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Nutritional ecology of nominally herbivorous fishes on coral reefs

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ABSTRACT: Nominally herbivorous acanthurids (surgeonfishes) and scarids (parrotfishes) have often been considered a 'homogeneous' functional group that consumes and digests algae. Recent work demonstrates that many of these fishes consume detritus. The objective of this study was to investigate the composition of dietary nutrients targeted by these and other fishes in terms of feeding behaviour, diet and short chain fatty acids (SCFA). We undertook a nutritional analysis of a range of species including detritivores, algivores, omnivores and planktivores from north eastern Australia. We calculated assimilation efficiencies for total protein amino acids (TAA), carbohydrate and lipid, and measured TAA in gut fluid along the intestine. Nutrients were assimilated similarly to their dietary proportions, with planktivores assimilating a high proportion of TAA, a moderate proportion of lipid and little carbohydrate. Omnivores assimilated moderate proportions of TAA and carbohydrate, and a low proportion of lipid. Algivores assimilated a low proportion of TAA and lipid, but a high proportion of carbohydrate. Detritivorous scarids and acanthurids differed significantly from algivores, assimilating a high proportion of TAA, a low proportion of carbohydrate and a moderate proportion of lipid. TAA levels in gut fluid of all species were highest in the anterior and lowest in the posterior intestine. Gut segments with highest TAA values were compared between dietary groups and followed a similar trend to TAA assimilation. Planktivores had high concentrations of TAA, while omnivores had intermediate, and algivores the lowest, concentrations. The highest gut fluid TAA concentrations were found in detritivorous scarids and acanthurids, and were significantly higher than in algivores. A significant negative correlation was found between anterior intestinal fluid TAA and posterior intestinal SCFA values. Detritivores had the highest levels of TAA but the lowest levels of SCFA. Planktivorous species had high levels of TAA and low to intermediate levels of SCFA. Omnivores had moderate levels of both TAA and SCFA. Algivores had low levels of TAA but high levels of SCFA. This indicates that there are major differences in the food resources targeted by detritivorous and algivorous fish species on coral reefs.

KEY WORDS: Amino acids · Assimilation · Carbohydrate · Coral reefs · Detritus · Herbivorous fishes · Lipid · Trophic strategies

INTRODUCTION

Coral reefs support a diverse assemblage of plant forms including macroscopic algae (usually foliose reds and fucoid browns) and the epilithic algal complex (EAC) that covers exposed surfaces of reef crests and flats (Steneck 1989, Choat 1991, Hixon 1997, Choat & Clements 1998). The EAC consists not only of filamentous and small turfing algae, but also aggregates of detritus and sediment particles, microbes (including bacteria, cyanobacteria, diatoms and dinoflagellates) and meiofauna (Hatcher 1983, 1988, 1997, Moriarty et al. 1985, Alongi 1989, Ducklow 1990, Hay & Steinberg 1992, Sorokin 1993, Choat & Clements 1998). In addition, the complex currents associated with coral reefs tend to accumulate macrozooplankton including salps, coelenterates and crustaceans at particular sites. All these elements represent food sources

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for the nominally herbivorous fishes of coral reefs (Choat et al. 2002). Despite a strong impression of behavioural and dietary uniformity, especially in those species feeding on the EAC, direct observation of gut contents demonstrates a high degree of dietary selectivity, including differences among EAC-grazing species that feed over precisely the same surfaces (Choat 1991, Purcell & Bellwood 1993, Choat et al. 2002).

Previous investigations have established the following: Species of reef fishes nominally classified as herbivores consume a wide range of dietary items including detrital aggregates, turfing and macroscopic algae and macrozooplankton (Wilson & Bellwood 1997, Wilson 2000, Choat et al. 2002). Food processing modes including feeding rates, gut transit frequencies and the presence of fermenting endosymbionts reflect a classification based on diet (Choat et al. 2004). In terms of relative abundances, nominally herbivorous fish faunas are dominated by species feeding on detrital aggregates in the reef environments investigated, while unambiguously herbivorous species are a minority at most sites (Choat et al. 2004, Wilson 2004).

Although a high degree of dietary selectivity has been demonstrated for dominant study taxa (primarily acanthurid, kyphosid and scarid fishes), the identity of the nutritional targets in each group has not been established. The purpose of the present study was to extend previous investigations (Choat et al. 2002, 2004) on the relationship between feeding behaviours, diet and microbial fermentation to identify the primary nutrients in diets and to estimate the assimilation of dietary macronutrients (protein, carbohydrates and lipid) in a suite of fish species. The species grazing the EAC, especially those taxa consuming mainly detrital aggregates, were of particular interest. Parallels may be drawn between coral reef EAC and its grazers and tropical freshwater systems (e.g. the rift lakes of Africa), where a diverse assemblage of grazing fishes feeds on the 'Aufwuchs' or periphyton, a thin layer of algae, detritus, microrganisms, and small invertebrates that covers rocky substrata (Bowen 1976, 1980, Reinthal 1990, Sturmbauer et al. 1992, Genner et al.1999). While there is an established body of literature that examines the composition and nutritional value of Aufwuchs, there is relatively little comparative information for coral reef systems (Choat & Clements 1998).

Previous insights into the nutritional strategies of coral reef fishes have come from the analysis of the products of intestinal microorganisms, i.e. short chain fatty acids (SCFA). These compounds are assimilated and used as a metabolic substrate by the fishes (Clements 1997, Mountfort et al. 2002). Analyses of SCFA in coral reef fishes have identified correlations between diet and SCFA levels in the intestine (Clements & Choat 1997, Choat & Clements 1998, Choat et al. 2002). Unequivocally herbivorous species have high concentrations of SCFA in their hindgut, indicative of the involvement of microbial fermentation in algal digestion. Species that consume detritus are characterised by low concentrations of SCFA, i.e. lower levels of intestinal microbial fermentation, but with a high proportion of isovalerate. Isovalerate is produced by the fermentative catabolism of the amino acid leucine, indicating high levels of leucine in the diet of those species (Choat & Clements 1998) Species that consume mixtures of plant and animal material have intermediate concentrations of SCFA.

Our primary objective herein is to investigate the relationships between dietary items, their nutritional composition and the pattern of intestinal fermentation in selected coral reef species covering the full dietary spectrum outlined above. The approach adopted involved 2 main tasks. First, the assimilation efficiencies were measured for total protein amino acids (TAA), carbohydrate and lipid. This was achieved by measuring these nutrients in the contents of the anterior alimentary tract (proxy for diet) and in the contents of the posterior alimentary tract (proxy for faecal matter). Secondly, TAA was measured in the gut fluid along the alimentary tract in previously defined regions and compared to published SCFA for the same species. Our predictions for the study species were as follows.

- Species feeding exclusively on macro- and turfing algae (algivores: *Naso unicornis*, *Kyphosus cinerascens*, *K. vaigiensis*, *Acanthurus lineatus*) and containing high levels of SCFA were predicted to display high levels of dietary and assimilated carbohydrates and correspondingly low levels of TAA. This would be equivalent to terrestrial vertebrate herbivores which use microbial fermentation to digest plant diets high in carbohydrate (Stevens & Hume 1995)
- Species feeding on animal matter (planktivores: *Naso hexacanthus*, *N. annulatus*, *Acanthurus mata*) were predicted to display the converse combination with high levels of dietary and assimilated TAA and low levels of carbohydrates. These species would be equivalent to terrestrial predators that consume animal diets high in protein
- Species in which diets are dominated by detrital aggregates and sediment particles (detritivores: *Chlorurus microrhinos*, *Scarus schlegeli*, *S. frenatus Acanthurus olivaceus*, *Ctenochaetus striatus*) were predicted to have high levels of dietary and assimilated TAA, indicative of a feeding habit dominated by protein scavenging. This prediction is supported by the high proportion of the SCFA isovalerate found in the intestinal fluid of these fishes, which is indicative of high dietary protein (Choat & Clements 1998)

• Omnivorous species (omnivores: *Naso tonganus*, *N. vlamingii*) were predicted to show intermediate levels of dietary carbohydrates and TAA

In the present study we use data on nutrient levels to examine dietary classification in nominally herbivorous coral reef fishes. In a separate study, we will use these nutrient data to explore how these fishes regulate nutrient intake and assimilation.

MATERIALS AND METHODS

Sample collection and processing. Samples were collected from a coral reef system at the northern end of the Great Barrier Reef, Australia (14° 42′ S, 145° 30′ E) in the austral summers of 1997 and 1998. Sampling was restricted to adult fishes collected by spearing from reef crest, flat and slope habitats on mid-shelf reefs surrounding Lizard Island and the adjacent outer barrier reefs (Day, Hicks and Noname reefs), where fishes were also collected from open water habitats (location details in Choat et al. 2002 and Gust et al. 2002). Fishes were immediately killed by pithing and placed on ice for transport to the laboratory. Specimens were collected after 11:00 h to ensure that gastrointestinal tracts were full. The 14 taxa collected comprised 9 species of acanthurids (*Acanthurus lineatus*, *A. mata*, *A. olivaceus*, *Ctenochaetus striatus*, *Naso annulatus*, *N. hexacanthus*, *N. tonganus*, *N. unicornis*, *N. vlamingii*), 2 species of kyphosids (*Kyphosus cinerascens*, *K. vaigiensis*), and 3 species of scarids (*Chlorurus microrhinos*, *Scarus frenatus*, *S. schlegeli*). Data on TAA and ash content of EAC algae and detritus are from Crossman et al. (2001) and in the present study are further analysed for carbohydrate and lipid.

Standard length, total length and total weight were measured for each fish in the laboratory (Table 1). Digesta were collected from the anterior and posterior

Table 1. Number (n), standard length (SL), weight (W) and diet of study species

Species	n	SL (mm) $\bar{x}$	SL (mm) Range	W (g) $\bar{x}$	W (g) Range	Diet composition	Trophic group	Source
Acanthurus lineatus	10	191	178–203	319	220–398	Filamentous rhodophytes and chlorophytes	Algivore	Robertson & Gaines (1986)
						Thallose and filamentous rhodophytes		Choat et al. (2002)
A. mata	5	327	301–359	999	780–1304	Zooplankton	Planktivore	Randall et al. (1990)
A. olivaceus	8	194	160–210	352	214–493	Detritus, diatoms and algae on sand	Detritivore	Myers (1989)
						Algal detritus		Choat et al. (2002)
Ctenochaetus striatus	8	182	157–198	297	182–363	Detritus, diatoms, cyanobacteria	Detritivore	Myers (1989)
						Algal detritus		Choat et al. (2002)
Naso annulatus	5	530	489–568	4076	3256–5000	Zooplankton, crinoids	Planktivore[a]	Clements & Choat (1995)
						Zooplankton, filamentous chlorophytes thallose rhodophytes		Choat et al. (2002)
N. hexacanthus	5	487	450–515	2358	1886–2738	Zooplankton	Planktivore	Myers (1989)
						Zooplankton		Choat et al. (2002)
N. tonganus	7	397	201–465	1613	288–1994	Fleshy algae, benthic invertebrates	Omnivore	Myers (1989)
						Fleshy algae, benthic invertebrates		Choat & Clements (1995)
						Thallose chlorophytes and rhodophytes, filamentous rhodophytes		Choat et al. (2002)
N. unicornis	5	276	236–321	724	416–1104	Thallose phaeophytes	Algivore	Robertson & Gaines (1986)
						Thallose phaeophytes, filamentous rhodophytes		Choat et al. (2002)
N. vlamingii	5	330	315–370	1002	804–1271	Zooplankton, fish faeces	Omnivore	Myers (1989), Robertson (1982)
						Zooplankton, filamentous chlorophytes, thallose rhodophytes		Choat et al. (2002)
Kyphosus cinerascens	5	322	254–342	1244	577–1529	Thallose and filamentous rhodophytes	Algivore	Myers (1989), Randall et al. (1990)
						Thallose rhodophytes, filamentous chlorophytes, thallose and filamentous phaeophytes		Choat et al. (2002)
K. vaigiensis	5	299	290–312	896	864–892	Thallose phaeophytes and chlorophytes	Algivore	Clements & Choat (1997)
						Thallose phaeophytes and chlorophytes		Choat et al. (2002)
Chlorurus microrhinos	4	307	276–335	1069	743–1368	Epilithic algae, living coral	Detritivore	Bellwood & Choat (1990)
						Detritus		Choat et al. (2002)
Scarus frenatus	10	238	194–285	539	253–920	Epilithic algae	Detritivore[b]	Bellwood & Choat (1990)
S. schlegeli	5	211	195–255	365	260–590	Epilithic algae, sand surface	Detritivore	Bellwood & Choat (1990)
						Detritus		Choat et al. (2002)

[a] This species does consume some algae but was designated planktivore as zooplankton and animal material make up >50% of its diet (Choat et al. 2002) and no identifiable algae were present in individuals collected for nutritional analysis

[b] Gut contents similar to *S. schlegeli* (J. H. Choat pers. obs.), therefore classed as detritivore

alimentary tract and frozen at –20°C for determination of assimilation efficiencies. Gut content of the stomach (or intestinal swelling in the scarids, which lack a stomach: Clements & Bellwood 1988) was considered a proxy for diet and the hindgut material was considered a proxy for faecal matter. Gut fluid (for amino acid analysis) was collected from 5 alimentary tract segments—the stomach (or intestinal swelling), and 4 intestinal segments. These 5 segments are referred to subsequently as Segments I to V, from anterior to posterior (Clements & Choat 1995). In acanthurids and scarids there was no clear differentiation along the intestine, so the intestine was divided into 4 segments of equal length (Segments II to V). *Kyphosus vaigiensis* has a distal chamber or caecum separated from the rest of the intestine by a sphincter (Clements 1997); this intestinal swelling was considered as Segment V. The remaining intestine for this species was divided into 3 sections of equal length (II–IV). No intestinal swelling was obvious in *K. cinerascens* (Clements 1997), so the intestine was sampled as described for acanthurids. To obtain gut fluid, fresh digesta were removed from each of the 5 gut segments, placed into Eppendorf tubes, and spun at 12 000 × *g* in an Eppendorf 5414 bench centrifuge for 10 to 15 min until a clear supernatant was obtained. The supernatant was decanted into new tubes, frozen, stored at –20°C, and subsequently transported to the University of Auckland. Gut fluid was removed from fresh digesta to avoid elevated amino acid levels found in gut fluid from frozen digesta (Crossman et al. 2000).

Analysis of the nutritional profiles of detritus feeding acanthurids and scarids identified 2 technical problems. In the stomach samples of *Acanthurus olivaceus* and *Ctenochaetus striatus* it was found that the muscular gizzard was filled with coarse calcareous material required for the trituration of detritus. There was insufficient gut fluid for amino acid analysis, and hence no amino acid data were obtained for this segment. In addition, the retention of inorganic material in the gizzard resulted in substantial underestimation of the levels of dietary macronutrients in the anterior intestinal region and hence the assimilation efficiencies. For this reason assimilation profiles could not be determined in these acanthurids.

In scarids, Choat et al. (2002) found masses of endogenous cells in the anterior gut along with a large quantity of detrital material. These unusual cells appeared to be secreted from glandular material near the pharyngeal jaws (Choat et al. 2002). The intact nature of these endogenous cells suggests that they may play a role in the digestive process. It is likely that these cells would have contributed to the nutrient levels measured, including the high levels of TAA in the solids from the anterior intestine. In order to obtain a more accurate picture of exogenous inputs of nutrients, gut fluid (which did not contain particulate or cellular material) was extracted and used to provide comparative assimilation profiles for all species.

In the laboratory frozen ingesta and digesta were dried to constant weight with a Dura-Drycan™ freeze drier. Wet weight was obtained prior to freeze-drying in order to calculate percentage water. The dried material was then ground to a fine powder in a ball mill (Retsch mixer mill MM2) and stored desiccated at –20°C. All mass measurements were determined to the nearest 0.01 mg on an analytical balance (Mettler AE 163, Mettler Instrumente). Proteins were extracted by accurately weighing approximately 20 mg of ground sample into Eppendorf tubes and adding 0.6 ml of 1 M NaOH. Tubes were then mixed by vortexing and placed on a rocker for 12 h (Montgomery & Gerking 1980). After extraction, tubes were centrifuged (12 000 × *g*, 5 min) to pellet particulate material, and supernatants were removed for amino acid analysis.

Analytical procedures. ***Amino acid analysis:*** Total nitrogen is used by many authors to measure protein, but will include a non-assimilable nitrogen component, while many spectrophotometric protein assays are subject to interference from compounds found in algae (Crossman et al. 2000). To circumvent these analytical problems, protein was measured by amino acid analysis. Another advantage of amino acid analysis is that it will detect peptides and free amino acids not detected by spectrophotometric protein assays.

Amino acid analysis was performed following the procedure of Crossman et al. (2000). Gut fluid or acidified protein extracts were loaded into hydrolysis tubes, dried under vacuum, and hydrolysed in 6 M HCl and 1% phenol at 150°C for 60 min under nitrogen using a PICO·TAG™ Work Station (Waters). Hydrolysed samples were derivatised with phenylisothiocyanate and quantified by reverse phase HPLC using a 421 amino acid analyser coupled to a 172 microbore HPLC (Applied Biosystems).

Ash, lipid and carbohydrate analysis: Ash content of the dried samples was determined by combusting 50 mg samples at 500°C in a Kotter kiln for 16 h.

Lipid was determined gravimetrically in a procedure modified from Folch et al. (1957). Samples of dried gut contents or EAC algae or detritus (40 mg) were mixed in 100 µl water and 1.5 ml of chloroform/methanol (1:2 v/v) and left to stand (10 min, 4°C). These were then centrifuged and the supernatants removed. A further 1.5 ml of chloroform/methanol (2:1 v/v) was added to the pellets and the samples processed as before. The 2 supernatant aliquots were pooled and 0.95 ml of 0.7% (w/v) NaCl solution was added. The samples were mixed thoroughly and then left to stand (30 min, 4°C).

Samples were then centrifuged and a measured portion of the lower chloroform/methanol phase removed and placed into pre-weighed glass vials. The chloroform was evaporated in a vacuum desiccator, and the vials re-weighed to determine lipid content.

Carbohydrate was measured using a reducing sugar assay modified from Englyst & Cummings (1988), whereby 10 to 20 mg of dry digesta, EAC algae, or detritus was weighed accurately into glass culture tubes with screw tops. Lipid was removed from samples containing >10% lipid. This was achieved by the addition of 4 ml of acetone to the tubes, which were then vortexed and left for 30 min. Tubes were then centrifuged at 1800 × *g* for 10 min. After centrifugation the lipid-rich supernatant was discarded and the tubes were allowed to dry in a 70°C oven for 20 min. Calcium carbonate was then removed from the samples. This involved wetting the samples with 50 µl of 80% ethanol, followed by 300 µl of 6 M HCl to remove the calcium carbonate. After dissolution the tubes were allowed to dry for 30 min in a 70°C oven. The carbohydrate in the samples was then hydrolysed. This was achieved by addition of 200 µl of 12 M sulphuric acid and heating the tubes in a 35°C shaking water bath for 1 h, followed by addition of 2.2 ml of water and incubation in a boiling water bath for a further 2 h. Cooled tubes were then centrifuged at 1800 × *g* for 10 min and the supernatant removed for colorimetry. This involved running blanks (25% saturated benzoic acid and 1 M sulphuric acid), fresh sugar standards (0.5, 1.0, 1.5, and 2.0 mg ml^{-1} glucose in a solution of 25% benzoic acid and 1 M sulphuric acid) and sample hydrolysates; duplicates of 1 ml from each solution were delivered to separate test tubes along with 0.1 ml of 0.5 mg ml^{-1} glucose (in 50% benzoic acid) and 0.1 ml 3.9 M sodium hydroxide, and then mixed by vortex. Next, 0.4 ml dinitrosalicylate solution (5.0 g 3,5-dinitrosalicylate acid, 8.0 g sodium hydroxide, and 150 g sodium/potassium tartrate in water to a final volume of 0.5 l) was added to each tube, mixed by vortex, and placed in a briskly boiling water bath for 10 min. Tubes were then cooled and 4.0 ml water added. The tubes were then mixed thoroughly by inversion and the absorbance read at 530 nm. Sugar concentration was determined in the samples by comparing sample absorbance to that of standards.

Assimilation efficiencies: Assimilation efficiency is the proportion of ingested food that is absorbed by the digestive system. Assimilation efficiencies were determined by comparing nutrient concentrations in the stomach (or anterior intestine) with faecal concentrations and using ash (a component assumed not to be digested or absorbed) to correct faecal nutrient concentrations, as described in Montgomery & Gerking (1980).

The grazing acanthurids *Ctenochaetus striatus* and *Acanthurus olivaceus* were not included, as preliminary analysis has shown that the methods used are not suitable for use with these fishes. Both these species have a muscular gizzard-like stomach in which ingested inorganic material is used to grind food (Horn 1989). This inorganic matter caused an underestimation of the nutritional value of stomach contents and resulted in negative assimilation efficiencies.

Statistical analysis. Principal components analysis (PCA) was performed on the 5 dependent variables: moisture, ash, amino acids, carbohydrate and lipid measured in stomach and anterior intestinal contents. As no moisture data were collected for EAC detritus and algae, these 2 independent variables were excluded from the analysis. The first 2 principal components were plotted and the 95% confidence ellipses plotted around the following *a priori* groups: herbivores, omnivores, carnivores, and algivores.

Kruskal-Wallis ANOVAs combined with Dunn's multiple comparison tests (Zar 1984) were used to test for differences in TAA, carbohydrate and lipid between the dietary groups detritivorous scarids, planktivores, omnivores and algivores.

Analysis of variance combined with Tukey's honestly significant difference (HSD) test (for unequal N) was used to test for differences in peak TAA concentration of gut fluid between the dietary groups detritivorous scarids, detritivorous acanthurids, planktivores, omnivores and algivores.

Pearson's correlation was used to examine the relationship between peak TAA concentration and peak SCFA concentration in the gut fluid.

RESULTS

The nutrient analysis of dietary (Segment I) and faecal (hindgut) material of the study species plus that of the epilithic algal community (detritus and algae), the main food source for many species, is presented in Table 2. Moisture, ash, amino acids, carbohydrate and lipid all varied in the diet between the study species. To explore the relationships between dietary nutrients and diet category (planktivores, omnivores, algivores and detritivorous scarids), data were analysed by principal components analysis. The results of the analysis are presented in Fig. 1. Data for the first 2 principal components (PC) explained 52.2% (PC 1) and 34.7% (PC 2) of the variation in the data, respectively. This shows a clear separation of algivores from planktivores, with algivores containing higher proportions of carbohydrate (28.8 to 42.1% vs. 4.7 to 7.9%) and planktivores greater proportions of amino acids (25.2 to 37.1% vs. 7.7 to 11%) and lipid (11.1 to 13.7% vs. 4.5

Table 2. Nutrient analysis of stomach or anterior intestinal contents and rectal material for study species. A.: anterior; –: no data

Species	Organ	Moisture (%)			Ash (% dry wt)			Amino acid (% dry wt)			Carbohydrate (% dry wt)			Lipid (% dry wt)		
		n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE	n	$\bar{x}$	SE
Planktivores																
Naso annulatus	Stomach	5	94.0	0.4	5	38.5	3.6	5	25.2	2.3	5	7.9	0.8	5	11.1	1.8
	Rectum	5	93.1	1.1	5	43.8	1.7	5	7.6	1.0	5	11.0	1.2	5	7.2	0.5
N. hexacanthus	Stomach	5	91.8	0.2	5	15.1	2.3	5	37.1	1.3	5	4.7	0.3	5	13.7	0.7
	Rectum	5	92.1	1.6	5	30.0	2.9	5	18.6	2.9	5	5.8	0.6	5	9.9	0.9
Omnivores																
Naso tonganus	Stomach	4	90.8	1.1	4	28.9	5.8	4	24.9	4.6	4	25.5	0.7	4	9.3	1.4
	Rectum	4	81.4	4.5	4	69.7	6.3	4	3.2	1.0	4	11.1	3.5	4	1.7	0.5
N. vlamingii	Stomach	5	90.5	0.8	5	33.7	3.2	5	15.7	4.2	5	14.8	2.2	5	8.1	0.9
	Rectum	5	89.5	0.7	5	47.5	1.2	5	5.3	0.4	5	15.7	1.7	5	5.3	0.5
Algivores																
Acanthurus lineatus	Stomach	5	82.9	3.1	5	41.4	2.1	5	11.0	1.1	5	28.8	2.5	5	5.9	0.7
	Rectum	5	81.7	1.6	5	48.3	2.1	5	9.0	0.9	5	23.1	1.8	5	4.9	0.7
Kyphosus cinerascens	Stomach	5	88.3	1.0	5	21.6	2.9	5	8.2	1.2	5	42.1	3.6	5	4.7	0.6
	Rectum	5	82.4	0.9	5	56.2	4.0	5	4.5	0.3	5	18.9	3.3	5	2.2	0.1
K. vaigiensis	Stomach	5	90.5	0.5	5	24.1	2.2	5	7.7	0.8	5	37.9	2.1	5	4.5	0.5
	Rectum	5	92.0	0.7	5	37.6	2.6	5	8.4	1.2	5	23.1	3.1	5	4.2	0.6
Naso unicornis	Stomach	5	86.0	1.2	5	32.2	4.4	5	8.3	1.1	5	30.3	2.2	5	6.7	0.7
	Rectum	5	89.1	1.3	5	46.4	2.5	5	6.0	0.3	5	20.1	2.3	5	4.6	0.1
Detritivorous scarids																
Chlorurus microrhinos	A. intestine	4	75.3	3.8	4	48.3	13.7	4	16.8	7.7	4	4.0	0.2	4	8.2	3.4
	Rectum	4	42.6	2.1	4	90.0	0.8	4	1.3	0.4	4	2.8	0.2	4	0.5	0.1
Scarus frenatus	A. intestine	5	79.6	0.8	5	19.7	5.4	5	44.9	4.3	5	4.6	0.2	5	17.5	1.3
	Rectum	5	65.8	1.4	5	79.2	1.2	5	4.3	0.4	5	6.2	0.3	5	1.6	0.1
S. schlegeli	A. intestine	5	74.5	1.4	5	55.9	6.8	5	22.1	3.8	5	4.0	0.2	5	7.5	1.1
	Rectum	5	51.2	1.9	5	90.1	1.7	5	2.4	0.4	5	2.7	0.2	5	0.8	0.2
Detritivorous acanthurids																
Acanthurus olivaceus	Stomach	5	43.3	4.4	5	90.6	0.6	5	0.6	0.2	5	2.3	0.4	5	0.4	0.1
	Rectum	5	50.6	3.5	5	90.5	9.5	5	2.1	0.5	5	1.8	0.4	5	0.8	0.1
Ctenochaetus striatus	Stomach	5	44.8	3.9	5	87.7	2.1	5	1.2	0.3	5	4.1	0.8	5	0.8	0.3
	Rectum	5	59.0	3.5	5	79.1	2.1	5	5.5	1.2	5	3.7	0.4	5	2.1	0.4
Epilithic algal community[a]																
	Detritus	–	–	–	43	81.0	1.2	49	2.1	0.2	6	7.1	1.1	6	0.8	0.2
	Algae	–	–	–	50	83.1	1.1	51	1.2	0.1	6	5.4	1.0	6	0.6	0.1

[a]Amino acid and ash values from Crossman et al. (2001)

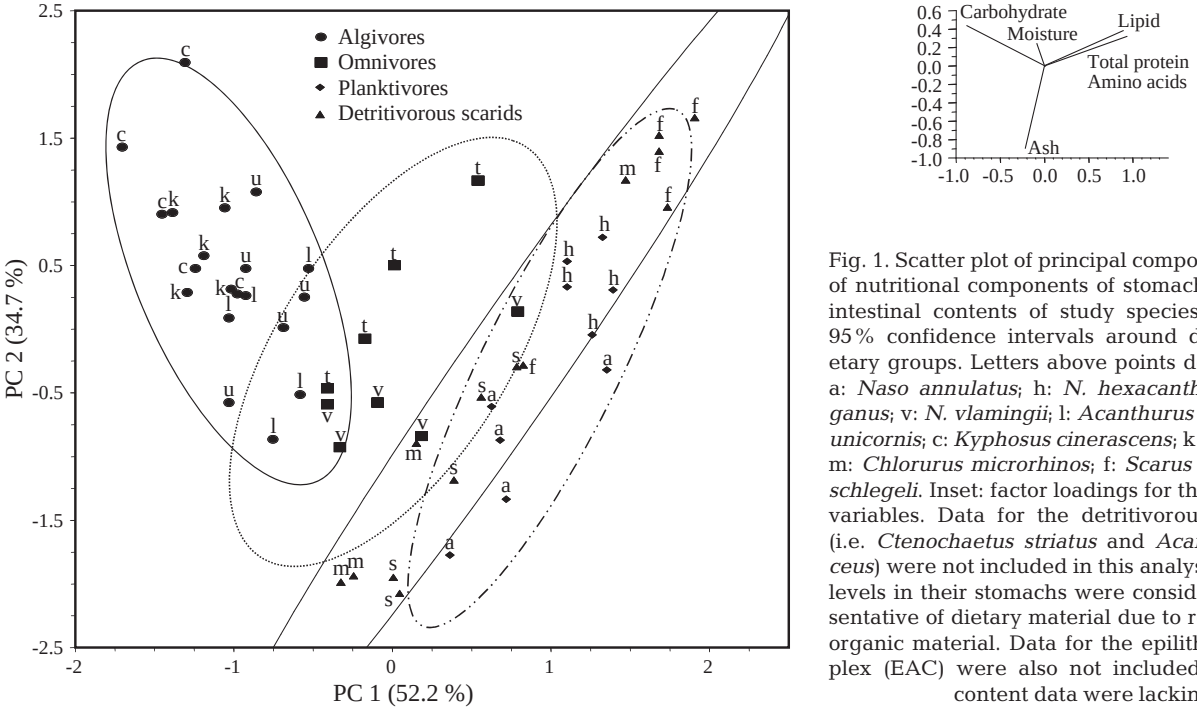

Fig. 1. Scatter plot of principal components analysis of nutritional components of stomach and anterior intestinal contents of study species. Ellipses are 95% confidence intervals around designated dietary groups. Letters above points denote species; a: *Naso annulatus*; h: *N. hexacanthus*; t: *N. tonganus*; v: *N. vlamingii*; l: *Acanthurus lineatus*; u: *N. unicornis*; c: *Kyphosus cinerascens*; k: *K. vaigiensis*; m: *Chlorurus microrhinos*; f: *Scarus frenatus*; s: *S. schlegeli*. Inset: factor loadings for the 5 nutritional variables. Data for the detritivorous acanthurids (i.e. *Ctenochaetus striatus* and *Acanthurus olivaceus*) were not included in this analysis, as nutrient levels in their stomachs were considered unrepresentative of dietary material due to retention of inorganic material. Data for the epilithic algal complex (EAC) were also not included, as moisture content data were lacking

to 6.7%) (Table 2). The omnivores overlap the distributions of the 2 previous categories with intermediate levels of amino acids (15.7 to 24.9%), carbohydrate (14.8 to 25.5%), and lipid (8.1 to 9.3%) (Table 2). Detritivorous scarids were distinct from algivores in having moderate to high levels of amino acids (16.8 to 44.9%) and lipids (7.5 to 17.5%) and low levels of carbohydrate (4.0 to 4.6%) (Table 2).

The macronutrient assimilation efficiencies of species are presented in Table 3. For some species, negative assimilation efficiencies were found. This was particularly evident in the detritivorous acanthurids *Acanthurus olivaceus* and *Ctenochaetus striatus*, for which negative assimilation values were very large. These species contain a gizzard like stomach in which they retain inorganic material for grinding dietary food particles (Horn 1989). It is likely that this material caused a substantial underestimation of diet quality, thus generating the large negative assimilation efficiencies. Therefore, these results were excluded from further analysis. Of the remaining species, 5 had negative assimilation values in 1 or more individuals; results for these species, including and excluding negative values are presented in Table 3. As it was unclear if these values were real or artefacts, negative values were retained in subsequent analyses so as not to bias the data set.

Table 3. Percentage assimilation of macronutrients present in the diet of study species

Species	n	Amino acid $\bar{x}$	Amino acid SE	Carbohydrate $\bar{x}$	Carbohydrate SE	Lipid $\bar{x}$	Lipid SE
Planktivores							
Naso annulatus	5	73.5	3.9	−23.1	15	39.0	8.2
	1[a]	70.8		70.8		38.2	
N. hexacanthus	5	75.4	3.8	38.9	5.8	63.4	5.5
Omnivores							
Naso tonganus	4	93.8	2.1	79.0	9.2	92.5	2.0
N. vlamingii	5	69.6	7.0	21.0	12	51.2	7.7
	4[a]	68.6	9.0	31.2	7.7	54.1	9
Algivores							
Acanthurus lineatus	5	25.6	12.8	27.7	10	24.7	12
	4[a]	36.4	8.9	18.4	6.0	32.4	12
Kyphosus cinerascens	5	77.5	4.0	83.7	1.7	80.2	3.8
K. vaigiensis	5	17.4	23	61.7	4.2	32.9	15.3
	3[a]	51.8	14	62.8	4.0	55.4	10.9
Detritivorous scarids							
Chlorurus microrhinos	4	93.2	2.2	59.0	13	94.8	1.8
Scarus frenatus	5	97.2	1.2	66.6	8.9	97.4	1.0
S. schlegeli	5	90.8	3.5	55.2	9.4	92.0	2.1
Detritivorous acanthurids							
Acanthurus olivaceus	5	−379	150	5.3	22	−132	62
	0[a]	–		–		–	
Ctenochaetus striatus	5	−660	269	−11	14.5	−336	163
	0[a]	–		–		–	

[a]Values excluding negative assimilation efficiencies

To determine which nutrients were predominately utilised by each dietary group, the proportions in which each nutrient was assimilated were calculated for each diet category (Fig. 2). The assimilation efficiencies of macronutrients generally followed their proportions in the diet. Planktivores and detritivorous scarids assimilated the highest proportion of amino acids and lowest proportion of carbohydrate, which were significantly different from the low proportion of amino acids and high proportion of carbohydrate assimilated by algivores (Dunn's test, $p < 0.05$, Fig. 2). The proportion of lipid assimilated was highest in detritivorous scarids and planktivores, but was only significantly different between detritivorous scarids and algivores (Dunn's test, $p < 0.05$, Fig. 2). The omnivores assimilated intermediate proportions of TAA, carbohydrate, and lipid, and were not significantly different from other dietary groups (Dunn's test, $p < 0.05$, Fig. 2).

Previous work on the nutritional ecology of the study species (Choat & Clements 1998) suggested a negative relationship between the level of fermentative digestion and dietary protein. To further examine the dietary strategies in the study species, the amino acid content of the gut fluid was measured and compared to published SCFA levels. This also removed the potential influence of endogenous cells secreted by detritivorous scarids, as these cells would have been removed by centrifugation. The amino acid content of the gut fluid varied along the gastrointestinal tract in all species. The highest amino acid values were measured in the anterior intestine, and then consistently dropped in following segments, with the lowest values in either the penultimate or distal gut segment (Fig. 3). In contrast, SCFA levels were lowest in anterior segments, with peak values occurring in posterior segments (Fig. 3, and Clements & Choat 1995).

The peak amino acid concentration of the gut fluid also varied between species, ranging from an average of 4.4 mg ml^{-1} in the algivorous acanthurid *Naso unicornis* to an average of 51.6 mg ml^{-1} in the scarid *Scarus frenatus* (Fig. 3). Peak amino acid concentrations of the gut fluid varied significantly (1-way ANOVA, $p < 0.001$) between the dietary groups planktivores, omnivores, algivores, detritivorous acanthurids and scarids. Tukey's HSD test for unequal n was used to locate differences between dietary groups (Fig. 4). Detritivorous

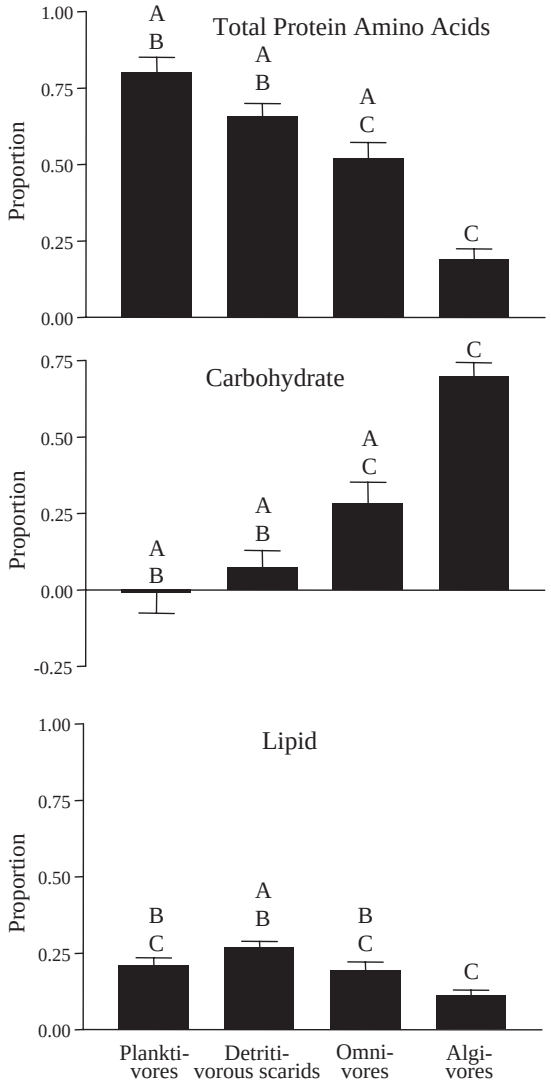

Fig. 2. Relative proportions (mean + SE) of each macronutrient assimilated by the 4 dietary groups. Planktivores (n = 10): *Acanthurus mata* and *Naso hexacanthus*; detritivorous scarids (n = 14): *Chlorurus microrhinos*, *Scarus frenatus* and *S. schlegeli*; omnivores (n = 9): *N. tonganus* and *N. vlamingii*; algivores (n = 19): *Acanthurus lineatus*, *N. unicornis*, *Kyphosus cinerascens* and *K. vaigiensis*. Kruskal-Wallis ANOVA showed that dietary groups varied significantly in total protein amino acids ($p < 0.001$), carbohydrate ($p < 0.001$) and lipid ($p < 0.001$). Different capital letters above bars represent significant differences ($p < 0.05$)

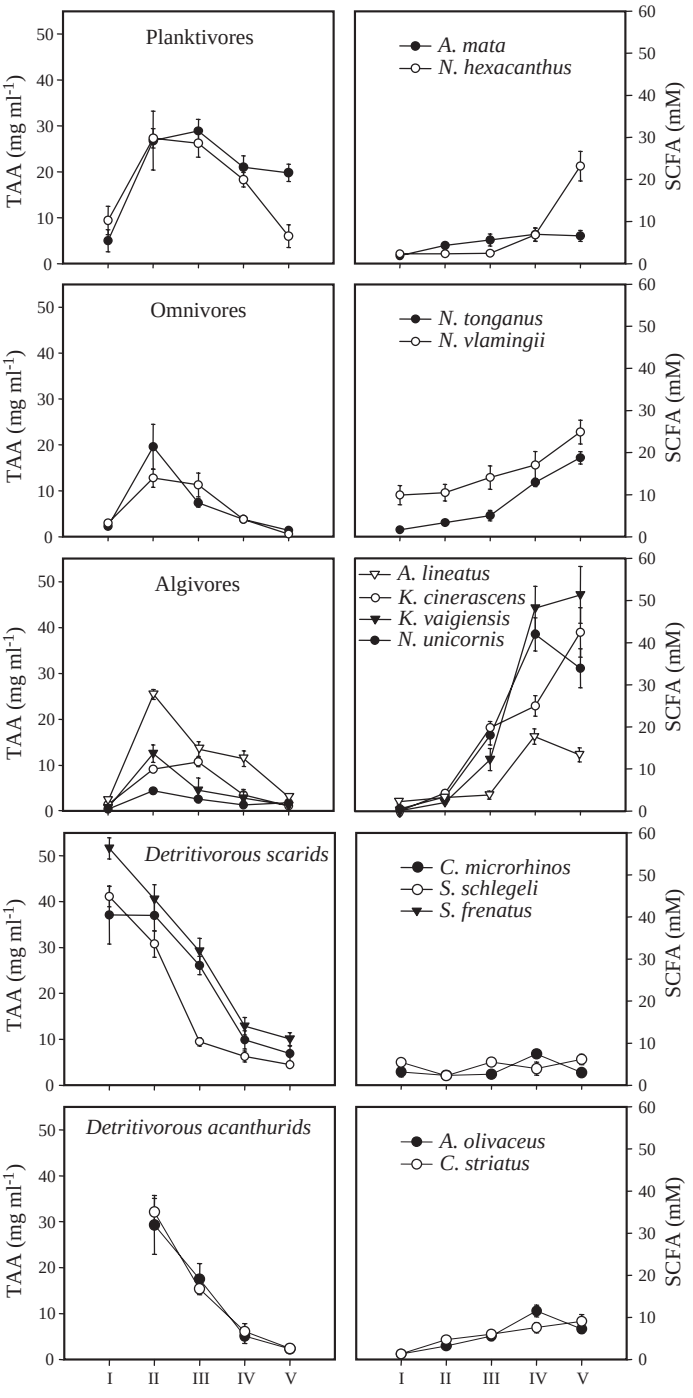

Fig. 3. Mean (±SE) concentrations of total protein amino acids (TAA) and total short chain fatty acids (SCFA) in fluid from the 5 gut segments (I to V) in the study species (SCFA data recalculated from Clements & Choat [1995, 1997]). Number of individuals measured for TAA and SCFA were: *Acanthurus lineatus* (TAA n = 6, SCFA n = 9), *A. mata*, *A. olivaceus* (TAA n = 5–8, SCFA n = 10), *Ctenochaetus striatus* (TAA n = 5, SCFA n = 10), *Naso hexacanthus* (TAA n = 5, SCFA n = 11), *N. tonganus* (TAA n = 5, SCFA n = 14), *N. unicornis* (TAA n = 5, SCFA n = 11), *N. vlamingii* (TAA n = 5, SCFA n = 14), *Kyphosus cinerascens* (TAA n = 5, SCFA n = 8), *K. vaigiensis* (TAA n = 5, SCFA n = 8), *Chlorurus microrhinos* (TAA n = 4, SCFA n = 4), *Scarus frenatus* (TAA n = 8, SCFA no data), and *S. schlegeli* (TAA n = 5, SCFA n = 7)

scarids had the highest values and were significantly different from all other groups; detritivorous acanthurids and planktivores had the next highest values, and were significantly different from omnivores and algivores but not from each other. There was no significant difference between omnivores and algivores.

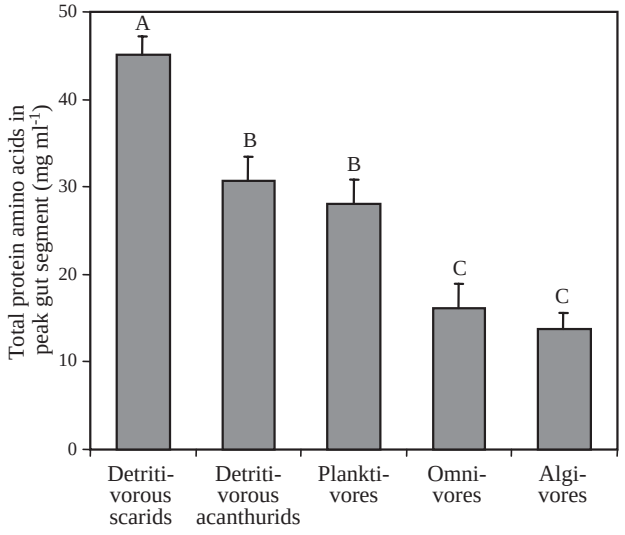

Fig. 4. Mean (+SE) peak total protein amino acid concentration in gut fluid of 5 dietary groups. For planktivores n = 10, omnivores n = 10, algivores n = 21, detritivorous scarids n = 17, detritivorous acanthurids (*Acanthurus olivaceus* and *Ctenochaetus striatus*) n = 12. Species composition of other groups as in legend to Fig. 2. 1-way ANOVA showed that TAA varied significantly between dietary groups ($p < 0.001$). Different capital letters above bars represent significant differences ($p < 0.05$)

Mean peak amino acid concentrations of the gut fluid (dependant variable) for species were plotted against literature values for mean maximum total SCFA concentrations in the gut fluid (Fig. 5). No value is plotted for *Scarus frenatus* as no published SCFA data exist for this species. This plot showed a strong negative correlation between peak amino acid values and maximum SCFA values. The trend was found to be significant by Pearson's correlation ($p < 0.001$, $r^2 = 0.739$). The different dietary groups separated along this correlation. Detritivorous scarids and acanthurids had the highest levels of TAA but the lowest levels of SCFA. Next were planktivorous species that were high in TAA and had low to intermediate levels of SCFA. Omnivores had moderate levels of both TAA and SCFA, and the last group contained 3 algivores that had low levels of TAA but high levels of SCFA.

The mole percent composition of amino acids in stomach or anterior intestinal material from dietary groups, and for EAC detritus and algae, is presented in Fig. 6, which also presents the mole percent composition of peak amino acids from gut fluid for the same dietary groups. The mole percent composition of amino acids in dietary material and the gut fluid are remarkably similar between the different trophic groups.

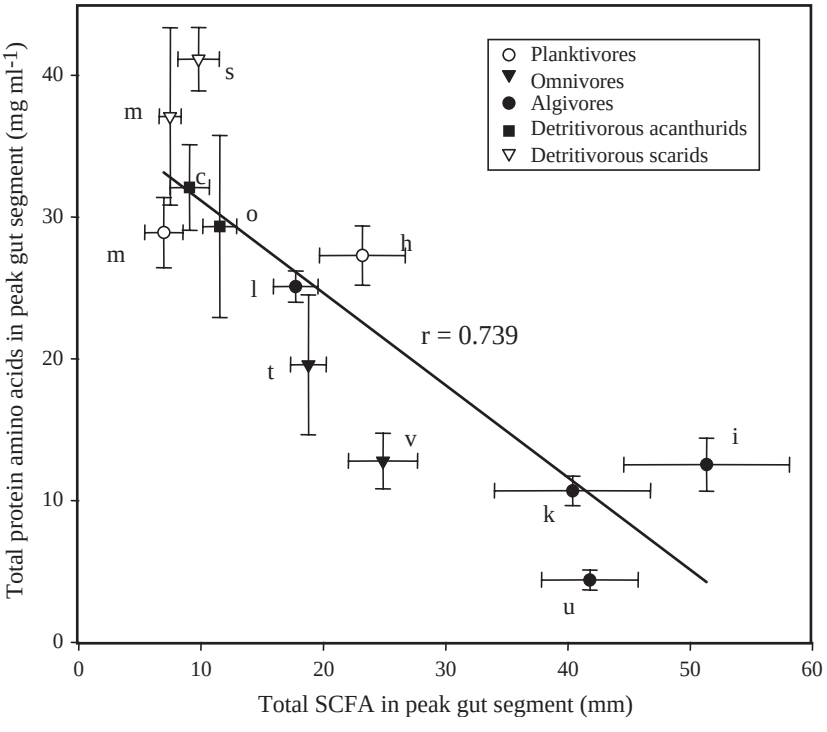

Fig. 5. Linear correlation between Peak TAA concentrations in gut fluid and maximum total SCFA concentrations in gut fluid of study species. Data are mean ± SE. Total SCFA data recalculated from Clements & Choat (1995, 1997) and Choat & Clements (1998). Letters next to data points designate species; c: *Ctenochaetus striatus*; o: *Acanthurus olivaceus*; other abbreviations as in legend to Fig. 1

DISCUSSION

In a series of studies on coral reef herbivores (Clements & Choat 1995, Choat & Clements 1998, Choat et al. 2002, Wilson et al. 2003) it has been argued (1) that marine herbivores display a greater diversity of diets, feeding behaviours and food processing modes than their terrestrial counterparts, and (2) that many of the species identified as herbivores are in fact closer to carnivores in terms of their nutritional profiles. This could not be confirmed until the dietary targets of the relevant species had been properly identified. The present study provides this confirmation by identifying the nutritional targets of a diverse range of nominally herbivorous fishes. Moreover, by establishing the pattern of nutrient levels and their assimilation within the alimentary tract, and by incorporating information on SCFA levels, this study provides a framework for comparing nutritional strategies in a range of grazing reef fishes.

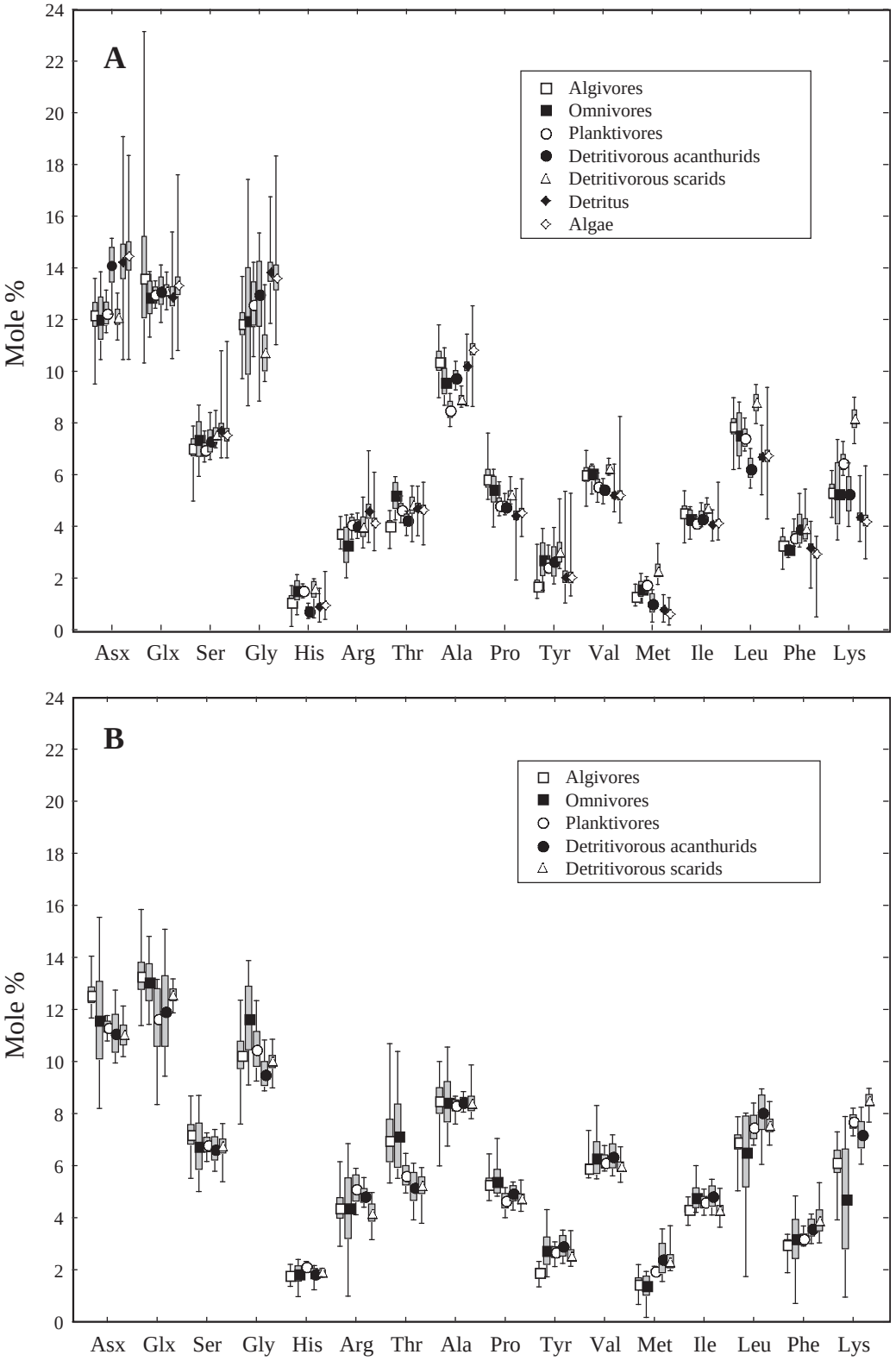

Fig. 6. Mean (symbols), 95% confidence intervals (boxes) and range (whiskers) of (A) mole % protein amino acids in stomach, anterior intestinal material and EAC detritus, and algae and (B) mole % of peak amino acids from gut fluid in different dietary categories: (A,B) algivores (*Acanthurus lineatus*, *Kyphosus cinerascens*, *K. vaigiensis*, *Naso unicornis*); omnivores (*N. tonganus*, *N. vlamingii*); planktivores ([A] *N. annulatus* [B] *A. mata*, [A,B] *N. hexacanthus*); (A,B) detritivorous acanthurids (*A. olivaceus*, *Ctenochaetus striatus*); detritivorous scarids (*Chlorurus microrhinos*, *Scarus frenatus*, *S. schlegeli*); and (A) EAC detritus and algae. Amino acid codes are Asx: aspartic acid and asparagine; Glx: glutamic acid and glutamine; Ser: serine; Gly: glycine; His: histidine; Arg: arginine; Thr: threonine; Ala: alanine; Pro: proline; Tyr: tyrosine; Val: valine; Met: methionine; Ile: Isoleucine; Leu: leucine; Phe: phenylalanine; Lys: lysine

TAA and SCFA as indicators of trophic status

The distribution of TAA and SCFA showed contrasting patterns (Fig. 3). For all species the highest amino acid values were measured in the anterior intestine, and consistently dropped in following segments, with the lowest values in either the distal or penultimate gut segment. This is indicative of protein digestion, whereby proteins and peptides are extracted from dietary solids into the fluid phase, then broken down by proteases into amino acids and assimilated across the gut wall (Stevens & Hume 1995). In contrast, the peak SCFA values measured by Clements & Choat (1995) were highest in the posterior intestine, consistent with the presence of fermentative microbial populations (Clements 1997). This inverse relationship indicates a nutritional continuum, with detritivores and planktivores with high TAA and low SCFA at one end, omnivores with intermediate levels of TAA and SCFA in the middle, and most algivores with low TAA but high SCFA at the other end.

The major dietary requirements of fishes are nitrogen and energy (Bowen et al. 1995). Fishes with high dietary TAA may predominantly metabolise this nutrient, as it can be used for both energy and as a source of nitrogen that is essential for growth (De-Silva & Anderson 1995). Fishes with lower levels of dietary TAA may increasingly rely on microbial fermentation (of algal carbohydrates) and the subsequent production of SCFA as an energy source, while saving dietary TAA for nitrogen requirements. The most striking contrasts are between genuine herbivores (with diets dominated by algae) and detritus feeding fishes. The former have diets and assimilation profiles dominated by carbohydrates, relatively low levels of TAA, and evidence of active microbial fermentation in the hindgut region. Detritus feeding fishes have levels of TAA in the anterior gut similar to fishes feeding on animal matter, high TAA assimilation rates, and reduced evidence of carbohydrate assimilation. The sources of the high TAA levels in detritus are undetermined, but it is likely that a major contributor is the high microbial biomass in coral reef sediments (Sorokin 1993).

Within those species feeding predominantly on algae, 2 contrasting strategies were apparent. Firstly, for those species targeting larger more biochemically complex algae a limited amount of dietary TAA is extracted in the anterior region of the alimentary tract by endogenous digestion. This nutrient source may be important for growth. However the important sources of energy in the diet are extracted from carbohydrate material, presumably through fermentative digestion in the hindgut (*Kyphosus cinerascens*, *K. vaigiensis*, *Naso unicornis*). Secondly, a number of herbivorous acanthurid fishes including *Acanthurus lineatus* harvest small, mainly red and green, filamentous algae (Clements & Choat 1995, Choat & Clements 1998). This material appears to be higher in assimilable TAA. These herbivores show a comparatively low commitment to accessing energy through fermentation (Choat et al. 2002).

Zooplanktivore diets were predictably characterised by high levels of TAA. The diet is however quite specific, comprising largely gelatinous zooplankton (mainly salps, Choat et al. 2002). Some zooplankton feeders (e.g. *Naso hexacanthus*) retain the capacity of extracting additional energy from the diet by fermenting the polysaccharides that make up salp body walls, and possibly crustacean chitin.

Amino acid composition of dietary material

Amino acid mole percentage composition can vary amongst sources of dietary protein (Friedman 1996). Feeding trials on aquaculture fishes have revealed that diets low in essential amino acids give poor growth (De-Silva & Anderson 1995). It is possible that the diets of algivorous fishes are poor in essential amino acids relative to the diets of carnivorous fishes, especially since plant proteins are often deficient in essential amino acids (e.g. Friedman 1996). However, the amino acid mole percent composition is remarkably similar in detritus and algae from EAC and in the Segment I contents and gut fluid from the dietary groups of planktivores, omnivores, algivores and detritivores. This suggests that amino acid mole percent composition is unlikely to play a role in the nutritional ecology of these fishes.

Strategies of fishes feeding on EAC

The most important results in this study were obtained for species feeding on the detrital aggregates and algae that occur within the EAC. These species make up a major component of coral reef grazing fish fauna (Choat et al. 2004), and were the primary focus of this study. Resolving the nutritional status of these species is crucial to our understanding of reef trophodynamics.

The 2 main elements of EAC, turfing algae and detrital aggregates, occur as fine-scale complex mixtures (Wilson & Bellwood 1997, Crossman et al. 2001). Levels of TAA, carbohydrate and lipid were low compared to dietary nutrients measured in the gut of fishes that feed on these resources. From the present study, 2 important findings emerged. Firstly, detritus-feeding fishes (primarly scarids and members of the genus

Ctenochaetus) consumed and assimilated a diet rich in total amino acids, fundamentally different to the high carbohydrate composition of diets dominated by plant material. Secondly, some species grazing over the same substratum ingested only algal material. This demonstrates that both detritus and algae feeding species are able to selectively target nutritious components of the EAC, and have evolved strategies to maximise their return from these.

The 2 major lineages exploiting the particulate components of the EAC (detritivorous scarids and detritivorous acanthurids) display contrasting morphologies and feeding behaviours. The oral jaws of scarids are characterised by a fused beak. Removal of EAC at the level of individual bites is non-selective (Bellwood & Choat 1990, Choat et al. 2002). As the EAC contains a significant proportion of living algal material (Crossman et al. 2001), selection of food items presumably occurs in the buccal cavity and pharynx of scarids, allowing them to concentrate detrital and associated bacterial elements. The dominant detritivorous acanthurid, *Ctenochaetus striatus*, achieves high selectivity of detrital and particulate material by combing it from algal turfs through a highly specialised dentition and oral jaws (Purcell & Bellwood 1993). Despite differing morphologies and feeding behaviours, both lineages show similar nutrient profiles along the alimentary tract, demonstrating their capacity to selectively ingest and assimilate protein rich material from the EAC.

In contrast, a number of acanthurids grazing the EAC (exemplified in this study by *Acanthurus lineatus*) feed exclusively on filamentous and turfing algae, with rhodophytes predominating (Choat et al. 2002). The concentration of TAA and SCFA levels in the gut fluid of *A. lineatus* indicates selection of an algal food resource relatively high in assimilable TAA, and a comparatively low commitment to accessing energy through fermentation. This species maintains a feeding territory characterised by fast growing polysiphonaceus red algae that it actively defends against other grazing species (Choat & Bellwood 1985, Choat 1991, Polunin & Klumpp 1992a). The consequences of differing feeding behaviours in scarids vs. *Ctenochaetus striatus* are clearly seen in their interactions with the territorial *A. lineatus*. *C. striatus* feeds within *A.lineatus* territories, eliciting no aggressive responses (Choat & Bellwood 1985). In contrast, scarids are subjected to immediate territorial aggression, a reflection of the fact that scarids remove algae, with gut content analysis suggesting that diet selection occurs in the pharynx (Choat et al. 2002). *C. striatus* on the other hand, combs the algal turf, extracting particulate matter without removing the algal complex (Purcell & Bellwood 1993).

Detritus and fisheries production on coral reefs

The emergent picture on coral reefs is that detritivorous fishes are abundant, and are therefore likely to have a substantial role in reef trophodynamics. Understanding this role will be crucial for calculating the energetics of coral reef food webs, including calculations of fishery production. Current fishery models have highlighted the importance of detritus based food webs, and the lack of information on fluxes and fates of detritus, although the energy flux from detritus to fishes is thought to be through the invertebrate food chain (Polunin & Klumpp 1992b, Polunin 1996). The potential consumption of detritus directly by grazing fishes has not been considered (e.g. Polunin & Klumpp 1992b, Polunin 1996, Van Rooij et al. 1998, Jackson et al. 2001).

CONCLUSION

The most significant finding of this study is that there are major differences in the dietary macronutrients targeted by nominally herbivorous fishes on coral reefs. Those feeding on detrital aggregates consume and assimilate a diet high in total protein amino acids and appear to have a limited capacity for fermentation. This is in contrast to algivorous species, which consume and assimilate diets high in carbohydrate and appear to have a high capacity for fermentation. Nominally herbivorous fishes are generally treated as an homogenous group in studies of reef trophodynamics. The results from this work underscore the diversity of food resources targeted by these fishes. This information needs to be incorporated into larger scale studies, and is likely to provide interesting insights into reef trophodynamics.

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LITERATURE CITED

Alongi DM (1989) Detritus in coral reef ecosystems: Fluxes and fates. Proc 6th Int Coral Reef Symp 1:29–36

Bellwood DR, Choat JH (1990) A functional analysis of grazing in parrotfishes (family Scaridae): the ecological implications. Environ Biol Fishes 28:189–214

Bowen SH (1976) Mechanism for digestion of detrital bacteria by the cichlid fish *Sarotherodon mossambicus* (Peters). Nature 260:137–138

Bowen SH (1980) Detrital nonprotein amino acids are the key to rapid growth of *Tilapia* in Lake Valencia, Venezuela. Science 207:1216–1218

Bowen SH, Lutz EV, Ahlgren MO (1995) Dietary protein as energy determinants of food quality: trophic strategies compared. Ecology 76:899–907

Choat JH (1991) The biology of herbivorous fishes on coral reefs. In: Sale PF (ed) The ecology of fishes on coral reefs. Academic Press, San Diego, CA, p 120–155

Choat JH, Bellwood DR (1985) Interactions amongst herbivorous fishes on a coral reef: influence of spatial variation. Mar Biol 89:221–234

Choat JH, Clements KD (1998) Vertebrate herbivores in marine and terrestrial environments: a nutritional ecology perspective. Annu Rev Ecol Syst 29:375–403

Choat JH, Clements KD, Robbins WD (2002) The trophic status of herbivorous fishes on coral reefs. I. Diet analyses. Mar Biol 140:613–623

Choat JH, Clements KD, Robbins WD (2004) The trophic status of herbivorous fishes on coral reefs. II. Food processing modes and trophodynamics. Mar Biol 145: 445–454

Clements KD (1997) Fermentation and gastrointestinal microorganisms in fishes. In: Mackie RI, White BA (eds) Gastrointestinal microbiology. Chapman & Hall, New York, p 156–198

Clements KD, Bellwood DR (1988) A comparison of the feeding mechanisms of 2 herbivorous labrid fishes the temperate *Odax pullus* and the tropical *Scarus rubroviolaceus*. Aust J Mar Freshw Res 39:87–108

Clements KD, Choat JH (1995) Fermentation in tropical marine herbivorous fish. Physiol Zool 68:355–378

Clements KD, Choat JH (1997) Comparison of herbivory in the closely-related marine fish genera *Girella* and *Kyphosus*. Mar Biol 127:579–586

Crossman DJ, Clements KD, Cooper GJS (2000) Determination of protein for studies of marine herbivory: a comparison of methods. J Exp Mar Biol Ecol 244:45–65

Crossman DJ, Choat JH, Clements KD, Hardy T, McConochie J (2001) Detritus as food for grazing fishes on coral reefs. Limnol Oceanogr 46:1596–1605

De-Silva SS, Anderson TA (1995) Fish nutrition in aquaculture. Chapman & Hall, London

Ducklow HW (1990) The biomass, production and fate of bacteria in coral reefs. Coral Reefs 25:265–289

Englyst HN, Cummings JH (1988) Improved method for measurement of dietary fiber as non-starch polysaccharides in plant foods. J Assoc Off Anal Chem 71:808–814

Folch J, Lees M, Sloane GY (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–507

Friedman M (1996) Nutritional value of proteins from different food sources: a review. J Agric Food Chem 44:6–29

Genner MJ, Turner GF, Hawkins SJ (1999) Foraging of rocky habitat cichlid fishes in Lake Malawi: coexistence through niche partitioning? Oecologia 121:283–292

Gust N, Choat JH, Ackerman JL (2002) Demographic plasticity in tropical reef fishes. Mar Biol 140:1039–1051

Hatcher BG (1983) Grazing in coral reef ecosystems. In: Barnes DJ (ed) Perspectives on coral reefs. Australian Institute of Marine Science, Townsville, p 164–179

Hatcher BG (1988) Coral reef primary productivity: a beggar's baquet. Trends Ecol Evol 3:106–111

Hatcher BG (1997) Organic production and decomposition. In: Birkeland C (ed) Life and death of coral reefs. Chapman & Hall, New York, p 140–174

Hay ME, Steinberg PD (1992) The chemical composition of plant–herbivore interactions in marine versus terrestrial communities. In: Rosenthal GA, Berenbaum MR (eds) Herbivores: their interactions with secondary metabolites. Evolutionary and ecological processes, Vol 2. Academic Press, San Diego, CA, p 371–413

Hixon MA (1997) Effects of reef fish on corals and algae. In: Birkeland C (ed) Life and death of coral reefs. Chapman & Hall, New York, p 230–248

Horn MH (1989) Biology of marine herbivorous fishes. Oceanogr Mar Biol Annu Rev 27:167–272

Jackson JBC, Kirby MX, Berger WH, Bjorndal KA and 15 others (2001) Historical overfishing and the recent collapse of coastal ecosystems. Science 293:629–638

Montgomery WL, Gerking SD (1980) Marine macroalgae as foods for fishes: an evaluation of potential food quality. Environ Biol Fishes 5:143–153

Moriarty DJW, Pollard PC, Alongi DM, Wilkinson CR, Gray JS (1985) Bacterial productivity and trophic relationships with consumers on a coral reef (MECOR I). Proc 5th Int Coral Reef Congr 3:457–462

Myers RF (1989) Micronesian reef fishes. Coral Graphics, Guam

Polunin NVC (1996) Trophodynamics of reef fisheries productivity. In: Polunin NVC, Roberts CM (eds) Reef fisheries. Chapman & Hall, London, p 113–135

Polunin NVC, Klumpp DW (1992a) Algal food supply and grazer demand in a very productive coral-reef zone. J Exp Mar Biol Ecol 164:1–15

Polunin NVC, Klumpp DW (1992b) A trophodynamic model of fish production on a windward reef tract. In: John DM, Hawkins SJ, Price JH (eds) Plant–animal interactions in the marine benthos, Spec Vol No. 46. Systematics Association, Clarendon, p 213–233

Purcell SW, Bellwood DR (1993) A functional analysis of food procurement in 2 surgeonfish species, *Acanthurus nigrofuscus* and *Ctenochaetus striatus* (Acanthuridae). Environ Biol Fishes 37:139–159

Randall JE, Allen GR, Steene RC (1990) Fishes of the Great Barrier Reef and Coral Sea. Crawford House Press, Bathurst, Australia

Reinthal PN (1990) The feeding habits of a group of herbivorous rock-dwelling cichlid fishes (Cichlidae: Perciformes) from Lake Malawi, Africa. Environ Biol Fishes 27:215–233

Robertson DR (1982) Fish faeces as fish food on a Pacific coral reef. Mar Ecol Prog Ser 7:253–265

Robertson DR, Gaines SD (1986) Interference competition structures habitat use in a local assemblage of coral reef surgeonfishes. Ecology 67:1372–1383

Sorokin YI (1993) Coral reef ecology. In: Lange OL, Mooney HA, Remmert H (eds) Ecological studies. Springer-Verlag, Berlin

Steneck RS (1989) Herbivory on coral reefs: a synthesis. Proc 6th Int Coral Reef Symp 1:37–49

Stevens CE, Hume ID (1995) Comparative physiology of the vertebrate digestive system, 2nd edn. Cambridge University Press, Cambridge

Sturmbauer C, Wolfgang M, Reinhard D (1992) Ecophysiology of Aufwuchs-eating cichlids in Lake Tanganyika: niche separation by trophic specialization. Environ Biol Fishes 35:283–290

Van Rooij JM, Videler JJ, Bruggemann JH (1998) High biomass and production but low energy transfer efficiency of

Caribbean parrotfish: implications for trophic models of coral reefs. J Fish Biol 53(Suppl A):154–178
Wilson SK (2000) Trophic status and feeding selectivity of blennies (Blenniidae: Salariini). Mar Biol 136:431–437
Wilson SK (2004) Growth, mortality and turnover rates of a small detritivorous fish. Mar Ecol Prog Ser 284:253–259
Wilson SK, Bellwood DR (1997) Cryptic dietary components of territorial damselfishes (Pomacentridae, Labroidei). Mar Ecol Prog Ser 153:299–310
Wilson SK, Bellwood DR, Choat JH, Furnas M (2003) Detritus in the epilithic algal matrix and its use by coral reef fishes. Oceanogr Mar Biol Annu Rev 41:279–309
Zar JH (1984) Biostatistical analysis, 2nd edn. Prentice Hall, Englewood Cliffs, NJ

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