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Algal food processing by *Stegastes nigricans*, an herbivorous damselfish: differences between an undisturbed and a disturbed coral reef site (La Réunion, Indian Ocean)

Thierry LISON DE LOMA [a,b*], **Mireille HARMELIN-VIVIEN** [a], **Odile NAIM** [a], **Marie-France FONTAINE** [a]

[a] Centre d'océanologie de Marseille, CNRS UMR 6540, station marine d'Endoume, Marseille, France
[b] Laboratoire d'écologie marine, université de la Réunion, 97715 Saint-Denis messag. cedex 9,
La Réunion, France

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Abstract – There has, as yet, been little study of nutrient disturbance and its consequences on the functional biology of primary consumers in coral reefs. The processing of epilithic algal communities by the damselfish *Stegastes nigricans* was investigated at La Réunion, at two sites of a fringing reef, oligotrophic for one and eutrophic for the other. Differences between sites were analyzed in quantifying organic matter, organic carbon, nitrogen, and organic 'nutrients' (carbohydrate, lipid and protein) in the algal food, the digestive contents and the faeces of *S. nigricans*. In the disturbed site, algal turfs and digestive contents presented significantly higher organic matter, organic carbon, nitrogen and organic nutrient values. Absorption efficiency of total matter was higher in fish from this site (26.8 %) than from the undisturbed site (16.3 %), as well for organic matter, organic carbon, nitrogen and organic nutrients. A larger mean total length was also measured for fish sampled in the disturbed site (11.4 cm versus 10.9 cm). The reinjection of organic nutrients by this herbivorous fish appeared to be enhanced in this site. It is suggested that eutrophication is the main disturbance factor accounting for the recorded differences. © 2000 Ifremer/CNRS/IRD/Éditions scientifiques et médicales Elsevier SAS

Herbivory / *Stegastes* / coral reef / eutrophication / La Réunion

Résumé – Transformation de la nourriture algale par *Stegastes nigricans*, un poisson demoiselle herbivore : différences entre un site non perturbé et un site perturbé sur un récif corallien (La Réunion, océan Indien). L'effet des perturbations trophiques et leurs conséquences sur la biologie fonctionnelle des consommateurs primaires des récifs coralliens a été peu étudié. Cet article présente la transformation des communautés algales épilithiques (gazons algaux) par le poisson demoiselle herbivore *Stegastes nigricans*, sur un récif frangeant de l'Ile de La Réunion dans deux sites du platier récifal interne : non perturbé et oligotrophe pour l'un, perturbé et eutrophe pour l'autre. Les différences entre les sites ont été analysées en quantifiant les teneurs en matière organique, carbone organique, azote total et 'nutriments' (glucides, lipides et protéines), dans la nourriture algale, les contenus digestifs et les fèces de *S. nigricans*. Dans le site perturbé, les gazons algaux et les contenus digestifs des poissons sont caractérisés par des pourcentages de matière organique et des teneurs en carbone organique, azote total et nutriments significativement plus élevés que dans le site non-perturbé. Les taux d'absorption totaux sont plus élevés chez les poissons de ce site (26,8 %) que chez les poissons du site non-perturbé (16,3 %). Le même résultat est observé pour les taux d'absorption de la matière organique, du carbone organique, de

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* Correspondence and reprints.
E-mail address: lison@univ-reunion.fr (T. Lison de Loma).

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l'azote total et des nutriments. La longueur totale des poissons prélevés est supérieure chez les poissons prélevés sur le site perturbé (11,4 cm contre 10,9 cm). La réinjection de nutriments dans l'écosystème par ce poisson herbivore est plus importante dans ce site. L'eutrophisation semble être le facteur principal qui explique les différences relevées entre les sites.

régime herbivore / *Stegastes* / récif corallien / eutrophisation / La Réunion

1. INTRODUCTION

Literature on marine herbivorous fish, even if recently increasing (cf review in Horn, 1989; Choat, 1991; Choat and Clements, 1998), is poor compared to that available on terrestrial herbivores. Until recently, studies on herbivorous fish at times concluded that real herbivores (i.e. macroalgal feeders as defined by Gerking, 1994) did not exist among fish, although substantial amounts of macroalgae were found in their stomachs (Moreno and Jara, 1984). One concept that prevailed was that fish were not digesting algae but their epiphytic organisms (Wheeler, 1980). Then it was demonstrated that fish could actually digest macroalgae (Anderson, 1987; Horn, 1989) and that fermentative digestion through the action of microorganisms could play an important role in this process (Rimmer and Wiebe, 1987; Clements, 1991). The feeding habits of territorial damselfishes have been well documented (Lassuy, 1980; Montgomery, 1980; Horn, 1989; Gerking, 1994), and it was widely accepted that they fed almost exclusively on the epilithic algal communities (EAC) within their territories. More recently, it has been shown that some damselfish species, including *Stegastes nigricans* (Lacepède, 1803), ingested more than just algae, namely detritus, i.e. non-living organic compounds, microbes and sediments (Wilson and Bellwood, 1997). In the epilithic algal matrix (EAM), the detritus fraction seemed to be the major source of organic matter in the territories, and the better represented component in the diet.

Many studies have demonstrated the key role of herbivores in biogeochemical cycles of coral reefs, particularly in recycling organic and inorganic carbon. Between 37 and 67 % of the daily benthic primary productivity may be reinjected into the reef ecosystem by herbivores (Polunin and Klumpp, 1992). These organisms represented a major link in the nitrogen cycle, as grazing by fish and invertebrates seemed to be an important factor in regulating nitrogen fixation on coral reefs (Wilkinson and Sammarco, 1983). Although not quantified, a regeneration path of ammonium through grazing was also clearly defined (Polunin and Koike, 1987; D'Elia and Wiebe, 1990).

At La Réunion (Indian Ocean), the Saint Gilles-La Saline reef flat is subject to submarine groundwater discharges (Join, 1991) enriched in nutrients (Cuet et al., 1988). Modifications of reef communities have been linked mainly to the impact of submarine groundwater discharges: intense growth of algae, degradation of coral assemblages, and modification of fish communities (Done, 1992; Naim 1993; Chabanet et al., 1995; Semple, 1997).

In this paper we present differences in the processing of EAM by *S. nigricans*, including absorption and reinjection of organic nutrients into the reef system, between a disturbed and an undisturbed site. High densities of the herbivorous black damselfish *S. nigricans* have been reported on the Saint Gilles-La Saline reef flat, where it is the most abundant herbivorous fish (Letourneur, 1992). This species appeared as a key species involved in the degradation of corals and in the maintenance of macroalgae (Naim, 1993). This territorial pomacentrid feeds on its own EAM in territories that develop on dead or sick coral, mostly *Acropora* sp. In the present work, the nutritive value of the EAM was compared between two sites differing by their eutrophication level. We also assessed the epilithic algal matrix processing by the fish and its absorption in both sites. The biochemical composition of faeces was finally compared between sites. Factors potentially explaining these differences are discussed.

2. MATERIALS AND METHODS

2.1. Study sites

The Saint Gilles-La Saline reef complex is located on the west coast of La Réunion (55°32′ E, 21°07′ S), Indian Ocean. This reef is exposed to intense hydrodynamic conditions resulting from south-east tradewinds and south-west dominant swells during austral winter, and from cyclonic swells during austral summer (Gabrié and Montaggioni, 1982). It is subjected to spatially uneven nutrient-enriched submarine groundwater discharges, resulting in an internal reef flat which is a mosaic of oligotrophic and eutrophic sites (Cuet et al., 1988; Naim, 1993). The choice of two sites on this reef (*figure 1*) was motivated by previous results on the chemical composition of reef waters and its impact on benthic communities. Benthic communities and nutrient enrichment patterns in these two sites of the reef complex are well known (*table I*). Trois-Chameaux (site U for undisturbed) is oligotrophic, has clear waters and relatively healthy coral communities. Planch'Alizés (site D for disturbed) is considered an eutrophic site, as higher levels of nutrients are presumably brought by submarine groundwater discharges in site D (Cuet et al., 1988). It has more turbid waters and is characterized by dense (at least during austral summer) macroalgal communities overgrowing coral colonies. Site U and site D are 2 km apart. Each site is approximately 100–150 m large to 160 m long. Ten stations were sampled in each site. They were located on the inner reef flat, where the highest *S. nigricans* densities are found. Site U and site D are in the same geomorphological zone (Montaggioni and Faure, 1980). Permanent transects are regularly surveyed in these two sites by the *laboratoire d'écologie marine* of the *université de La Réunion*, and there have been numerous studies on the effects of disturbance and eutrophication on several reef processes (Cuet et al., 1988; Done, 1992; Naim, 1993; Chabanet et al., 1995; Chazottes, 1996; Semple, 1997; Mioche and Cuet, 1999).

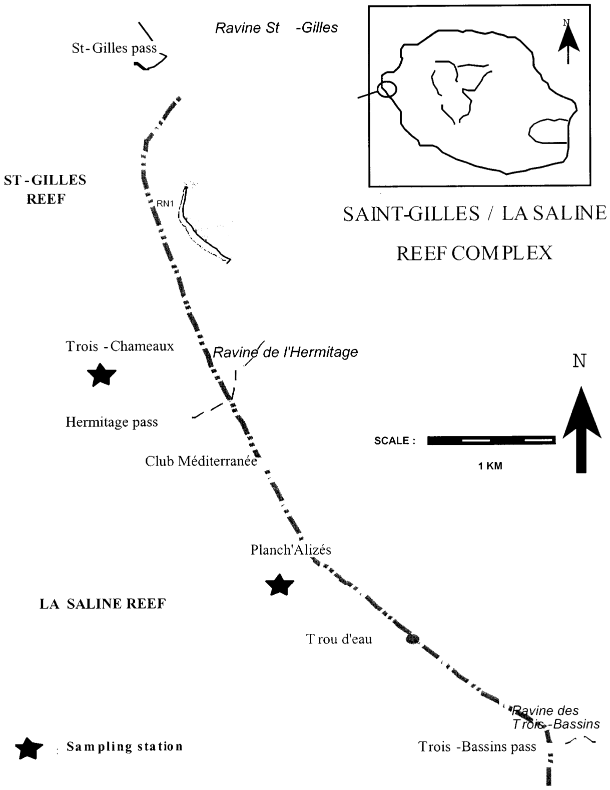

Figure 1. Sampling sites on Saint Gilles-La Saline fringing reef complex (site U: undisturbed site, site D: disturbed site, RN1: national road).

2.2. Sampling procedures

Sampling was carried out in summer. Ten stations were chosen randomly in each site. In each station, a damselfish territory was randomly selected.

To study the food processing by the fish, two sets of ten algal samples were collected, corresponding to each of ten territories. Within each territory the adult fish (length > 8 cm) was collected by rotenone (8 %) poisoning, early in the afternoon, when the gut is most replete (Letourneur et al., 1997). The faeces of each fish were collected in situ using a pipette, at the bottom of the territories, where *S. nigricans* defecates systematically.

All specimens and samples were immediately put on ice for transfer to the laboratory (transfer time: 10 min), where the total length of each fish was measured. The digestive tract was removed, split into six sections: stomach (S), four sections in the intestine (from fore to hindgut: I1, I2, I3, I4) and rectum (R). The content of each section was carefully collected. All samples were then frozen for later analysis. A first set of samples was dried to constant weight at 60 °C for 24 h, in order to perform organic matter and calcium carbonate measurements. A second set was freeze-dried before performing organic nutrient measurements in Marseille.

Table I. Comparison of chemical and biological features of site U (undisturbed) and site D (disturbed).[a]

	Features	Back reef zone	Inner reef flat
Site U	NO_3	0.73 ± 0.35 μM	0.71 ± 0.36 μM
	PO_4	0.23 ± 0.06 μM	0.39 ± 0.18 μM
	Salinity	35.12 ± 0.12	35.14 ± 0.11
	Benthic communities	No fleshy algae	High coral vitality
Site D	NO_3	3.23 ± 0.99 μM	0.96 ± 0.42 μM
	PO_4	0.36 ± 0.18 μM	0.33 ± 0.09 μM
	Salinity	34.68 ± 0.15	35.13 ± 0.11
	Benthic communities	High coverage of fleshy algae	High percentage of coral degradation

[a] From Chabanet et al., 1995. Values are mean ± SD.

2.3. Chemical analyses

In order to determine organic matter percentages, aliquots of dry matter were weighed, combusted at 535 °C for 5 h to remove organic matter, and weighed again.

Total carbon and nitrogen percentages of the freeze-dried samples were measured in a Leco CHN analyser by using subsamples of 10 mg. Organic carbon percentages were then calculated, using the calcium carbonate content of the samples. Calcium carbonate contents were quantified on burnt (535 °C for 5 h) subsamples by adding 1.7 mL HCl solution (4 N) and measuring the excess volume with a NaCl solution (0.5 N). C:N mass ratios and C:N atomic ratios were computed using organic carbon contents.

Carbohydrates were determined by heating weighed aliquots of freeze-dried matter in 5 mL of double distilled water, at 95 °C for 2 h. After centrifugation, 1 mL of the supernatant (hot water soluble fraction) and the dried residue (insoluble fraction) (Harmelin-Vivien et al., 1992) were treated by the phenosulfuric acid method (Dubois et al., 1956). Absorbances at 492 nm were determined spectrophotometrically and compared to a glucose standard curve. Total lipid fraction was determined by the Marsh and Weinstein (1966) modified method (Mayzaud and Martin, 1975), after lipid extraction by the Bligh and Dyer (1959) method, using 1.2 mL of distilled water, 1.5 mL of chloroform and 3.0 mL of methanol. After centrifugation, 1.5 mL of distilled water and 1.5 mL of chloroform were added to the supernatant. Absorbances at 360 nm were compared to a tripalmitic acid standard curve. Protein fraction was determined by the Lowry et al. (1951) method, after extraction during 12 h in a solution of NaCl, Tris and EDTA. Absorbances at 700 nm were compared to a beef albumin standard curve.

2.4. Data analyses

Total absorption efficiency and absorption efficiency of each organic compound (organic matter, organic carbon, nitrogen, carbohydrates, lipid and protein) were determined according to Montgomery and Gerking (1980), Edwards and Horn (1982), Horn and Neighbors (1984), and Targett and Targett (1990). Whereas the term ‘assimilation’ is used in these papers, the term ‘absorption’ is more convenient for the measured variable, as absorption defines the amount of compound that crosses the intestinal wall while assimilation stands for the amount of compound being incorporated in the body cells (Lawrence, 1975). The term ‘absorption’ will be used in the present paper, as it does not conflict with the energetic literature.

$$\text{total absorption efficiency (\%)} = (1.0 - \text{minimum\% ash in foregut/maximum\% ash in hindgut})\ 100 \tag{1}$$

$$\text{organic compound absorption efficiency (\%)} = (1.0 - (\text{min\% organic compound in hindgut/max\% organic compound in foregut})\ (\text{minimum\% ash in foregut/maximum\% ash in hindgut}))\ 100 \tag{2}$$

Methods employed to estimate absorption efficiency or digestibility have been discussed and their limits defined in a few papers (Galetto and Bellwood, 1994). The standard ash-marker method (formulae (1) and (2)) was chosen for reasons of comparability and feasibility.

Differences between sites and between the various sections of the digestive tract of fish were tested using repeated measures-analysis of variance (ANOVAR), or multivariate repeated measures ANOVA (MANOVAR) when the sphericity condition of the repetition levels (here, the sections of the digestive tract) was not verified (Potvin et al., 1990; von Ende, 1993). In case a significant 'between section' difference was found, a posteriori comparisons of means using the Student–Newman–Keuls test were carried out within each site. Differences between the two sites, in terms of fish size, absorption efficiencies, biochemical composition of EAM and faeces, and loss of organic nutrients between rectal contents and faeces were tested using Student t-tests on independent samples. Differences between absorption efficiencies of organic nutrients within a site were tested using Student t-tests on dependent samples. All statistical procedures were performed at a 0.05 confidence level.

3. RESULTS

3.1. Chemical composition of the epilithic algal matrix (EAM)

Epilithic algal matrix in the disturbed site (site D) contained significantly (*table II*) more organic matter than EAM in the undisturbed site (site U). The same pattern was observed for organic carbon and nitrogen percentages, with significantly higher values in EAM found in site D than in site U. C:N mass ratios measured in algal food were also significantly higher in site D than in site U (*table II*). The C:N atomic ratios were 11.6 ± 0.12 in site D and 11.2 ± 0.53 in site U. The EAM differed significantly in three biochemical characteristics between sites (*table III*). The concentrations of soluble carbohydrate, lipid and protein were higher in site D than in site U. EAM contained similar amounts of insoluble sugar in site D and in site U.

3.2. Fish size

Fish sampled in the disturbed and nutrient-enriched site D were significantly larger ($t = 3.45$, $P < 0.001$) than fish collected in the undisturbed and oligotrophic site U, with a mean total length of 11.4 ± 0.6 cm for site D fish versus 10.9 ± 0.5 cm for site U fish.

3.3. Food transformation

Percentages of organic matter in each section of the digestive tract were significantly higher in fish from site D than in those collected in site U (*figure 2*), except for stomach contents where the difference was not significant at a 5 % level, but the probability ($P = 0.058$) was near this level. Percentages of organic matter displayed a similar variation along the digestive tract, in fish from both sites. The values rapidly and significantly decreased in the first half of the tract, then slightly increased in the rectum (*figure 2*). Organic carbon and nitrogen percentages both showed a similar pattern to organic matter values along the digestive tract (*figure 3*), with significant decreases in the first part of the tract, and increases in

Table II. Mean comparisons of organic matter, organic carbon (C) and nitrogen (N) percentages, and C:N mass ratios in algal turfs and in the faeces of *S. nigricans*, between site D (disturbed) and site U (undisturbed).[a]

	Algal turfs				Faeces			
Organic matter	48.8 ± 1.3	44.3 ± 2.1	5.5	*	21.8 ± 2.9	17.8 ± 1.8	3.4	*
C	16.9 ± 1.5	14.4 ± 0.7	4.5	*	5.5 ± 0.2	4.8 ± 0.2	7.7	*
N	1.70 ± 0.15	1.50 ± 0.1	3.2	*	0.55 ± 0.08	0.51 ± 0.09	1.0	n.s.
C:N	9.96 ± 0.10	9.61 ± 0.43	2.4	*	10.2 ± 1.5	9.7 ± 1.6	0.7	n.s.

[a] $n = 10$; t = Student test value; P = probability; * = $P < 0.05$; n.s. = non significant difference.

Table III. Mean comparisons of soluble carbohydrate, insoluble carbohydrate, lipid and protein concentrations in algal turfs and in the faeces of *Stegastes nigricans*, between site D (disturbed) and site U (undisturbed).[a]

	Algal turfs				Faeces			
Soluble carbohydrate	96.9 ± 7.8	82.2 ± 4.9	4.8	*	22.7 ± 3.2	18.3 ± 3.9	2.6	*
Insoluble carbohydrate	48.8 ± 13.0	52.7 ± 16.8	−0.5	n.s.	15.4 ± 3.1	16.2 ± 3.8	−0.5	n.s.
Lipid	26.1 ± 5.0	20.4 ± 2.6	3.0	*	9.4 ± 1.1	9.0 ± 1.5	0.6	n.s.
Protein	35.9 ± 1.5	27.5 ± 5.3	4.5	*	14.4 ± 1.6	11.4 ± 2.9	2.8	*

[a] $n = 10$; t = Student test value; P = probability; * = $P < 0.05$; n.s. = non significant difference. Values are in mg of equivalent g^{-1} of freeze-dried matter.

the last part. Percentages were also significantly higher in each gut section in fish from site D than in fish from site U, except for nitrogen values in the stomach where the difference was not significant. The C:N mass ratio remained low compared to the C:N mass ratio calculated in the EAM, with values ranging from 4.9 ± 1.0 to 3.1 ± 0.5 in site U, and from 5.3 ± 0.4 to 4.3 ± 0.3 in site D. The ratio was significantly higher in site D than in site U. The C:N atomic ratios varied between 3.7 ± 0.6 and 5.7 ± 1.1 in site U, and between 5.1 ± 0.3 to 6.2 ± 0.5 in site D. Soluble and insoluble carbohydrate values were always higher in fish from site D whatever the section of the digestive tract considered (*table IV*). Most of the differences were significant in fish guts between sites. A significant decrease in soluble and insoluble carbohydrate concentrations was observed along the digestive tract of fish from both sites. The insoluble/soluble ratio showed a significant increase in the first half of the intestine of fish from both sites, then stabilized at a high level as far as the rectum (*figure 4*). Significant differences between sites were observed in all sections, except in the stomach, for lipid and protein concentrations and in the rectum for proteins, concentrations being higher in fish from the disturbed site (*table IV*). Lipid and protein concentrations decreased greatly from the stomach to the intestine, then increased significantly from hind gut levels to the rectum in fish from both sites.

3.4. Faeces chemical composition

Percentages of organic matter in faeces were significantly higher in site D than in site U (*table II*). The faeces also differed significantly in organic carbon contents between sites, whereas no significant difference in nitrogen contents was observed. No significant difference in C:N mass ratios was found between sites. C:N atomic ratios were higher, with respectively 11.9 ± 1.8 and 11.4 ± 1.9 in site D and site U. Organic nutrient composition of faeces was similar between sites in insoluble carbohydrates and lipids (*table III*). Soluble carbohydrate and protein concentrations were higher in faeces produced by fish living in the disturbed site than in those produced in the undisturbed site.

Organic matter and nutrient concentration differences between rectal content and faeces were larger in site D than in site U. The difference in organic matter percentage between rectum and faeces was 24.0 ± 3.7 % in site D and 10.5 ± 2.5 % in site U, values

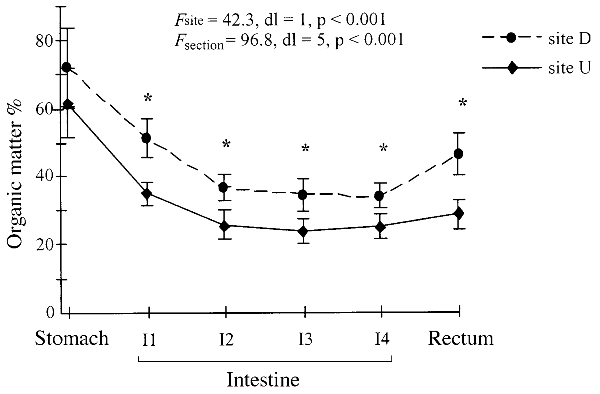

Figure 2. Variations of mean organic matter percentages in the contents of the different sections of the gastro-intestinal tract of *S. nigricans*: stomach, intestine, rectum for fish sampled in site D (disturbed) and site U (undisturbed) (F_{site}: result of the ANOVAR for the site factor, $F_{section}$: result of the MANOVAR for the section factor, P: probability level, *: significant difference between sites at $P < 0.05$, error bars: ± SD).

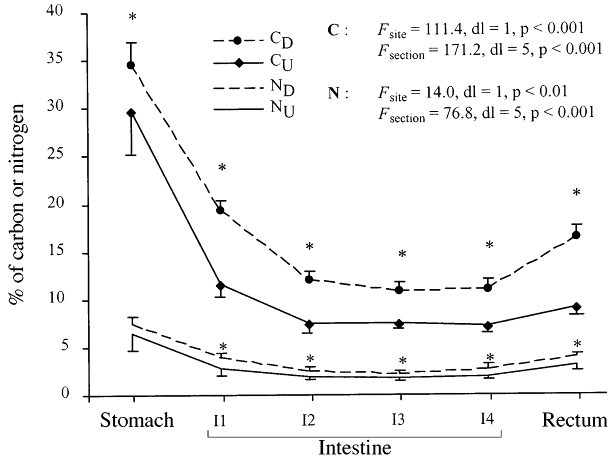

Figure 3. Variations of mean organic carbon and nitrogen percentages in the different sections of the gastro-intestinal contents of *S. nigricans*: stomach, intestine, rectum for fish sampled in site D (disturbed) and site U (undisturbed) (F_{site}: result of the ANOVAR for the site factor, $F_{section}$: result of the MANOVAR for the section factor, P: probability level, *: significant difference between sites at $P < 0.05$, error bars: ± SD).

being significantly different between the sites ($t = 9.09$, $P < 0.001$). The difference in lipid contents was 58.1 ± 4.2 mg.g^{-1} in site D versus 45.6 ± 11.9 mg.g^{-1} in site U, with a significantly higher value in site D ($t = 2.97$, $P < 0.01$).

3.5. Absorption efficiencies

The total absorption efficiency and absorption efficiencies of organic carbon, nitrogen, lipids and proteins by *S. nigricans* were significantly higher in fish collected in the nutrient-enriched disturbed site D, while no significant difference between sites was observed for organic matter and carbohydrate absorption efficiencies, even if higher in fish living in the disturbed site (*table V*). In both sites, lipid and protein absorption efficiencies were higher than both carbohydrate absorption efficiencies, but the differences were only significant in site D ($F = 5.34$, $P < 0.01$) between insoluble carbohydrate and lipid absorption efficiency ($P < 0.001$) and between insoluble carbohydrate and protein absorption efficiency ($P < 0.01$). No significant difference was observed between organic carbon and nitrogen absorption efficiencies in both sites.

4. DISCUSSION

Herbivorous fish play a key role in the transfer of material and energy in coral reef food webs by transforming algal matter and releasing it as faeces, which constitute a major food resource for detritivores, and a source of minerals for benthic communities (Geesey et al., 1984; Polunin and Koike, 1987). The present study performed on La Réunion reef flats contributed to the knowledge of the biology of *Stegastes nigricans*, with results on food and faeces chemical composition, and absorption efficiencies of several compounds for this species at La Réunion. It also brought significant differences in the fish biological processes between sites. The potential causes accounting for these differences will be discussed hereafter.

Clear differences found between sites in the food, the digestive contents and the faeces of *S. nigricans*, justified a posteriori the small number of fish sampled (ten fish per site).

Inside territories of both sites, EAM contained mean values of 46 % of organic matter, 3.2 % of protein and 2.3 % of lipid. These values, low in protein and lipid, and close to those of red algae, are consistent with others found in the literature (Montgomery and Gerking, 1980; Edwards and Horn, 1982; Horn and

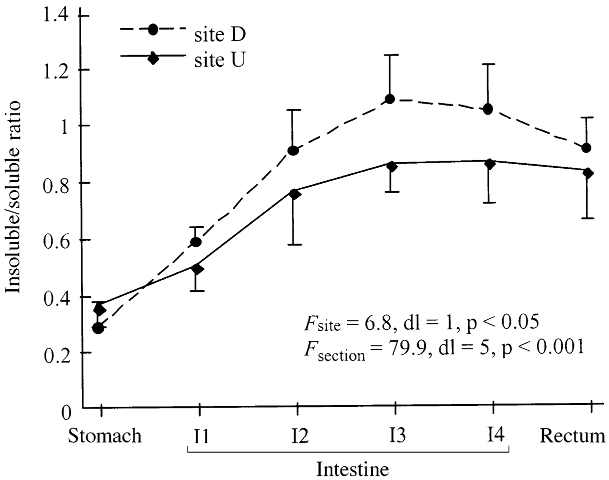

Figure 4. Variations of the insoluble/soluble ratio in the different sections of the gastrointestinal contents of *S. nigricans*: stomach, intestine, rectum for fish sampled in site D (disturbed) and site U (undisturbed) (F_{site}: result of the ANOVAR for the site factor, $F_{section}$: result of the MANOVAR for the section factor, P: probability level, error bars: ± SD).

Table IV. Mean (± SD) soluble carbohydrate, insoluble carbohydrate, lipid and protein concentrations at different sections of the digestive tract of *Stegastes nigricans* in site D (disturbed) and site U (undisturbed).[a]

		Soluble carbohydrate	g	Insoluble carbohydrate	g	Lipid	g	Protein	g
Site D	S	51.3 ± 14.4	bc	13.1 ± 2.5	c	137.7 ± 15.6	a	167.5 ± 13.8	a
	I1	62.6 ± 6.9	a	36.0 ± 4.1	b	85.4 ± 12.3	b	100.6 ± 11.2	b
	I2	53.2 ± 9.4	b	47.5 ± 11.0	a	53.6 ± 10.3	d	66.7 ± 8.6	c
	I3	44.9 ± 9.3	bcd	47.1 ± 6.2	a	46.9 ± 8.0	d	55.8 ± 7.7	d
	I4	42.9 ± 8.1	cd	43.3 ± 5.8	a	49.5 ± 6.3	d	64.6 ± 8.1	cd
	R	38.4 ± 5.9	d	34.1 ± 5.5	b	67.5 ± 4.8	c	94.0 ± 7.2	b
Site U	S	38.6 ± 4.5	b	13.7 ± 3.7	c	124.9 ± 32.3	a	165.5 ± 20.7	a
	I1	52.5 ± 18.2	a	25.8 ± 9.8	b	61.6 ± 9.1	b	72.8 ± 13.7	b
	I2	48.5 ± 10.6	a	36.1 ± 11.3	a	40.7 ± 8.8	cd	45.3 ± 9.2	d
	I3	36.8 ± 8.0	b	31.7 ± 10.1	ab	33.8 ± 6.0	d	42.4 ± 4.1	d
	I4	33.3 ± 6.6	b	28.7 ± 7.9	ab	39.1 ± 10.9	d	47.3 ± 9.9	d
	R	30.8 ± 5.9	b	24.2 ± 7.9	b	54.6 ± 11.7	bc	90.3 ± 10.0	c
F_{site}	P	7.8, **		14.0, ***		21.3, ***		19.1, ***	
F_{section}	P	13.0, ***		38.5, ***		42.5, ***		136.2, ***	

[a] S = stomach; I1-I4 = intestine; R = rectum; F_{site} = result of the ANOVAR for the site factor; $F_{section}$ = result of the MANOVAR for the section factor; P = probability level; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; g = result of Student–Newman–Keuls means comparison tests with $P < 0.05$. Means with a letter in common are statistically equivalent; a indicating the highest means, $n = 10$. Values are in mg of equivalent g^{-1} of freeze-dried matter.

Neighbors, 1984). The nitrogen contents of food measured in both sites (1.7 % and 1.5 %) compared closely with nitrogen contents measured in other turf algae, such as *Enteromorpha* spp. (1.73 %, Menzel, 1959; 1.87 %, Nicotri, 1980) and *Ceramium* sp. (1.71 %, Nicotri, 1980), which are major components of EACs and damselfish food in different parts of the reef world (Lassuy, 1980, 1984; Hixon and Brostoff, 1981; Sammarco, 1983; Scott and Russ, 1987). They were lower than those measured in algal turf-food of *Plectroglyphidodon lacrymatus* in Papua-New Guinea (2.5 %, Polunin, 1988). The C:N mass ratio was lower in the EAM at La Réunion (10:1) than in Papua-New Guinea (15:1, Polunin, 1988). However theis ratio was higher than previous estimates of EACs detritus fraction C:N ratios (6:1, Wilson and Bellwood, 1997), and close to those of periphyton in a coral reef of the Philippines (9:1 to 17:1, Klumpp et al., 1992). However the C:N mass ratios of algae found in the literature varied greatly between species (7:1 to 78:1, Atkinson and Smith, 1983). The ratio found at La Réunion indicated a high quality food, as only food with a C:N ratio of 17:1 or lower would provide an adequate protein supply (Russel-Hunter, 1970). The C:N atomic ratios were comparable to the ratio found for EACs at Pandora Reef (Russ and McCook, 1999). This reef was an inshore reef subject to increased inorganic nutrient concentrations, similar to the situation of fringing reefs at La Réunion.

Absorption efficiencies greatly varied according to organic nutrients, lipid absorption being the highest, followed by protein absorption, soluble and then insoluble carbohydrate absorption. The insoluble/soluble ratio increased from algal food to faeces, proving that soluble carbohydrates were assimilated prior to insoluble carbohydrates (Handa et al., 1972; Harmelin-Vivien et al., 1992). The fact that C:N ratios did not vary much along the tract suggests that organic carbon and nitrogen were absorbed following the same pattern. This suggestion was confirmed by the similar C and N absorption efficiencies found in both sites (*table V*). These values were lower than the digestibilities found for *S. nigricans* fed green alga *Enteromorpha* (Galetto and Bellwood, 1994), but this might be due to the presence of other less digestible algal species in the natural diet of *S. nigricans* at La Réunion. Nitrogen absorption efficiencies were close to 61 %, the average value found for *Stegastes lividus* absorption efficiencies (Lassuy, 1984). Total absorption efficiencies calculated for *S. nigricans* in this study (16 % and 27 %) were comparable to the values estimated by Galetto and Bellwood (1994) for the same species on the Great Barrier Reef (20 %), and by Montgomery and Gerking (1980) in two damselfishes living in the Gulf of California, the Cortez damsel *Eupomacentrus rectifraenum* (Gill) (20 %) and the giant blue damsel *Microspathodon dorsalis* (Gill) (24 %). Protein absorption efficiencies in this study (52 % and 59 %) were lower than those found in the Cortez damsel (57 %) and the giant blue damsel (67 %), but stayed close. Lipid absorption efficiencies were higher than those found by these authors (54 % in site U and 62 % in site D versus 46 % in *E. rectifraenum* and 56 % in *M. dorsalis*). Carbohydrate absorption efficiencies were also higher than those calculated by Montgomery and Gerking (1980) (between 46 % and 55 % in site D versus 37 % in *E. rectifraenum* and 44 % in *M. dorsalis*). Nevertheless, these authors did not directly measure carbohydrate concentrations, but deduced them by difference after lipid and protein measures, which may have induced some biases in further calculations. The modification of organic matter percentages and organic nutrient concentrations along the gut provided information on the absorption mechanisms of *S. nigricans*. Absorption of compounds easy to assimilate (soluble carbohydrates, proteins and lipids) mainly occurred in the first half of the tract, a phenomenon also reported by Polunin (1988) in the reef-dwelling jewel damsel *Plectroglyphidodon lacrymatus* (Quoy and Gaimard), another herbivorous pomacentrid fish.

Conspicuous differences in organic nutrient concentrations were observed between the rectum faecal matter and the faeces collected in situ in both sites. As faeces were collected soon after their ejection by fish, these differences might be due to either a terminal absorption in the rectum, just before the ejection of faeces, or a rapid leaching of organic nutrients after their emission. This result might induce a bias in the calculation of absorption efficiencies by classical methods, in which efficiencies were estimated between values in algal food and values in faeces (Montgomery and Gerking, 1980; Edwards and Horn, 1982; Horn and Neighbors, 1984). Rapid and massive dissolution of organic nutrients in surrounding waters, indicated by differences in organic nutrient concentrations between rectal contents and faeces, might have caused a loss of organic nutrients and organic matter in faeces, inducing an overestimation of the real absorption.

Differences between the disturbed site D and the undisturbed site U were found, not only in the nutritive value of food of the black damselfish *S. nigricans*, but also in its gastro-intestinal and faeces contents, and in nutrient aborption efficiencies. The algal turfs (EAM) growing in *S. nigricans* territories on which this fish fed, were richer in terms of organic matter, organic carbon and nitrogen and organic nutrients in the disturbed site than in the undisturbed reef site. Sampling procedures and light exposure being similar in both sites, the differences observed between sites could be explained by several ecological factors such as the nutrient levels and the turbidity of reef waters, as well as species composition and sediment load of the EAM. The increase in turbidity observed in site D should normally slow down primary production. Contrary to this hypothesis, higher organic matter percentages and organic nutrient concentrations were found in the site D turfs. The higher sediment loads trapped by macroalgae noted in site D by Semple (1997) did not result in lower organic concentrations

Table V. Mean absorption efficiencies in *Stegastes nigricans* collected in the undisturbed site (U) and in the disturbed site (D), of soluble carbohydrates (SC), insoluble carbohydrates, lipids, proteins, organic carbon (C), nitrogen (N), organic matter and total absorption efficiency.[a]

	Site U	Site D	*t*	*P*
Soluble carbohydrate	51.9 ± 12.6	55.1 ± 8.7	0.62	n.s.
Insoluble carbohydrate	46.0 ± 12.9	49.2 ± 8.4	0.63	n.s.
Lipid	54.0 ± 8.8	61.7 ± 3.7	2.43	*
Protein	52.1 ± 7.1	59.1 ± 6.2	2.21	*
C	48.5 ± 3.1	59.6 ± 3.5	7.17	*
N	48.7 ± 6.9	60.9 ± 6.5	3.86	*
Organic matter	46.6 ± 5.2	52.3 ± 7.5	1.87	n.s.
Total efficiency	16.3 ± 2.8	26.8 ± 6.3	4.60	*

[a] $n = 10$; t = Student test value; P = probability; * = $P < 0.05$; n.s. = non significant difference. Values are % ± SD.

in site D turfs. Eventually, the differences observed between the two sites are best reflected in differences of nutrient levels of reef waters and species composition of the EAM. Site D, defined as eutrophic in several papers compared to the oligotrophic site U (Cuet et al., 1988; Naim, 1993; Chabanet et al., 1995; Semple, 1997; Mioche and Cuet, 1999). The differences observed in the EAM were in agreement with the results published by McGlathery (1995) that showed an increase in nitrogen content in marine plants growing in nutrient-enriched reef sites. Recent papers on the effects of increased availability of nutrients on benthic algal production showed that algal tissue nitrogen increased drastically after increased nutrient supply (Schaffelke, 1999), and that the yield to grazers on coral reefs also increased (Russ and McCook, 1999). We can assume that potential differences in species composition of the turfs between sites (study in process) could also be linked to nutrient enrichment, as eutrophication was the major factor that accounted for the differences in macroalgae communities observed between the two sites studied (Naim, 1993; Semple, 1997).

Accordingly, organic matter percentages and organic nutrient concentrations along the digestive tract of fish feeding on enriched food were always higher than in fish from the undisturbed site. The lack of difference observed in stomach contents could be explained by the abundance of internal fluids in this part of the gut. This could also account for the disparity in organic matter percentages between algae and stomach contents noted in both sites. In site D, algae contained 49 % of organic matter and stomach contents 72 %, and at site U they were respectively 44 % and 62 %. Thus, a difference of 23 % in organic matter percentage between algae and stomach existed at site D, and a difference of 18 % appeared at site U, suggesting an enrichment in the stomach at site D compared to site U. A higher production of protein and lipid-rich internal fluids in fish from site D could induce such findings, and could thus explain the higher absorption efficiencies measured at site D. This hypothesis is reinforced by the fact that proteins and lipids are the only organic nutrients following this stomach-enrichment pattern. This phenomenon was also reported for the Cortez damselfish (Montgomery and Gerking, 1980), but the authors explained it as the result of mucus secretion by cells lining the buccal cavity, oesophagus or stomach or as the result of cell debris. Higher absorption efficiencies were measured at site D. Could this result be related to the fact that fish presented larger mean size, as absorption could provide more available anabolic compounds (such as proteins) to the fish? The determination of the protein/energy ratio (Horn et al., 1986) and the study of fish growth in both sites would give us more information about this point.

In the disturbed site D, difference in concentrations between rectal content and faeces was higher than in the undisturbed site, suggesting that more organic nutrients were reinjected in the field through dissolved matter by leaching from faeces. Leached organic nutrients might be recycled through the action of microorganisms. Recycling of nutrients is thought to play a major role in the maintenance of high organic productivity on coral reefs (D'Elia and Wiebe, 1990), as the enrichment of reef waters in organic nutrients is directly available to other vegetal or animal organisms (Sakata, 1990). Klumpp et al. (1987) defined damselfish territories as high primary productivity zones. One of their hypotheses to explain that phenomenon resided in the fertilization of algal turfs through the production of detritus by the fish itself. As reinjection of organic nutrients in the environment by the herbivorous fish *S. nigricans* was more intense in the eutrophic disturbed site, this phenomenon might reinforce the effect of eutrophication on La Réunion coral reefs.

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REFERENCES

Anderson, T.A., 1987. Utilization of algal cell fractions by the marine herbivore the luderick, *Girella tricuspidata*. J. Fish. Biol. 31, 221–228.

Atkinson, M.J., Smith, S.V., 1983. C:N:P ratios of benthic marine plants. Limnol. Oceanogr. 28, 568–574.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Chabanet, P., Dufour, V., Galzin, R., 1995. Disturbance impact on reef fish communities in Reunion Island. J. Exp. Mar. Biol. Ecol. 188, 29–48.

Chazottes, V., 1996. Etude expérimentale de la bioérosion et de la sédimentogénèse en milieu récifal: effets de l'eutrophisation (île de la Réunion, Océan Indien). C.R. Acad. Sci. Paris 323, 787–794.

Choat, J.H., 1991. The biology of herbivorous fishes on coral reefs. In: Sale, P.F. (Ed.), The Ecology of Fishes on Coral Reefs. Academic Press, New York, pp. 120–155.

Choat, J.H., Clements, K.D., 1998. Vertebrate herbivores in marine and terrestrial environments: a nutritional ecology perspective. Ann. Rev. Ecol. Syst. 29, 375–403.

Clements, K.D., 1991. Endosymbiotic communities of two herbivorous labroid fishes, *Odax cyanomela* and *O. pullus*. Mar. Biol. 109, 223–229.

Cuet, P., Naim, O., Faure, G., Conan, J.Y., 1988. Nutrient-rich groundwater impact on benthic communities of La Saline fringing reef (Reunion Island): preliminary results. Proc. 6th Intern. Coral Reef Symp. 2, 207–212.

D'Elia, C.F., Wiebe, W.J., 1990. Biogeochemical nutrient cycles in coral-reef ecosystems. In: Dubinsky, Z. (Ed.), Coral Reefs. Ecosystems of the World. Elsevier Science, Amsterdam, pp. 49–74.

Done, T.J., 1992. Phase shifts in coral reef communities and their ecological significance. Hydrobiologia 247, 121–132.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.

Edwards, T.W., Horn, M.H., 1982. Assimilation efficiency of a temperate-zone intertidal fish (*Cebichthys violaceus*) fed diets of macroalgae. Mar. Biol. 67, 247–253.

Gabrié, C., Montaggioni, L.F., 1982. Sediments from fringing reefs of Reunion Island, Indian Ocean. Sedim. Geol. 31, 281–301.

Galetto, M.J., Bellwood, D.R., 1994. Digestion of algae by *S. nigricans* and *Amphiprion akindynos* (Pisces: Pomacentridae), with an evaluation of methods used in digestibilty studies. J. Fish. Biol. 44, 415–428.

Geesey, G.G., Alexander, G.V., Bray, R.N., Miller, A.C., 1984. Fish fecal pellets are a source of minerals for inshore reef communities. Mar. Ecol. Prog. Ser. 15, 19–25.

Gerking, S.D., 1994. Plant-eating fish. In: Gerking, S.D. (Ed.), Feeding Ecology of Fish. Academic Press, San Diego, pp. 57–84.

Handa, N., Yanagi, K., Matsunaga, K., 1972. Distribution of detrital materials in the Western Pacific Ocean and their biochemical nature. Mem. Inst. Ital. Idrobiol. 29 (suppl.), 53–71.

Harmelin-Vivien, M.L., Peyrot-Clausade, M., Romano, J.C., 1992. Transformation of algal turf by echinoids and scarid fishes on French Polynesian coral reefs. Coral Reefs 11, 45–50.

Hixon, M.A., Brostoff, W.N., 1981. Fish grazing and community structure of Hawaiian reef algae. Proc. 4th Intern. Coral Reef Symp. 2, 507–514.

Horn, M.H., 1989. Biology of marine herbivorous fishes. Oceanogr. Mar. Biol. Ann. Rev. 27, 167–272.

Horn, M.H., Neighbors, M.A., 1984. Protein and nitrogen assimilation as a factor in predicting the seasonal macroalgal diet of the Monkeyface Prickleback. Trans. Am. Fish Soc. 113, 388–396.

Horn, M.H., Neighbors, M.A., Murray, S.N., 1986. Herbivore responses to a seasonally fluctuating food supply: growth potential of two temperate intertidal fishes based on the protein and energy assimilated from their macroalgal diets. J. Exp. Mar. Biol. Ecol. 103, 217–234.

Join, J.L., 1991. Caractérisation hydrogéologique du milieu volcanique insulaire. Le Piton des Neiges (Ile de la Réunion). Thèse de Doctorat, Université de Montpellier, France.

Klumpp, D.W., McKinnon, D., Daniel, P., 1987. Damselfish territories: zones of high productivity on coral reefs. Mar. Ecol. Prog. Ser. 40, 41–51.

Klumpp, D.W., Salita-Espinosa, J.S., Fortes, M.D., 1992. The role of epiphytic periphyton and macroinvertebrate grazers in the trophic flux of a tropical seagrass community. Aquat. Bot. 43, 327–349.

Lassuy, D.R., 1980. Effects of farming behaviour by *Eupomacentrus lividus* and *Hemiglyphidodon plagiometopon* on algal community structure. Bull. Mar. Sci. 30, 304–312.

Lassuy, D.R., 1984. Diet, intestinal morphology, and nitrogen assimilation efficiency in the damselfish *Stegastes lividus* in Guam. Env. Biol. Fish. 10, 183–193.

Lawrence, J.M., 1975. On the relationships between marine plants and sea urchins. Oceanogr. Mar. Biol. Ann. Rev. 13, 213–286.

Letourneur, Y., 1992. Dynamique des peuplements ichtyologiques des platiers récifaux de l'île de la Réunion. Thèse de Doctorat, Université Aix-Marseille II, France.

Letourneur, Y., Galzin, R., Harmelin-Vivien, M., 1997. Temporal variations in the diet of the damselfish *Stegastes nigricans* (Lacepède) on a Réunion fringing reef. J. Exp. Mar. Biol. Ecol. 217, 1–18.

Lowry, O.H., Rosebroug, N.J., Farr, A.L., Randall, R.J., 1951. Protein mesurement with the Folin reagent. J. Biol. Chem. 193, 265–275.

McGlathery, K.J., 1995. Nutrient and grazing influences on a subtropical seagrass community. Mar. Ecol. Prog. Ser. 122, 239–252.

Marsh, D.W., Weinstein, D., 1966. Simple charring method for determination of lipids. J. Lip. Res. 7, 574–576.

Mayzaud, P., Martin, J.L.M., 1975. Some aspect of biochemical and mineral composition of marine plankton. J. Exp. Mar. Biol. Ecol. 17, 297–310.

Menzel, D.W., 1959. Utilization of algae for growth by the Angelfish *Holacanthus bermudensis*. J. Cons. Int. Explor. Mer. 29, 308–313.

Mioche, D., Cuet, P., 1999. Métabolisme du carbone, des carbonates et des sels nutritifs en saison chaude sur un récif frangeant soumis à une pression anthropique (Ile de la Réunion, Océan Indien). C.R. Acad. Sci. Paris 329, 53–59.

Montaggioni, L.F., Faure, G., 1980. Les récifs coralliens des Mascareignes (Océan Indien). Coll. Trav. Centre Univ. Franc. Oc. Ind., Université de la Réunion.

Montgomery, W.L., 1980. Comparative feeding ecology of two herbivorous damselfishes (Pomacentridae: Teleostei) from the gulf of California, Mexico. J. Exp. Mar. Biol. Ecol. 47, 9–24.

Montgomery, W.L., Gerking, S.D., 1980. Marine macroalgae as foods for fishes: an evaluation of potential food quality. Env. Biol. Fish. 5, 143–153.

Moreno, V.J., Jara, T., 1984. Ecological studies on fish fauna associated with *Macrocystis pyrifera* belts in the South of the Fuegian Islands, Chile. Mar. Ecol. Prog. Ser. 15, 99–107.

Naim, O., 1993. Seasonal responses of a fringing reef community to eutrophication (Reunion Island, Western Indian Ocean). Mar. Ecol. Prog. Ser. 99, 137–151.

Nicotri, M.E., 1980. Factors involved in herbivore food preference. J. Exp. Mar. Biol. Ecol. 42, 13–26.

Polunin, N.V.C., 1988. Efficient uptake of algal production by a single resident herbivorous fish on the reef. J. Exp. Mar. Biol. Ecol. 123, 61–76.

Polunin, N.V.C., Klumpp, D.W., 1992. Algal food supply and grazer demand in a very productive coral-reef zone. J. Exp. Mar. Biol. Ecol. 164, 1–15.

Polunin, N.V.C., Koike, I., 1987. Temporal focusing of nitrogen release by a periodically feeding herbivorous reef fish. J. Exp. Mar. Biol. Ecol. 111, 285–296.

Potvin, C., Lechowicz, M.J., Tardif, S., 1990. The statistical analysis of ecophysiological response curves obtained from experiments involving repeated measures. Ecology 71 (4), 1389–1400.

Rimmer, D.W., Wiebe, W.J., 1987. Fermentative microbial digestion in herbivorous fishes. J. Fish. Biol. 31, 229–236.

Russ, G.R., McCook, L.J., 1999. Potential effects of a cyclone on benthic algal production and yield to grazers on coral reefs across the central Great Barrier Reef. J. Exp. Mar. Biol. Ecol. 235, 237–254.

Russel-Hunter, W.D., 1970. Aquatic Productivity. Macmillan, New York.

Sakata, T., 1990. Microflora in the digestive tract of fish and shell-fish. In: Lésel, R. (Ed.), Microbiology in Poecilotherms. Elsevier Science, Amsterdam, pp. 171–176.

Sammarco, P.W., 1983. Effects of fish grazing and damselfish territoriality on coral reef algae. I. Algal community structure. Mar. Ecol. Prog. Ser. 13, 1–14.

Schaffelke, B., 1999. Short-term nutrient pulses as tools to assess responses of coral reef macroalgae to enhanced nutrient availability. Mar. Ecol. Prog. Ser. 182, 305–310.

Scott, F.J., Russ, G.R., 1987. Effects of grazing on species composition of the epilithic algal community on coral reefs of the central Great Barrier Reef. Mar. Ecol. Prog. Ser. 39, 293–304.

Semple, S., 1997. Algal growth on two sections of a fringing coral reef subject to different levels of eutrophication in Reunion Island. Oceanol. Acta 20, 851–861.

Targett, T.E., Targett, N.M., 1990. Energetics of food selection by the herbivorous parrotfish Sparisoma radians: roles of assimilation efficiency, gut evacuation rate, and algal secondary metabolites. Mar. Ecol. Prog. Ser. 66, 13–21.

von Ende, C.N., 1993. In: Scheiner, S.M., Gurevitch, J. (Eds.), The Designs and Analysis of Ecological Experiments. Chapman & Hall, New York.

Wheeler, A., 1980. Fish-algal relations in temperate waters. In: Price, J.H., Irvine, D.E.G, Farnham, W.F. (Eds.), The Shore Environnement, Vol. 2: Ecosystems. Academic Press, London, pp. 677–698.

Wilkinson, C.R., Sammarco, P.W., 1983. Effects of fish grazing and damselfish territoriality on coral reef algae. II: nitrogen fixation. Mar. Ecol. Prog. Ser. 13, 15–19.

Wilson, S., Bellwood, D.R., 1997. Cryptic dietary components of territorial damselfishes (Pomacentridae, Labroidei). Mar. Ecol. Prog. Ser. 153, 299–310.