest that terrestrial run-off may contribute to the detritus collected from *Salarias patzneri* territories. Detritus from terrestrial sources is characterised by long-chain fatty acids (Eglinton and Hamilton 1967) and long-chain hydrocarbons with a definite odd predominance (e.g. Shaw et al. 1979, Saliot et al. 1988). The CPI_{20-35} of detritus collected during both seasons has a slightly odd predominance, and detritus collected in the winter is characterised by long-chained hydrocarbons. Furthermore, unusually high percentages of the even-numbered alkenes in detritus samples may be indicative of terrestrial input, as similar alkenes have previously been recorded from marsh grass (Canuel et al. 1997). However, evidence of terrestrial input to detritus in *S. patzneri* territories is weak, because there are no long-chained fatty acids in any of the detrital samples. It is more likely that longer chain alkanes are derived from algae and bacteria, which are known to produce small amounts of these alkanes, and have CPI similar to those found in detrital samples (Clark and Blumer 1967, Han and

Calvin 1969, Gelpi et al. 1970). Terrestrial run-off and coastal plants are therefore unlikely to be significant contributors of organic detritus in *S. patzneri* territories.

3.4.2 Seasonal aspects of input.

Seasonal variation can have a profound affect on the sources of organic detritus in the marine environment (Mayzaud et al. 1989, Canuel and Martens 1993). In the present study, an increase in the percentage of PUFA and higher contribution of diatoms and dinoflagellates in summer detritus samples are a good indication of increased inputs from microalgae during this season. Feeding intensity of blennies is also greater during this season (Chapter 1), which increases the turnover rate of detritus during this season. In contrast, lower feeding rates by blennies in the winter will reduce detrital turnover rates, resulting in a longer time for lipid diagenesis. Prolonged diagenesis of lipids results in a reduction of unsaturated fatty acids (Haddad et al. 1991) and a higher percentage of long chain hydrocarbons (Canuel and Martens 1996), which were characteristic of winter detritus. Thus, the relatively higher contribution of microalgae to detritus collected during the summer is likely to be a consequence of seasonal increases in microalgal activity and increased turnover rates of sediments during this season.

Productivity and biomass of bacteria on coral reefs also can change seasonally (Moriarty et al. 1985, Johnstone et al. 1990, Hansen et al. 1992) and based on vaccenic acid content it was estimated that bacterial biomass was usually greater in detrital samples collected during the summer. An increase in the percentage of odd fatty acids and a slightly higher stanol/sterol ratio during the summer also suggests a relatively greater percentage and activity of bacteria during this season (Canuel and

Martens 1993). Thus, bacteria, like microalgae, are possibly more important components of detritus in the summer.

Overall, similarities in the lipid profiles of detritus and algal samples, combined with the dominance of lipids characteristic of algae, suggest that the primary source of detritus in *Salarias patzneri* territories is filamentous algae (Figure 3.6). However, the presence of specific biomarkers indicates there are also significant contributions from several types of microalgae, coral mucus and bacteria. These additional sources of detritus may add essential fatty acids, proteins and micronutrients, which improve the nutritional value of detritus relative to filamentous algae. The combination of different detritus sources likely makes detritus in *S. patzneri* territories a potentially valuable dietary resource. Subtle differences in the lipid profiles of detritus and filamentous algae also mean that it may be possible to determine the source of dietary lipids for *S. patzneri,* and this is the subject of Chapter 4.

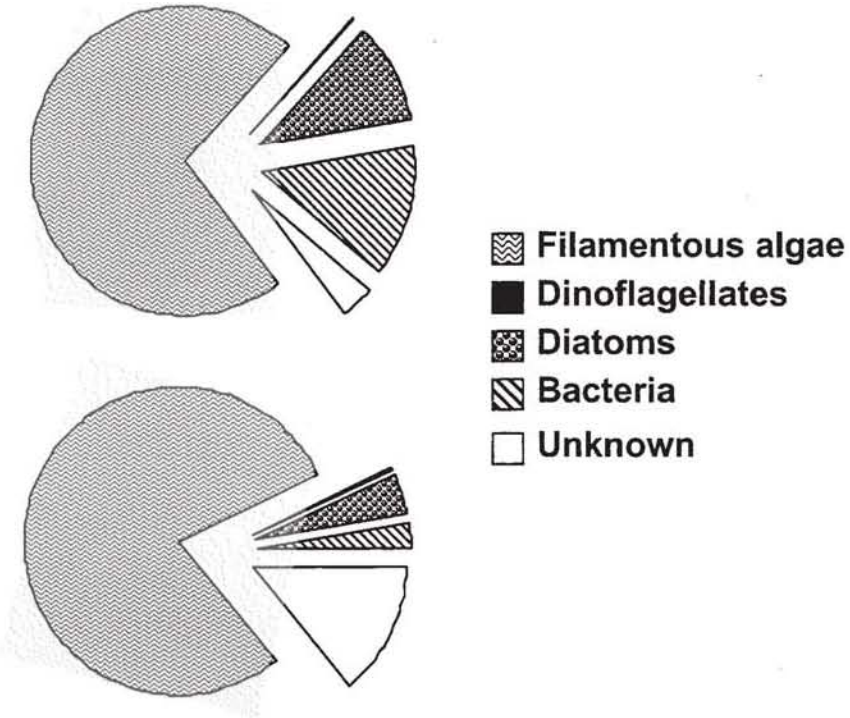

Figure 3.6 Estimated contributions of filamentous algae and microbes to the detritus collected from *Salarias patzneri* territories. Pie segments represent the percentage contribution of the different sources to the organic matter in detrital samples. Data calculated from specific lipid biomarkers.

Chapter 4. Sources of dietary lipids in the coral reef blenny *Salarias patzneri.*

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4.1 Introduction

When assessing the trophic status of marine fishes, many studies initially investigate the gut contents or diet selection (e.g. Horn 1983, Horn et al. 1982, 1986, Clements and Choat 1997, Wilson and Bellwood 1997, Ojeda and Munoz 1999) and in some cases digestive processes (e.g. Clements and Choat 1995, Choat and Clements 1998). The first chapter of this thesis has shown that salariin blennies predominantly ingest detrital aggregates and relatively small quantities of filamentous algae. A representative blenny species, *Salarias patzneri*, has been shown to feed selectively on detrital particles <125μm (Chapter 1), and biochemical analysis of detritus <125μm collected from *S. patzneri* territories has established that it is of a nutritional value capable of sustaining fish growth (Chapter 2). However, identification of gut contents, feeding preferences and nutritional assessment of dietary components does not provide a sufficient basis to assign trophic status unequivocally. A comprehensive assessment of trophic status must also consider assimilation.

Numerous studies have demonstrated that coral reef fishes assimilate nutrients from algae and/or detritus ingested in the field (e.g. Bruggeman et al. 1994a,b, Ferreira et al. 1998). However, as many of these fishes are likely to ingest a combination of algae and detrital aggregates (Choat 1991), the source of assimilated nutrients is unclear. Studies conducted under laboratory conditions have determined that fish are capable of assimilating nutrients from algae (e.g. Galetto and Bellwood 1994, Sturm and Horn 1998), however, the ability of coral reef fish to absorb nutrients

from a diet based solely on detrital aggregates is yet to be established. By comparing the relative amount of fatty acid biomarkers in detrital and algal samples with fatty acids in blenny tissues, this chapter will assess if lipids in fish tissues are derived from detrital aggregates or filamentous algae.

In fish, lipids rather than carbohydrates are the major source of energy reserves (Cowey & Sargent 1977). Lipids can be obtained directly from the diet or synthesed *de novo* by fish, although some fatty acids are considered essential and must be obtained through the diet (Bell et al. 1986). By comparing the fatty acid content of fish with available dietary items it is possible to get an indication of which fatty acids and dietary items are being assimilated. Experiments with specific fatty acid biomarkers and ratios of fatty acids have previously been used to demonstrate the movement of energy from microalgae (Fraser et al. 1989, Klungsoyr et al. 1989), bacteria (Ederington et al. 1995, Goedkoop et al. 1998) and copepods (Gatten et al. 1883) through the food chain. These biomarkers and ratios also have identified components of fish diets (Fraser et al. 1989, St John & Lund 1996), and therefore comparisons of fatty acid biomarkers in detritus and filamentous algae with fish tissues may help identify which of these EAM components fishes assimilate.

In this chapter I will compare the essential fatty acids and dietary biomarker content of fish tissues with those in detritus and filamentous algae collected from the territories of a representative blenny species, *Salarias patzneri.* This chapter will assess qualitatively the extent to which detritus or filamentous algae satisfy the lipid requirements of this fish. This analysis will provide a new perspective on the assimilation of detritus and filamentous algae by blennies and provide a quantitative estimate of the extent to which ingested detritus may be used as a dietary resource by coral reef fishes.

4.2 Methods

Six *Salarias patzneri* were collected from the Lizard Island lagoon (14° 42'S, 145° 30'E) during August 1998 (Austral winter) and another six during February 1999 (Austral summer). All *S. patzneri* were mature males, with a minimum total length of 35mm. They were collected using the anaesthetic clove oil and sexed by examination of the genital papillae. A sample of the detritus and algae from within each *S. patzneri* territory was collected 10-15 minutes before each fish was caught. Detritus and algal samples were collected using an underwater sampler (Purcell 1996), following the technique outlined in Chapter 2. This collection provided particulate samples <125μm that were predominantly composed of detrital aggregates, a combination of non-living amorphous organic matter with heterotrophic and autotrophic microbes (cf. Bowen 1979). The algal samples contained predominantly filamentous algae from the Rhodophyta, Chlorophyta and Phaeophyta (see Chapter 2). After collection, the fish were dissected to remove the alimentary canal, preventing contamination from ingested items; then, all samples and fish were frozen and freeze-dried to obtain dry weights. Lipids from the freeze-dried samples were extracted, separated and analysed following the procedures outlined in Chapter 3.

Potential dietary biomarkers and essential fatty acids that were present in *Salarias patzneri* tissues, detrital and algal samples were compared using MANOVA. Sample type (*S. patzneri*, detritus and algae) and seasons (summer and winter) were entered as fixed factors and data were arcsine or $\log_{10}(x + 1)$ transformed before analysis to meet the assumptions of MANOVA (Zar 1999). Two-way ANOVAs, in conjunction with Tukey's HSD, were used as posthoc tests to assess any significant

differences. Sequential Bonferonni corrections were applied to alpha levels to minimise the probability of type 1 error, whilst maintaining high statistical power (Rice 1989). Fatty acid percentages or ratios that differed significantly between detritus and algae samples were presented graphically, as they had the potential to determine the source of dietary lipids for *S. patzneri*.

4.3 Results

The total lipid and fatty acid content of *Salarias patzneri* did not vary greatly between samples collected during the summer and winter (Table 4.1). The predominant fatty acids in fish collected during the summer were: 16:0, 22:6ω3, 20:5ω3, 22:5ω3, 18:1ω9 and 16:1ω7, whereas in the winter 16:0, 22:6ω3, 20:5ω3, 16:1ω7, 22:5ω3 and 18:1ω9 were the major fatty acids in descending order of abundance. Polyunsaturated fatty acids (PUFA) were the main fatty acids in fish samples collected during both seasons, accounting for approximately half of all the fatty acids. Detrital samples collected from *S. patzneri* territories during the summer and winter were dominated by the fatty acids 16:0 and 16:1ω7, and together they accounted for approximately 60% of all fatty acids in these samples. Algal samples collected from *S. patzneri* territories were dominated by 16:0, 14:0 and 18:1ω9, which combined accounted for approximately 70% of all fatty acids in algal samples. Saturated fatty acids were the main type of fatty acids in both detrital and algal samples.

Table 4.1 Fatty acids in *Salarias patzneri* tissues and detrital and filamentous algae samples collected from the epilithic algal matrix within *S. patzneri* territories. Values for individual fatty acids are the mean % of total fatty acids from six samples. Figures in parenthesis are standard errors. Trace indicates fatty acid was present at concentrations <0.1%.

	Salarias patzneri		Detritus		Algae	
	Summer	Winter	Summer	Winter	Summer	Winter
Tot. lipids mg.g^{-1}dry wt	55.2 (7.1)	55.2 (3.7)	4.0 (0.5)	5.0 (1.6)	8.1 (1.0)	6.9 (1.6)
Fat. acids % Tot. lipids	21.8 (2.3)	24.4 (4.4)	22.0 (2.0)	14.5 (5.1)	18.6 (3.3)	17.5 (1.9)
13:0	0	0	0	0	0.7 (0.2)	0.2 (0.1)
14:0	3.2 (1.2)	5.2 (0.9)	10.2 (0.7)	8.4 (1.0)	7.4 (0.6)	7.7 (0.8)
i15:0	trace	0	0.7 (0.1)	0.4 (0.1)	0.7 (0.2)	0.4 (0.1)
a15:0	trace	trace	0.2 (0.1)	0.2 (0.1)	trace	0.2 (0.1)
15:0	0.5 (0.1)	0.7 (0.1)	1.3 (0.2)	1.1 (0.2)	3.4 (0.7)	1.5 (0.3)
16:1ω7	7.3 (1.6)	9.0 (0.9)	14.9 (0.9)	13.6 (1.6)	5.7 (0.9)	5.8 (0.6)
16:0	23.5 (1.7)	24.7 (0.5)	44.3 (0.9)	48.4 (1.4)	53.5 (4.4)	56.0 (0.8)
17:0	0.4 (0.1)	0.3 (0.1)	0.4 (0.1)	0.7 (0.2)	0.5 (0.1)	0.3 (0.1)
$_{18}$PUFA	2.2 (0.4)	3.7 (0.4)	1.9 (0.2)	0.9 (0.3)	1.4 (0.4)	1.2 (0.1)
18:2ω6	2.5 (0.1)	3.1 (0.3)	2.5 (0.2)	3.5 (0.7)	4.0 (0.7)	4.1 (0.3)
18:1ω9	8.5 (1.0)	8.3 (0.6)	6.5 (0.5)	9.3 (1.2)	7.1 (0.6)	10.0 (0.6)
18:1ω7	2.3 (0.1)	1.9 (0.2)	4.3 (0.4)	4.9 (0.7)	6.2 (0.6)	6.0 (0.5)
18:0	3.5 (0.4)	2.7 (0.3)	4.3 (0.9)	5.2 (0.9)	1.5 (0.1)	2.1 (0.2)
20:5ω3	10.9 (1.3)	9.3 (0.7)	2.6 (0.3)	1.4 (0.3)	3.0 (1.0)	2.1 (0.3)
20:4ω6	4.3 (0.4)	5.1 (0.2)	3.7 (0.4)	1.4 (0.2)	3.7 (1.2)	2.1 (0.4)
22:6ω3	20.3 (2.4)	16.7 (1.3)	1.7 (0.2)	0.2 (0.1)	0.6 (0.6)	trace
22:5ω3	9.6 (0.6)	8.6 (0.4)	0	0	0.1 (0.1)	0
ΣSaturate	31.2 (2.2)	33.6 (1.2)	60.5 (1.0)	63.8 (0.7)	67.2 (4.4)	67.8 (0.8)
ΣMono	18.0 (0.7)	19.3 (0.7)	25.8 (0.5)	27.9 (0.5)	19.0 (0.5)	21.8 (0.4)
ΣPoly	49.9 (2.9)	46.5 (1.7)	12.4 (1.0)	7.4 (0.8)	12.7 (3.9)	9.5 (0.8)
ΣBranch	0.2 (0.1)	trace	0.9 (0.2)	0.7 (0.2)	0.8 (0.2)	0.6 (0.1)
16:1/16:0	0.29(0.06)	0.36(0.03)	0.34(0.02)	0.28(0.03)	0.12(0.03)	0.10(0.01)
ω3/ω6	6.06(0.74)	4.28(0.38)	0.68(0.06)	0.35(0.07)	0.41(0.07)	0.35(0.05)

Essential fatty acids and fatty acids that have been used previously as indicators of diet (Dunstan et al. 1988, Virtue et al. 1993, Ederington et al. 1995, St John and Lund 1996, Goedkoop et al. 1998) and where identified in *Salarias patzneri* tissues, detritus

and algal samples are listed in Table 4.2. The percentage/ratio of these fatty acids was significantly different in *S. patzneri* tissues, detrital and algal samples (Pillai's trace $P<0.001$), and between samples collected during the summer and winter (Pillai's trace $P=0.004$). However, there was no significant interaction between sample type and season. Posthoc ANOVAs, indicated significant differences in the percentage/ratio of all essential fatty acids and dietary biomarkers between the different sample types (Table 4.2). Of these, only the 16:1ω7/16:0 ratio and percentages of 18:2ω6 and 22:6ω3 were significantly different in detrital and algal samples and therefore potentially useful in assessing the source of *S. patzneri* dietary lipids.

MANOVA and Tukey's HSD test determined that the ratio of 16:1ω7 to 16:0 and percentage of 18:2ω6 in *Salarias patzneri* tissues and detritus samples were both statistically similar. However, the 16:1ω7/16:0 ratio in these samples was significantly higher than in algal samples and the percentage of 18:2ω6 in *S. patzneri* and detritus was significantly lower than in algal samples (Figure 4.1).

The percentage of 22:6ω3 in *S. patzneri* tissues also was significantly greater than in detritus samples, which was greater than in algal samples (Figure 4.1). The percentage/ratios of the remaining fatty acids were similar between detrital and algal samples. The percentage of 20:5ω3, 20:4ω6 and the ω3/ω6 ratio were, however, significantly greater in *S. patzneri* tissues than detritus and algae and the sum of branched fatty acids was significantly lower in *S. patzneri* tissues than either detritus or algae. The ω3/ω6 ratio and percentage of 22:6ω3 in all sample types was also greater in samples collected during the summer.

Table 4.2. Essential fatty acids and fatty acid ratios that have previously been related to diet and were identified in *Salarias patzneri* tissues, detritus and filamentous algae. Values are summary statistics from ANOVAs comparing fatty acid content/ratio in *S. patzneri* tissues, detritus and algae samples. * Indicates significant difference after sequential Bonferonni adjustment to alpha levels.

	Type		Season	
	$F_{2,30}$	p	$F_{1,30}$	p
18:2ω6	5.1	0.012*	2.9	0.095
20:5ω3	76.2	<0.001*	4.8	0.036
20:4ω6	10.8	<0.001*	6.7	0.014
22:6ω3	265.9	<0.001*	10.9	0.002*
Σ Branched [1,2]	18.3	<0.001*	2.6	0.114
16:1/16:0 [3,4]	24.8	<0.001*	0.003	0.960
ω3/ω6 [5]	344.2	<0.001*	12.0	0.002*

References relating fatty acid to diet: 1. Goedkoop et al. (1998), 2. Ederington et al. (1995), 3. St John and Lund (1996), 4. Virtue et al. (1993), 5. Dunstan et al. (1988).

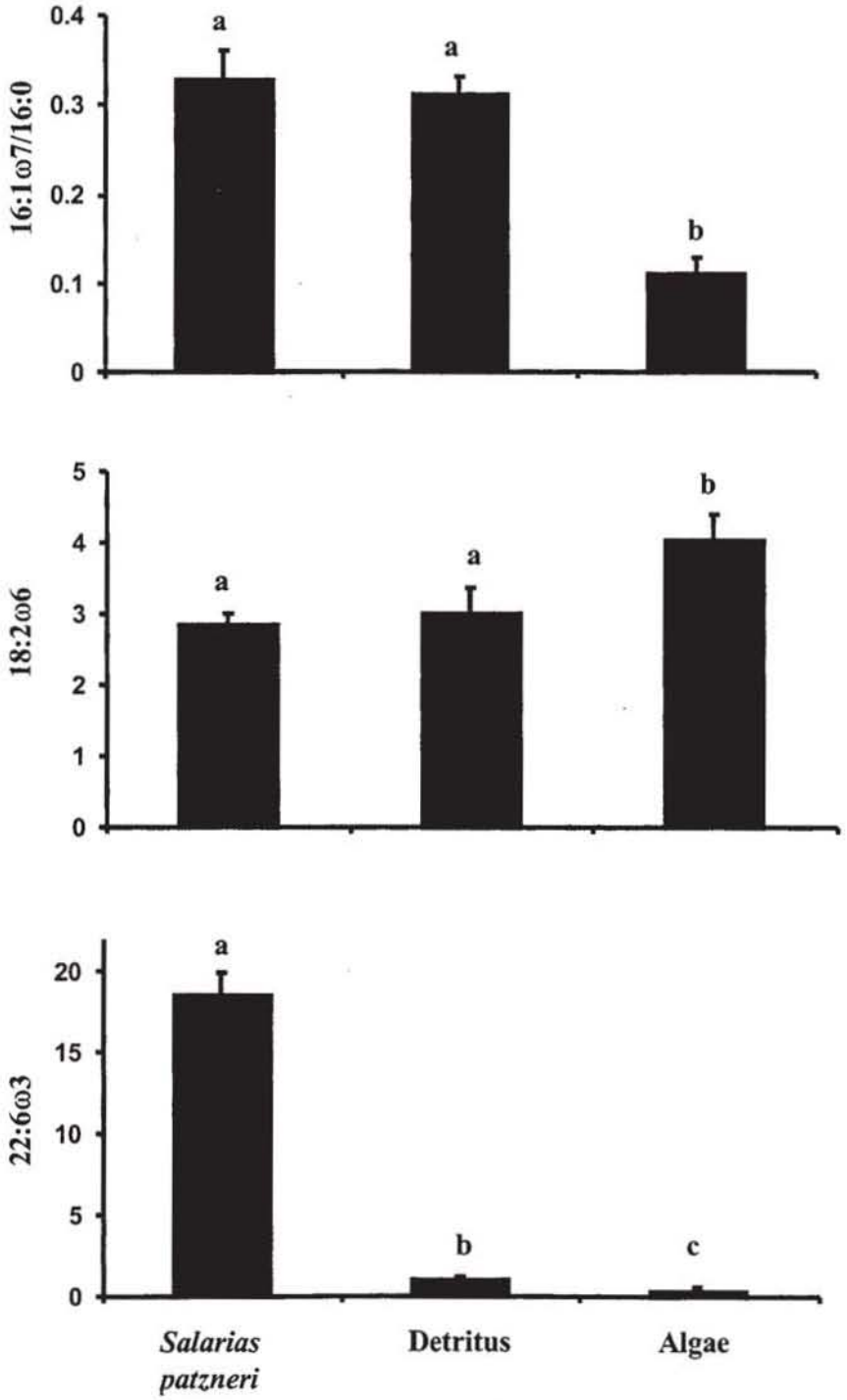

Figure 4.1. Mean essential fatty acids and fatty acid biomarkers of potential use in discriminating between detritus and filamentous algae sources of lipids for *Salarias patzneri*. Values for 22:6ω3 and 18:6ω3 are percentages of total fatty acids, 16:1ω7/16:0 is a ratio. Error bars are standard errors based on 12 samples. Letters above each column represent statistically similar groups, based on Tukey's HSD test.

4.4 Discussion

Together, the similarity in the ratio of 16:1ω7 to 16:0 and percentage of 18:2ω6 in *Salarias patzneri* tissues and detrital samples strongly suggest that the dietary lipids in *S. patzneri* tissues are primarily assimilated from detritus. Furthermore, the significantly lower ratio of 16:1ω7 to 16:0 and higher percentage of 18:2ω6 in algal samples suggest that the direct contribution of filamentous algae to lipids within *S. patzneri* tissues is limited.

The ratio of 16:1ω7 to 16:0 has been used previously as an indicator of diet in krill (Virtue et al. 1993) and fish (Fraser et al. 1989, St John and Lund 1996) and high levels of 16:1ω7 in body tissues used as dietary biomarkers in copepods (Graeve et al. 1994) and midges (Goedkoop et al. 1998). Furthermore, North Sea cod larvae kept in aquaria and fed diets with different 16:1ω7 to 16:0 ratios changed their fatty acid ratios over a period of only 13 days, after which the ratio of 16:1ω7 to 16:0 in larvae and diets were essentially the same (St John and Lund 1996). The ratio of 16:1ω7 to 16:0 is therefore a proven dietary marker, and the similarity of the ratio of these two fatty acids in detritus and *Salarias patzneri* tissues provides excellent evidence that detrital lipids are assimilated by *S. patzneri*.

An increase in the relative concentration of 16:1ω7 in *Salarias patzneri* tissues and detritus compared to the filamentous algae could be a result of the presence of diatoms in detritus samples. Diatoms are known to have high concentrations of 16:1ω7 (Volkman et al. 1989, Viso and Marty 1993) and may account for up to 18% of the organic matter in sediments collected from *S. patzneri* territories (Chapter 3). High concentrations of 16:1ω7 in acanthurids that feed on the EAM also has been used to implicate diatoms as a dietary resource for these fish (Montgomery et al.

1999). Furthermore, larval herring increase the ratio of 16:1ω7 to 16:0 in their body tissues when diatoms were more prevalent in an enclosed food chain (Fraser et al. 1989). High concentrations of 16:1ω7 in detritus and *S. patzneri* tissue relative to algae may, therefore, be an indication that diatoms are an important constituent of *S. patzneri* diet. The ratio of 16:1ω7 to 16:0 in *S. patzneri* tissues and detrital samples is, however, much lower than usually observed in diatom cultures (Volkman et al. 1989, Viso and Marty 1996). The similarity of this ratio in *S. patzneri* tissues and detrital samples therefore infers that it is the detrital aggregates and diatoms within these aggregates that are assimilated, rather than just the diatoms.

The detritus collected from *Salarias patzneri* territories also contained fatty acids that are indicative of bacteria (Chapter 3). Many bacteria have a high 16:1ω7 content (Wilkinson 1988) and may be a source of dietary lipids for *S. patzneri*. Bacteria also are characterised by branched fatty acids, which have been used previously as trophic biomarkers to indicate the movement of energy from bacteria through the food chain (Ederington et al. 1995, Goedkoop et al. 1998). Bacteria have been proposed as a dietary resource for detritivorous reef fishes (Choat and Clements 1998), a suggestion that is supported by the presence of branched fatty acids in *S. patzneri* tissues. Thus, lipids from bacteria are assimilated; however, these bacteria biomarkers accounted for less than 0.1% of the total fatty acids in *S. patzneri* tissues, much less than the 0.6-0.9% seen in detritus or 0.6 to 0.7% seen in filamentous algae samples. Furthermore, bacteria are unlikely to contain polyunsaturated fatty acids (Jantzen and Bryn, 1985), which accounted for approximately half of the total fatty acids in *S. patzneri* tissues. Consequently, bacteria are probably not a major source of dietary lipids for *S. patzneri*.

The fatty acid 18:2ω6, in conjunction with others, has been used previously as a dietary marker in crustaceans, fish, seals and river dolphins (Napolitano 1999) and has been used to identify seagrass and angiosperm detritus in the diet (Napolitano 1999). 18:2ω6 is usually a minor component of diatoms (Volkman et al. 1989, Viso and Marty 1996) and is often more prevalent in macroalgae (Johns et al. 1979, Vascovsky et al. 1996). A relatively low percentage of 18:2ω6 in *S. patzneri* tissues and detritus samples and a significantly higher percentage in algal samples therefore supports the 16:1ω7/16:0 data, which suggests that detrital aggregates are the major source of dietary lipids for this species.

The major PUFA in *Salarias patzneri* tissues was 22:6ω3 and the percentage of this fatty acid in *S. patzneri* tissue was significantly greater than in detritus or algal samples. In many marine fish 22:6ω3 appears to accumulate in tissues (Watanabe 1982), which would explain the relatively high concentration of this fatty acid in *S. patzneri* samples. This highly unsaturated fatty acid also may be produced by fish via the elongation and desaturation of other ω3 fatty acids (Kanazawa et al. 1979). However, it is possible that many marine fish lack the enzymes necessary for this conversion and rely solely on dietary sources of 22:6ω3 (Bell et al. 1986). In detrital samples, likely sources of 22:6ω3 are microalgae (Viso and Marty 1996) and copepods (Lee et al. 1971); however, the absence of copepod biomarkers 20:1 and 22:1 (Gatten et al. 1983, Clarke et al. 1987) in detritus or *S. patzneri* tissues suggests that 22:6ω3 is derived primarily from microalgae. The high percentage of 22:6ω3 in *S. patzneri* tissues compared to detrital and algal samples also suggests that this fatty acid is absorbed from ingested detrital aggregates and or algae and that it accumulates in fish tissue.

In summary, these results strongly suggest that detrital aggregates are the major source of dietary fatty acids for the blenny, *Salarias patzneri*. Similarities in the ratio of 16:1ω7 to 16:0 and the percentage of 18:2ω6 in *S. patzneri* tissues and detrital samples indicate that fatty acids from detritus and microalgae, particularly diatoms, within detrital aggregates are assimilated by *S. patzneri*. These results suggest that detritus and associated microalgae may be important dietary constituents for fish that feed on the EAM. The ingestion and assimilation of detrital aggregates by fish may therefore, represent a major pathway for the transfer of energy on coral reefs.

Chapter 5. Multiscale habitat associations of detritivorous blennies (Blenniidae: Salariini).

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5.1 Introduction

Fish that feed on epilithic algae and associated detritus provide an essential link between primary production and secondary consumers on coral reefs (see reviews by Horn 1989, Choat 1991). Because of their importance to coral reef ecology the distribution of fish that feed on primary resources has been studied widely. However, many of these studies have tended to concentrate on the large, conspicuous species and excluded the smaller, cryptic fish (e.g. Russ 1984a,b, Meekan and Choat 1997). Small fish with total lengths <100 mm are the most diverse and abundant size class of fishes on coral reefs (Munday and Jones 1998, Akerman and Bellwood 2000). Furthermore, as small fish have a higher mass specific metabolic rate than larger-bodied fish (Clarke and Johnston 1999) and generally higher turnover rates, their high abundance suggests that they may play an important role in the transfer of energy on coral reefs (Akerman and Bellwood 2000).

A number of different factors are likely to influence the distribution patterns of coral reef fishes (Williams 1991). The availability of resources, in particular dietary resources, is one factor that may have a central role in determining the distribution of herbivorous and detritivorous fishes (Williams et al. 1986). To satisfy high energy requirements, herbivorous and detritivorous fishes often ingest large amounts of algae or detritus and feed selectively on protein rich material (Bowen et al. 1995). Spatial variation in the availability and quality of dietary resources may therefore influence the abundance and distribution of the fish that feed on them. Previous studies have

examined the relationship between algal availability and the abundance of herbivorous fishes (e.g. Russ 1984c, Hart et al. 1996), however; the influence of detrital quality and availability on the distribution of coral reef fishes is yet to be established. This is despite recent studies that have found that the quality and quantity of detritus can vary across reef zones (Purcell and Bellwood 2001) and with levels of wave exposure (Crossman et al. In press).

Apart from broad-scale patterns of detritus quality, fine-scale distributions of food resources may be particularly important for small detritivores, as many of these fish are strongly site attached (Nursall 1977, Horn 1989, Goncalves and Almada 1998) and rely on resources within territories to satisfy dietary requirements. The type of microhabitat utilized by these fish must, therefore, be capable of accumulating enough detritus to satisfy their relatively high energetic requirements, as well as provide adequate shelter. Variation in the availability of suitable habitat resources at both broad and fine scales may therefore shape the distribution of fish. Furthermore, the influence of a habitat resource is likely to vary with spatial scale (Syms 1995), and it is therefore important to examine fish distributions and their relationships with habitat resources at a range of spatial scales.

Blenniid fish from the tribe Salariini are small (5-17 cm total length), cryptic fishes (Randall et al. 1990), which feed on the epilithic algal matrix, primarily ingesting detrital aggregates (Chapter 1). On high latitude reefs, blennies are an abundant component of fish assemblages (Townsend and Tibbetts 2000) that, in conjunction with other small fish, are responsible for removing large amounts of material from the epilithic algal community (Hatcher 1981). In this environment, salariin blennies represent an abundant and ecologically significant group of small

detritivores; however, their significance on tropical coral reefs remains to be determined.

In this chapter I will quantify the distribution and abundance of salariin blennies. These data will be compared to patterns of availability of trophic resources and used to evaluate the importance of these small detritivorous fishes to coral reef trophodynamics. Estimates of blenny abundance and biomass will be compared with those of other herbivorous and detritivorous fishes, providing information on the relative contribution of blennies to herbivorous/detritivorous reef fish assemblages.

5.2 Methods

Visual censuses were conducted on the fringing reef around Lizard Island (14° 42'S, 145° 30'E) at three locations of differing degrees of wind and wave exposure (Figure 5.1). Exposed locations were subjected either directly or obliquely to the prevailing southeasterly winds. Sheltered locations were in the lee of the island but were occasionally exposed to northwesterly swells. The most sheltered location was inside the lagoon. Within each location three sites were selected haphazardly and at each site; three transects were visually censused on the top and three on the side of the reef. The reef tops were at a depth of 1-2 m at mean high tide and the sides at a depth of 2-5 m at mean high tide. Transects (50x2 m) were censused for approximately 20 minutes, by swimming at a constant rate along an underwater tape measure. The transect width and census time were predetermined following a pilot study that compared precision of blenny density estimates at different swimming speeds and transect widths. Visual censuses were conducted at all locations, sites and zones during August 1998 (Austral winter). All censuses were performed between 10:00 and

17:00, as a pilot study had shown that time of day and tidal height had no significant influence on estimates of blenny densities during these hours.

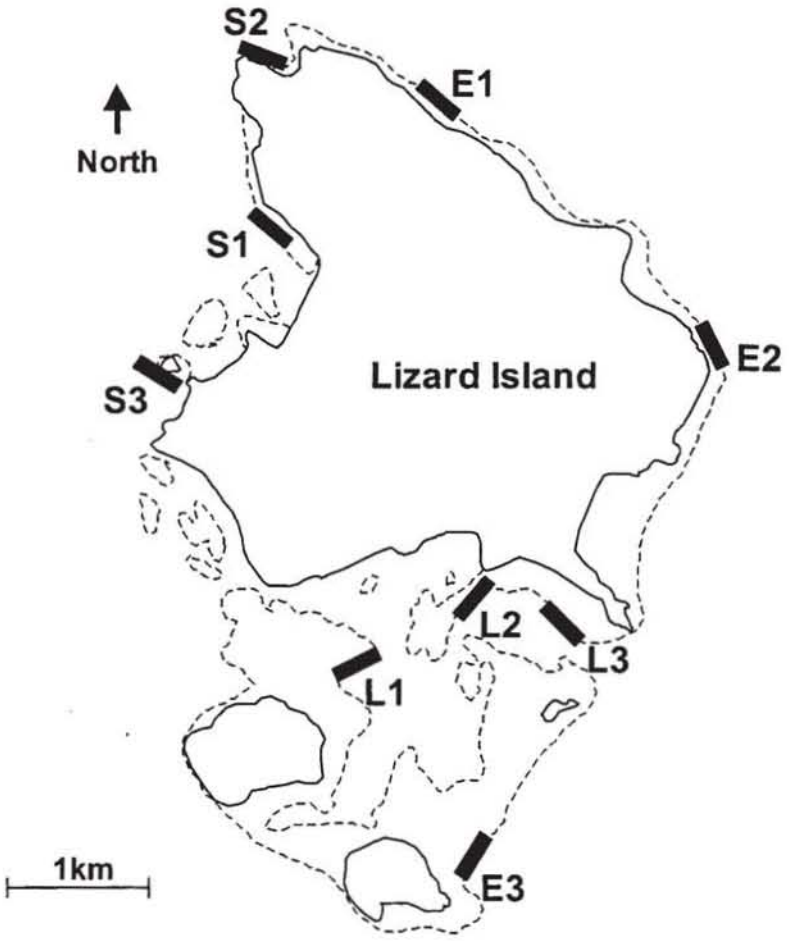

Figure 5.1 Map of Lizard Island showing sites in exposed (E1,E2,E3), sheltered (S1,S2,S3) and lagoon (L1,L2,L3) locations.

Blennies within transects were identified to species level, their total length estimated and the microhabitat on which they were first observed recorded. The types of microhabitat available to blennies were assessed using point intercept transects. For all blenny census transects, available microhabitat was estimated by recording the type of substratum at every marked metre of the transect tape. This provided 50 microhabitat readings for each transect. Microhabitats were classified into: massive, branching, corymbose, encrusting, soft and other corals, as well as rock, rubble, sand and miscellaneous. The different coral categories were based on growth forms described in Veron (1986), and each coral category was further classified as either living or dead. The miscellaneous category included macroalgae, clams and sponges.

To estimate the total biomass of blennies, total length estimates for each blenny seen on transects was converted to fish weight using length-weight relationships obtained from a representative sample of blenny species collected during the course of the study. The accuracy of fish length estimates was assessed throughout the study by estimating the length of fish in areas adjacent to sampling sites, then capturing the fish and measuring total length to the nearest mm with Vernier calipers. For 190 fish, with measured total lengths between 15 and 70 mm, estimated length was 98 ± 1% of the actual fish length.

To investigate the relative importance of blennies on coral reefs, I compared their density and biomass at exposed and sheltered locations to those of other detritivorous/herbivorous fishes. Each fish species was assigned a functional group based on published gut content analyses (Sano et al. 1984, Wilson and Bellwood 1997, Chapter 1) and their feeding behavior. Those species with more than 50% detrital material in their guts were classified as detritivores and those with less than 50% detritus, but greater than 50% algae, were classified as grazers. Densities of

territorial pomacentrids were estimated from Meekan et al. (1995), whilst density and size estimates of other species were provided by J.H. Choat (unpublished data). Sites used by Meekan et al. (1995) and J.H. Choat corresponded to those surveyed in this study; however these sites were surveyed at different times. Given the temporal differences between these studies and my own, abundance estimates should therefore be considered as approximations, which were used to suggest the relative importance of blennies as a trophic group on coral reefs. Length-weight relationships from Kulbicki et al. (1993) and L. Bay (unpublished data) were used to convert estimated fish lengths into biomass.

5.2.1 Statistical analyses

The distributions of the six most abundant blenny species at Lizard Island were compared between zones (top and side) and among locations (exposed, lagoon and sheltered) using MANOVA. Before comparisons were made, data within each site were pooled and the mean number of each species on the top of each site and side of each site treated as a replicate. These data were then $\log_{10}(x + 1)$ transformed to meet the assumptions of MANOVA. Significant differences in MANOVA were further investigated using a two-way ANOVA for each species, with location and zone as fixed factors. Significance levels were corrected using sequential Bonferroni adjustments (Rice 1989), which reduced the probability of a type 1 error. The mean abundance of each species at different zones and locations was also examined graphically to help interpret ANOVA results. To compare the total density and biomass of salariin blennies, a two-way ANOVA, with reef location and zone as fixed factors, was used. Total biomass data were square-root transformed to meet the

assumptions of ANOVA and any significant differences evaluated using Tukey HSD tests.

Fine-scale distribution patterns of blennies were examined using selectivity indices to compare the relative availability and usage of different types of microhabitat. Indices and 95% percent confidence intervals were calculated using the methods described by Manly et al. (1993) (sampling design II, protocol A). For each blenny species, indices were calculated only for microhabitats where three or more individual fish were seen to use that microhabitat. The remaining microhabitats were pooled into the "other" category. Blennies were considered to use a microhabitat that was at a significantly greater level than was proportionally available if the lower bound of the 95% confidence interval exceeded 1.

5.3 Results

5.3.1 Broad scale habitat associations

Fifteen species of salariin blenny from eight genera were identified during visual surveys of the reefs around Lizard Island (Table 5.1). The relative abundance of the six most common blenny species varied amongst reef locations and zones. Four of the species were found predominantly at either the lagoon or exposed reefs, whilst the other two species were distributed more evenly between the lagoon, exposed and sheltered locations (Figure 5.2). All six species of blenny were found on the tops of reefs, and four of the species used this zone almost exclusively (Figure 5.2). In contrast, only two species were found in relatively high numbers on the side of reefs ,and both of these species were also regularly seen on reef tops. MANOVA, conducted on the relative abundance of the six blenny species, confirmed that there

were differences in distribution patterns, with a significant interaction between zone and location (Pillai's trace $P<0.001$).

Table 5.1 Salariin blennies observed whilst conducting visual surveys at Lizard Island. Mean total lengths calculated from visual estimates of fish seen during surveys. SE = Standard Error.

Species	**Number observed**	**Mean total length ± SE (cm)**	**Favoured location/zone**
Atrosalarias fuscus	37	7.2 ± 0.3	Lagoon/Top & Side
Blenniella periopthalmus	1	8.0	Exposed/Top
Cirripectes chelomatus	13	6.7 ± 0.4	Lagoon/Top
Cirripectes filamentous	5	5.4 ± 0.2	Lagoon/Top
Cirripectes polyzona	8	5.3 ± 0.2	Exposed/Top
Cirripectes stigmaticus	153	8.6 ± 0.2	Exposed/Top
Crossosalarias macrospilus	2	5.0 ± 0.5	Exposed &Lagoon/Side
Ecsenius aequalis	1	4.0	Lagoon/Side
Ecsenius bicolor	15	5.2 ± 0.2	Exposed/Side
Ecsenius mandibularis	6	4.4 ± 0.2	Lagoon/Side
Ecsenius stictus	126	4.1 ± 0.1	Exposed/Side
Exallias brevis	3	11.0 ± 0.6	Exposed/Top
Glyptoparus delicatulus	29	3.2 ± 0.1	Exposed/Top
Salarias fasciatus	22	8.1 ± 0.5	Lagoon/Top
Salarias patzneri	188	4.1 ± 0.1	Lagoon/Top

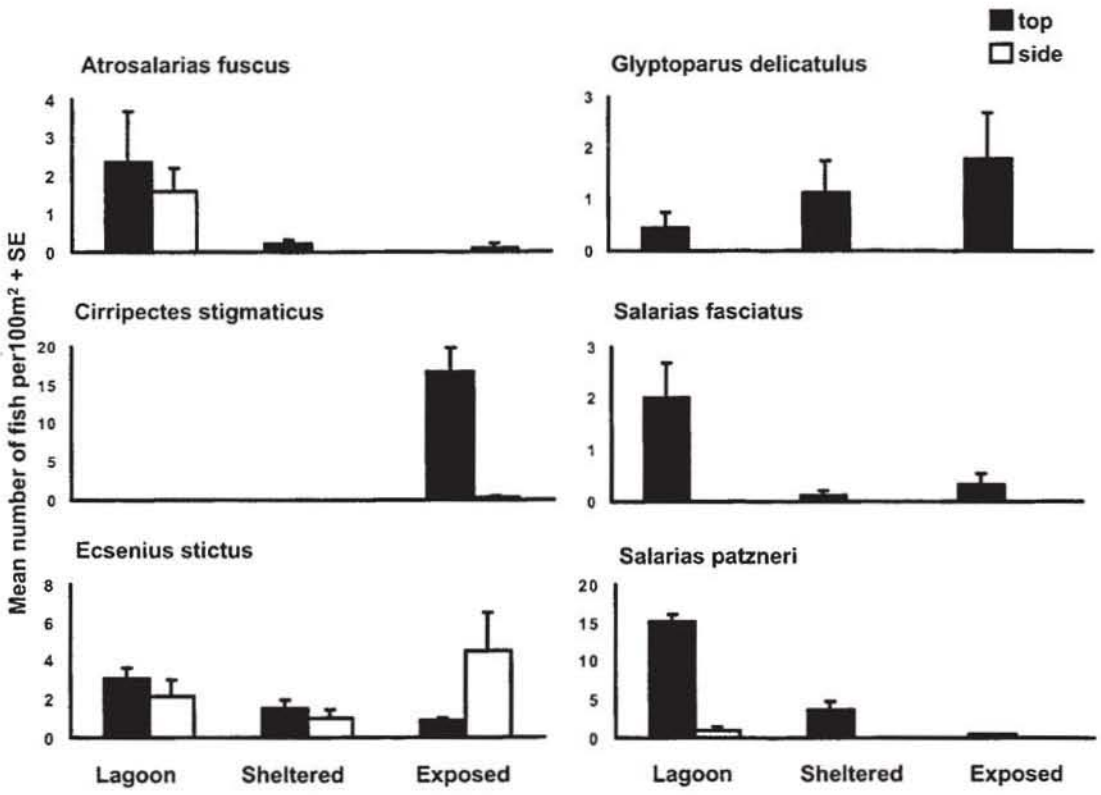

Figure 5.2 Broad scale distribution patterns of the six most abundant species of salariin blenny at Lizard Island. Error bars are standard errors calculated from 3 sites for each combination of zone and location. Note the different scales on y axes.

Salarias patzneri was the most abundant blenny species and was mostly found on the top of lagoon reefs (Figure 5.2). *Salarias fasciatus* had a similar distribution pattern to *S. patzneri*, with the highest levels of abundance also occurring on the top of lagoon reefs. The relative density of *S. patzneri* is, however, an order of magnitude greater than *S. fasciatus*. The other highly abundant species at Lizard Island was *Cirripectes stigmaticus,* which was observed predominantly on the top of exposed reefs (Figure 5.2). Two-way ANOVAs comparing the relative density of individual species found significant interactions between locations and zones for each of the aforementioned species (Table 5.2). Significant interactions can be attributed to higher densities on the top of lagoon reefs for *S. patzneri* and *S. fasciatus*, and exposed reef tops for *C. stigmaticus* (Figure 5.2). No interactions were detected for the three other blenny species, although there were significantly more *Glyptoparus delicatulus* on the top of reefs and significantly more *Atrosalarias fuscus* in the lagoon (Table 5.2). Of the six species investigated *E. stictus* was the only species for which there was no significant difference detected in broad-scale distributions.

Table 5.2 Summary of significant differences in the broad-scale distribution of six species of salariin blennies. Results are from 2-way ANOVAs comparing the relative abundance of each species at different reef locations and zones. ANOVAs were used as a post hoc investigation of a significant MANOVA interaction between Zone and Location (Pillai's trace $P<0.001$).

Species	Significant factor	df	F	P
Atrosalarias fuscus	Location	1,12	6.2	0.01
Cirripectes stigmaticus	Zone x Location	2,12	25.8	0.001
Ecsenius stictus	None			
Glyptoparus delicatulus	Zone	1,12	8.6	0.01
Salarias fasciatus	Zone x Location	2,12	6.0	0.01
Salarias patzneri	Zone x Location	2,12	64.3	<0.001

When densities for all blenny species were combined (Figure 5.3) the total abundance was significantly higher on the tops of reefs ($F=39.3_{1,12}$ $P<0.001$) and at the lagoon and exposed locations ($F=11.1_{2,12}$ $P=0.002$). There was no significant interaction between zone and location. The weight of individual blennies, calculated from length estimates and the relationships in Table 5.3, was used to estimate the total biomass of blennies at different zones and locations. There was a significant interaction when the biomass of blennies at different zones and locations were compared ($F= 14.7_{2,12}$ $P=0.001$). Tukey's HSD test confirmed graphical comparisons (Figure 5.3) that the interaction was due to a significantly greater biomass of blennies on the top of exposed reefs.

Table 5.3 Length-weight relationships for salariin blennies. W= weight (g) TL= total length (cm). Equations based on weight and length measurements of blennies collected from the Great Barrier Reef, Australia.

Species		n	r^2
Atrosalarias fuscus	$W=0.013TL^{2.92}$	73	0.98
Cirripectes spp	$W=0.023TL^{2.81}$	8	0.98
Ecsenius spp	$W=0.015TL^{2.60}$	49	0.93
Glyptoparus delicatulus	$W=0.01TL^{2.88}$	10	0.91
Salarias fasciatus	$W=0.015TL^{2.87}$	13	0.97
Salarias patzneri	$W=0.007TL^{3.09}$	127	0.97

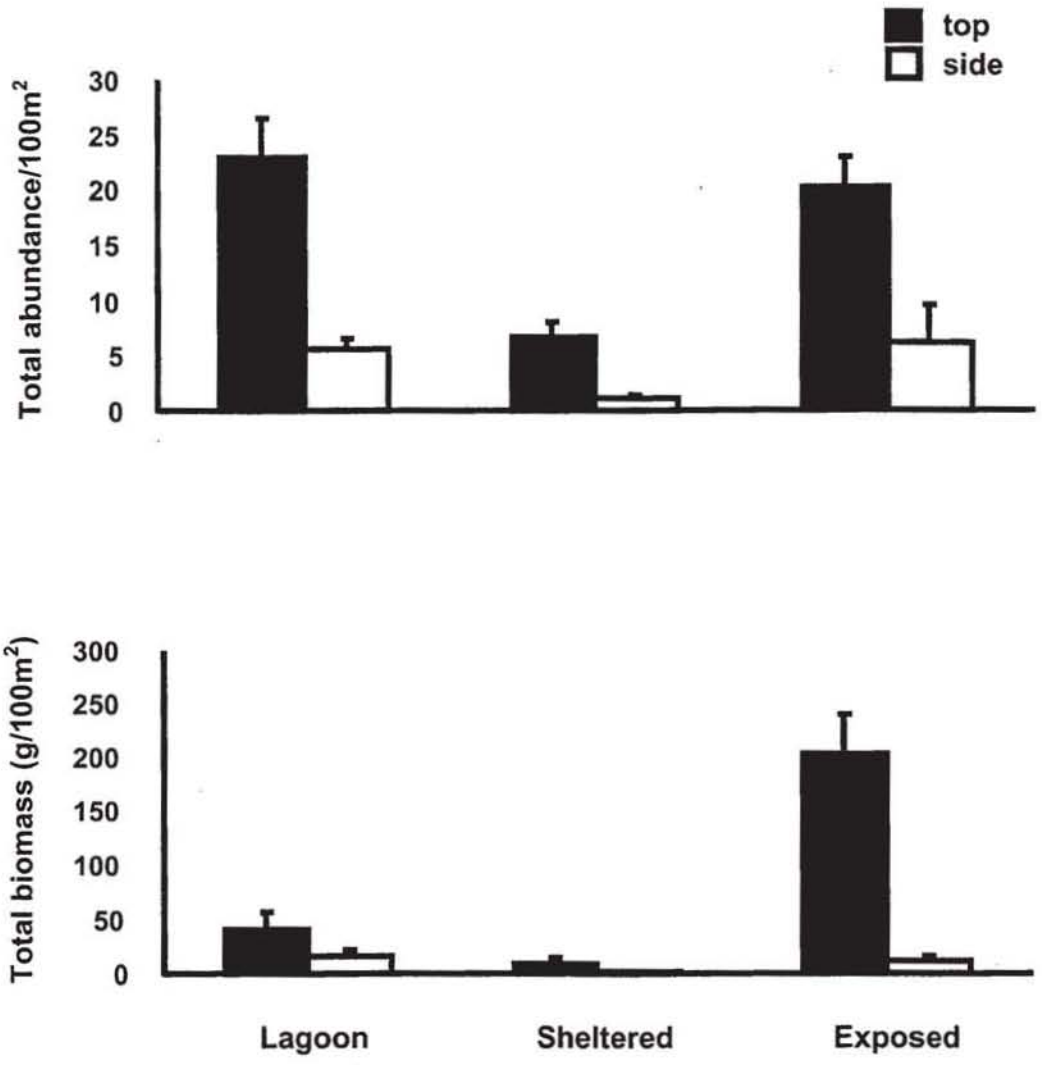

Figure 5.3 Broad-scale patterns of mean total abundance and total biomass of salariin blennies on Lizard Island. Error bars are standard errors calculated from 3 sites for each combination of zone and location.

5.3.2 Fine scale habitat associations

Each of the six blenny species investigated in this study was found to use one or two microhabitats at a higher level than expected based on microhabitat availability (Figure 5.4). Preferences for microhabitats varied between species; however, in all species at least one dead coral growth form was over-represented. *Salarias patzneri*, *Glyptoparus delicatulus* and *Ecsenius stictus* all showed a positive selection for dead massive corals, whilst *Atrosalarias fuscus* and *Salarias fasciatus* selected dead branching corals. In addition, *E. stictus* displayed a preference for living massive corals and *A. fuscus* for living branching corals; however, their use of these microhabitats was less frequent than that of the corresponding dead growth forms (Figure 5.4). *Cirripectes stigmaticus* showed preferences for corymbose corals, living and dead, as well as encrusting corals.

5.3.3 Blenny abundance and biomass relative to other detritivorous/herbivorous fishes.

Density and biomass estimates of all functional fish groups were highest on the top of exposed reefs (Figure 5.5). Salariin blennies, which were classified as territorial detritivores, were an important constituent of this functional group, accounting for approximately 60% of the density and 21% of biomass estimates on exposed reef tops. Overall, the territorial detritivores were found to be a substantial component of the total detritivorous/herbivorous fish community. On the top of exposed reefs they contributed 37% to the total density and 26% to the total biomass of detritivorous/herbivorous fishes.

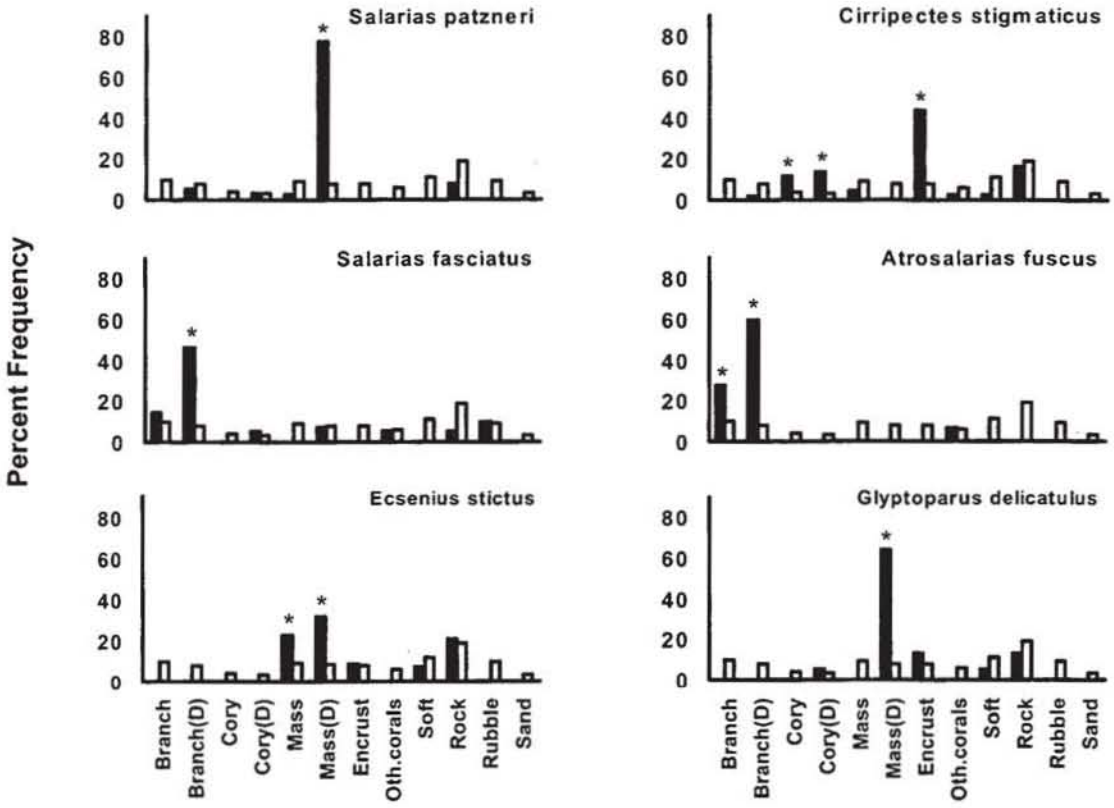

Figure 5.4 Microhabitat associations of the six most abundant salariin blennies at Lizard Island. Percentage values for microhabitat availability and fish presence were calculated from 54 transects. White bars represent percentage of microhabitat available, and black bars represent percentage of fish found in that microhabitat.

* indicates positive selectivity based on selection ratios with 95% confidence intervals. Branch = branching coral, Cory = corymbose coral, Mass = massive coral, Encrust = encrusting coral, Oth.corals= other corals, (D) indicates the coral is dead.

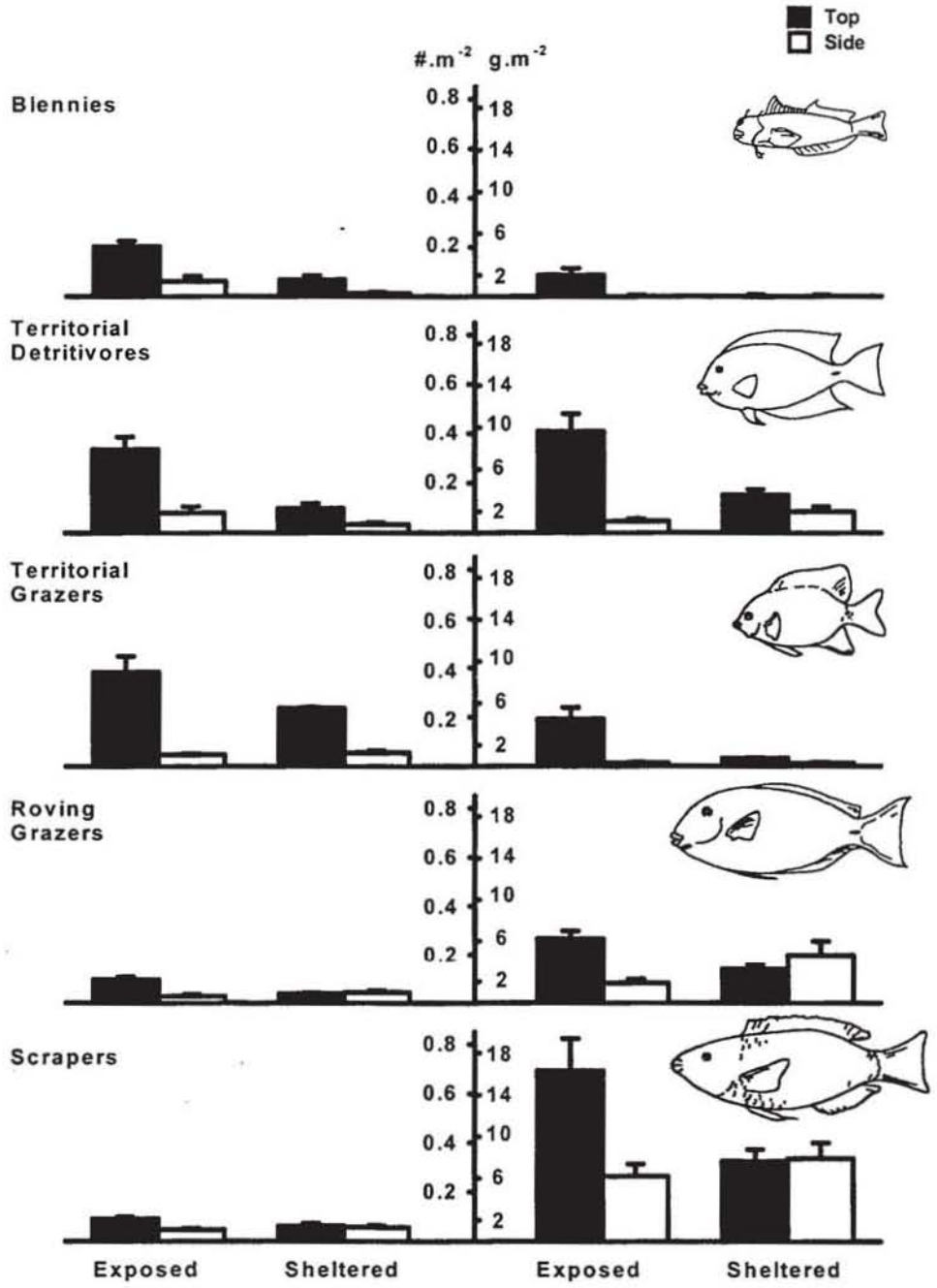

Figure 5.5 Relative abundance and biomass of functional groups feeding on detritus and/or algae. Functional group composition: Territorial detritivores (salariin blennies, *Stegastes nigricans*, *S. fasciolatus*, *Dischistodus* spp and *Ctenochaetus* spp). Territorial grazers (*Pomacentrus* spp, *Plectroglyphidodon lacrymatus*, *Stegastes apicalis*, *Acanthurus lineatus*). Roving grazers (all acanthurids with the exception of *Ctenochaetus* spp and *Acanthurus lineatus*). Scrapers (all scarids). Mean values were calculated from n = 2 to 4 sites for each taxa within a functional group. Standard errors for taxa within a functional group were then summed to produce error bars.

5.4 Discussion

The major dietary items ingested by the six species of blenny investigated in this study were detrital aggregates (Chapter 1). In order to satisfy dietary requirements it is expected that detritivorous fishes, such as blennies, will feed selectively at locations rich in protein (Bowen et al. 1995). Of the six blenny species investigated in this study, four were found at significantly higher densities on reef tops and, when blennies were considered as a single group, their density and biomass is highest on the top of reefs at all locations. On an exposed reef at Lizard Island, Purcell and Bellwood (2001) found that although the C:N ratio of detritus did not differ between reef zones, the percentage of organic detritus in sediments was greatest on the crest. Blennies feeding at this location therefore have access to sediments with a higher percentage of organic detritus and nitrogen.

The presence of sediments with a high percentage of organic detritus on exposed reef tops at Lizard Island coincides with high estimates of blenny abundance and biomass in this environment. High levels of blenny abundance on coral reefs have been documented previously by Odum and Odum (1955), who reported a prevalence of small blennies on the reef ridge at Eniwetok, and more recently by Townsend and Tibbetts (2000), who estimated high densities and biomass of blennies on the rim of reefs on the southern Great Barrier Reef. The association of high blenny abundance with exposed reef tops is therefore consistent over a large geographical range of coral reefs. Furthermore, the acanthurid, *Ctenochaetus striatus*, which has a similar diet and feeding technique to many salariin blennies (see Chapter 1), is also found in high numbers on exposed reef crests (Russ 1984b, Choat and Bellwood 1985). Thus, exposed reef crests seem to be the favoured habitat for a range of detritivorous fishes. This preference may be partially related to the higher organic content of sediments at this location, as organic content can be used as an indicator of the nutritional value of

detritus (Bowen 1987). However, other factors such as competition, recruitment, and predation also are likely to influence the broad scale distribution of detritivores. Competition and predation pressures, as well as recruitment levels, are known to vary spatially (Williams 1991), and the effect of these processes on blenny distributions may be substantial.

On a finer spatial scale, each of the blenny species investigated in this study displayed a preference for dead coral microhabitats. This association with dead corals may be related to the suitability of these microhabitats for accumulating food, as coral skeletons are generally overgrown with filamentous algae that incorporate a matrix of material, including large amounts of detrital aggregates (Chapter2). Although all species showed a preference for dead coral microhabitats, the type of coral microhabitat used by blennies varied among species. This variation may reflect the suitability of different microhabitats as a refuge from predators, as many small fish rely on specific shelter sites (Munday and Jones 1998). Predation can play an important role in the distribution of fish on coral reefs (Stewart and Jones 2001), and the use of microhabitats that act as suitable predator refuges can effect survival and persistence of small fish (Beukers and Jones 1998). Overall, on a fine scale, it appears that the distribution of blennies may be influenced by the availability of dead coral microhabitats that act as sites of detrital accumulation as well as provide suitable refuge sites. However, as with broad-scale fish distributions, factors other than resource availability and predation are likely to influence the fine-scale distribution of blennies, and, although not addressed in this study, these processes may have a significant effect on the abundance of blennies in different microhabitats.

Comparisons of salariin blenny numbers and biomass with other detritivorous/herbivorous fishes were used to examine the relative importance of

blennies as a trophic group. Blennies represented a substantial part of the territorial detritivore functional group. On the top of exposed reefs, where the abundance and biomass of all functional groups were highest, blennies accounted for more than half of the density and approximately a fifth of the biomass estimates of territorial detritivores. Overall, territorial detritivores also accounted for approximately a third of the density and a quarter of the biomass estimates of the detritivore/herbivore fish assemblages on exposed reef tops. Temporal differences between blenny censusing and that of other detritivorous/herbivorous fish studies dictate that conclusions must be tentative; however, the contribution by blennies and other detritivorous fishes appears to be substantial and suggests that detrital feeding fish are an important component of coral reef food webs. The contribution of blennies to detritivorous/herbivorous fish assemblages may be even greater on higher latitudinal reefs of the Great Barrier Reef, where blenny biomass relative to other detritivorous/herbivorous fishes (see Townsend and Tibbetts 2000) was even higher than on the reefs surrounding Lizard Island. This difference suggests that the relative importance of blennies may vary with geographic location.

In summary, most salariin blennies displayed distinct distribution patterns at both broad and fine scales. On a broad scale, blenny numbers and biomass are greatest on the exposed reef top where the relative amount of detritus in sediments is high. On a finer scale, they are associated with dead corals that may act as places of detrital accumulation or shelter sites. Detritus availability may not be the sole factor driving blenny distribution patterns, as other processes, such as competition and predation, are also likely to play an important role in blenny distribution. However, this study did show that at both large and small spatial scales blenny abundance and biomass is strongly associated with the availability of their primary dietary resource, detritus. On

the top of exposed reefs, where the density and biomass of all functional groups were highest, blennies were found to be a major component of the territorial detritivore functional group. This functional group represented approximately a third of the density and a quarter of the biomass estimates for the detritivorous/herbivorous fish community. The substantial contribution of small territorial detritivores, such as blennies, to the detritivorous/herbivorous fish community suggests that these fishes are an important component of the fish assemblages that are responsible for secondary production on coral reefs.

Overview

In aquatic ecosystems, energy is transferred through trophic food webs that are based on grazing by herbivores and/or the consumption of detritus by detritivores (Begon et al. 1990). On coral reefs, the prevalence of supposedly herbivorous fishes and invertebrates with high consumption rates has led to the assumption that herbivory is the primary mode of energy transfer in this ecosystem (Hatcher 1983a, 1997). A significant proportion of the energy produced by coral reef algae is, however, converted into detritus and passes through detrital pathways (Arias-Gonzalez et al. 1997). Consequently, detrital pathways are likely to play an important role in the coral reef trophodynamics. This study has shown that salariin blennies use detritus as their primary dietary resource and that these fish are a significant component of coral reef fish assemblages that feed on the EAM.

On the Great Barrier Reef, Australia, salariin blennies ingest predominantly detrital aggregates and comparatively small quantities of filamentous algae (Chapter 1). The predominance of detritus in the guts of a representative species, *S. patzneri,* was consistent between seasons and throughout post-settlement ontogeny, suggesting that detritus is the primary dietary resource of both juvenile and adult blennies throughout the year. *Salarias patzneri*, feeds preferentially on particles <125μm, which are of a size that is typical of nutritionally valuable amorphic detritus, whilst avoiding filamentous algae. Comparisons of the fatty acids in *S. patzneri* tissues, with those in detrital aggregates <125μm and filamentous algae, indicated that detrital aggregates are assimilated by this blenny (Chapter 4). Gerking (1994) defined a detritivorous fish as "one that ingests a predominant amount of detritus in its diet", with a caveat that ingestion does not necessarily mean absorption. *Salarias patzneri*

can, therefore, unequivocally be classified as a detritivorous fish and other salariin blennies that predominantly ingest detrital aggregates are also likely to be detritivores.

Biochemical comparisons of detrital aggregates with filamentous algae collected from the EAM within *Salarias patzneri* territories indicated that protein to energy ratios were slightly higher in the detrital aggregates and were above the ratio that Bowen (1979) identified as critical for fish growth (Chapter 2). As a substantial proportion of the organic matter in the EAM was also in the form of detrital aggregates (41-44%), detritus represents a high quality dietary resource that is readily available for *S. patzneri* in the summer and winter. Visual analysis of detrital aggregates found that they were mainly amorphic detritus, with small amounts of diatoms, cyanobateria and invertebrates (Chapter 2). Amorphic detritus has no structure or form, making it difficult to identify its source. However, based on the high productivity of algal turfs (Larkum 1983), much of which are not consumed by herbivores (Hatcher 1981, Klumpp and Polunin 1990, Polunin and Klumpp 1992), it is believed that a large percentage of the detritus on coral reefs is derived from the epilithic algal community (Hansen et al. 1992). This hypothesis is supported by the similarity of fatty acid, hydrocarbon and sterol profiles of detritus and filamentous algae collected from the same EAM (Chapter 3). Subtle differences in these profiles do, however, indicate that filamentous algae are not the sole source of amorphic detritus. Lipid biomarkers suggest that microalgae, coral mucus and bacteria are also important sources of amorphic detritus.

The addition of micronutrients, fatty acids and protein from microalgae, coral mucus and bacteria to detrital aggregates is likely to enhance the overall diversity and concentration of nutrients in detrital samples that are primarily derived from filamentous algae. For detritivorous fishes, the most important dietary component, in

terms of somatic growth, may be protein (Bowen et al. 1995). Items identified as likely components of detritus in *Salarias patzneri* territories are all potential sources of protein, and their possible contribution to the protein content of detritus is estimated in Table 6.1. This table does not represent all of the potential sources of protein, because some detrital sources were not quantified visually or by lipid biomarkers, making calculations of their potential protein input unfeasible.

Table 6.1 Sources of protein in detrital samples collected from the EAM within *Salarias patzneri* territories.

Source	**Protein content of source % dry weight**	**% of Organic matter from detritus samples**	**Protein input ($mg.g^{-1}$ of organic matter)**
Bacteria	63 [1]	1-10 (Ch. 3)	6-63
Diatoms	16-18 [2]	1.5-1.9 (Ch. 2)	2-3
Diatoms		4-18 (Ch. 3)	6-32
Blue green algae	37-52 [3]	1.9-3.2 (Ch. 2)	7-16
Copepods	55-58 [4]	0.2-0.4 (Ch. 2)	1-2
Filamentous algae	2.8-13.2 [5]	7.6-10.4 (Ch. 2)	2-14
Filamentous algae		< 86 (Ch. 3)	< 24-113
TOTAL			18-226

1. Simon and Azam (1989), 2. Lourenco et al. (1998), 3. Vargas et al. (1998), 4. Alonzo et al. (2001), 5. Montgomery and Gerking (1980)

Calculations in Table 6.1 suggest that the three major sources of protein in the detritus collected from *Salarias patzneri* territories are; bacteria, diatoms and filamentous algae. All three of these items are likely to be used as a protein source by *S. patzneri*, as there was no evidence that blennies preferentially assimilated a particular component of detrital aggregates (Chapter 4). It would therefore be remiss to attribute the nutritional quality of detrital aggregates to a single detrital component

or source, because it is the combination of nutrients supplied from various origins that makes detritus an ideal dietary resource.

The potential sources of protein in table 6.1 and sources of detritus in Chapter 3 are however based on the standing biomass of organisms and detritus. Microbes, like bacteria, have high rates of productivity, particularly in tropical waters (Moriarty et al. 1985), and although their standing biomass is relatively small, microbes are continually producing protein. Variation in the dynamics of different microorganisms may therefore effect their contribution to detrital nutrients and dietary value to detritus consumers. Furthermore, we need to know more about the dynamics of detritus production. Algae is obviously a major source of detritus, however, we need to know the rates at which detritus accumulates and the process that are involved in detrital production. Combined with information on detritivore consumption rates, information on detritus accumulation rates will help to clarify the importance of detritus and detritivorous fish to coral reef trophodynamics.

Salariin blennies represent a substantial portion of detritivorous/ herbivorous fish assemblages on coral reefs (Chapter 5), and the relatively high abundance and ingestion rates of blennies suggest they play an important role in coral reef trophodynamics. Furthermore, longevity estimates of two blenny species from different locations on the Great Barrier Reef suggest these two species live for only one or two years (Wilson unpublished data). In contrast, larger and more conspicuous fish from the families Acanthuridae and Scaridae may live for 10-20 years (Choat and Axe 1996, Choat et al. 1996). Relatively short longevity estimates for blennies infer higher turnover rates of these fish relative to acanthurids and scarids, and therefore detritus converted to blenny biomass may be passed on to higher trophic levels at a faster rate. Consequently, salariin blennies may play a more important role in the

transfer of energy from detritus to higher trophic levels than larger, longer-lived fish that feed on the EAM.

The high abundance of blennies, with relatively high metabolic rates, infers that the detrital aggregates on which they feed are an energetically and nutritionally valuable dietary resource. This inference is supported by biochemical analyses that indicate detrital aggregates are capable of supporting fish growth and that these aggregates are of similar and possibly greater nutritional value than filamentous algae. As detrital aggregates are a major organic component in the EAM, these results suggest detritus may contribute significantly to the diet of other fishes that feed on the EAM. Detritus may therefore have a much more direct role in coral reef fish diets than previously indicated, and specialist detrivitoves, such as blennies, as well as many of the fishes we have traditionally classified as herbivores, may satisfy large portions of their dietary requirements via the ingestion and assimilation of detritus.

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