

## NUTRITIONAL ECOLOGY

# Nutritional ecology of marine herbivorous fishes: ten years on

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## Summary

1. Marine herbivorous fishes are considered to be of critical importance in determining the biological structure of shallow reef environments, and by implication have key roles in carbon flux in reef ecosystems. Despite this, the nutritional processes that underpin these critical ecological roles have received comparatively little attention.

2. Here we give an overview of recent progress in the nutritional ecology of marine herbivorous fishes, and then examine two recent paradigms that we consider important in the development of the field: (i) the role of temperature in latitudinal gradients of diversity and abundance, and (ii) the impact of these fishes on coral reefs. Our aim is to illustrate how an integrative nutritional ecology approach can enrich insights gained from studies of fish herbivory, and to emphasize the distinctive differences between herbivory in marine and terrestrial systems.

3. We argue that much of the work on trophic biology in marine herbivorous fishes has focused on the ecological impacts of fishes on reefs, the deterrent properties of marine algae, and the morphological and mechanical aspects of ingestion. This has come at the expense of two of the elements necessary for an integrative understanding of feeding ecology, that is, food composition and the physiological processes involved in nutrient extraction and utilization. Together, these factors have hindered the development of the nutritional framework for analysing food resources, feeding patterns and evolutionary trends that has proved successful for terrestrial vertebrate herbivores.

4. The reef grazing and algal secondary metabolite paradigms, while both extremely productive, have failed to develop the clear predictive framework for diet choice required in broader contexts such as reef management and understanding the evolution of herbivory. The lack of a focus on nutritional factors has led to premature conclusions on the influence of temperature on algal digestion, both at the level of digestive processes and the biogeography of marine faunas.

5. Some marine herbivorous fishes appear to be bending the 'rules' of hindgut fermentation, especially with respect to temperature and fermentation substrates, and so the study of nutritional ecology in these animals has potential to generate novel insights for the field of vertebrate nutrition in general.

**Key-words:** biogeography, diet, digestion, fermentation, herbivory, marine/terrestrial comparisons, physiological constraints, temperature

## Introduction

Marine herbivorous fishes (Choat & Clements 1998) are considered to be critically important in determining the biological structure of shallow reef environments (Gaines & Lubchenco 1982; Horn 1989), and by implication have key roles in the flow of carbon through tropical reef ecosystems.

Understanding these roles requires information on the nutritional relations between these fishes and their environments – that is, a nutritional ecology perspective is needed (Foley & Cork 1992). The evolutionary diversity of herbivorous fishes, their dietary resources and their gastrointestinal symbionts makes them a potentially powerful model system for nutritional ecology in general (Clements & Raubenheimer 2006).

In 1998 we reviewed the literature on the nutritional ecology of marine fishes, and concluded that the field was poorly

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developed in contrast with the literature on terrestrial vertebrate herbivores (Choat & Clements 1998). Specifically, the study of terrestrial vertebrates was better advanced in terms of integrating three key elements in nutritional ecology: (i) the biochemical composition of foods, (ii) the coordinated behavioural and sensory responses associated with food detection and ingestion, and (iii) the physiological processes associated with extracting and utilizing nutrients from these foods. This integration provided a model approach to the study of nutritional ecology in terrestrial vertebrates that included information on food resources, digestive processes and their ecological and evolutionary consequences (e.g. Van Soest 1994). In fish, by contrast, the first and last of the above three factors in particular were poorly understood. This deficiency has hindered the development of the framework for analysing food resources, feeding patterns and evolutionary trends that has proved so successful for terrestrial herbivores (e.g. Parra 1978; Slansky & Rodriguez 1987; Van Soest 1994; Raubenheimer & Simpson 1998; Sakaguchi 2003; Clauss *et al.* 2007; Karasov & Martínez del Río 2007; Felton *et al.*, 2009; Parker *et al.*, 2009; Torregrossa & Dearing, 2009).

This special issue on nutritional ecology provides an opportunity to examine the state of the field a decade later, in terms of both the areas that we felt were important in 1998 and subsequent developments. We open with a brief review of progress in the research themes that we highlighted in 1998. Thereafter, we examine two recent paradigms that we consider particularly relevant in the development of the field: the role of temperature in latitudinal gradients of diversity and abundance in marine herbivorous fishes, and the impact of herbivorous fishes on coral reefs. Our aim here is to illustrate how an integrative nutritional ecology approach can enrich insights gained from studies of fish herbivory. As with Choat & Clements (1998), since our focus is primarily ecological we will not draw heavily on the aquaculture literature, although we note that the 'nutritional' part of nutritional ecology is far better developed in aquaculture than for wild species (e.g. Halver & Hardy 2002).

## Advances since 1998

In this section we provide an update on research themes reviewed by Choat & Clements (1998), under the same section headings that we used previously.

### DIET QUALITY: IMPLICATIONS FOR THE FEEDING OF HERBIVORES

#### (i) Positive and negative choices

Studies of terrestrial vertebrate herbivores have examined food selection in the context of an interaction between the nutritional properties of plants and the properties of the herbivore's digestive system. In contrast, marine studies have had a heavy focus on the deterrent properties of the main plants involved, algae and seagrasses (Choat & Clements 1998). For example, Mantyka & Bellwood (2007) suggested

that the macroalgal attributes that may explain patterns of consumption by herbivorous fishes include morphology, growth rates, competitive ability, and structural and chemical defences. Targett & Arnold (1998) claimed that they could successfully predict the response of herbivorous fishes to particular algal species by combining Horn's (1989) framework of marine herbivorous fish gut types (more on which below) with data on phlorotannin characteristics.

More recent studies have indicated that, in addition to the gut environment of the herbivore, characteristics of the plant other than just secondary metabolites were necessary to predict herbivore responses (e.g. Targett & Arnold 2001; Van Alstyne, Whitman & Ehlig 2001). Sotka and Whalen (2008) further suggested that explaining variation among fish species in susceptibility to polyphenolics simply in terms of their gut characteristics has been of minimal usefulness in understanding physiological and biochemical tolerance to these compounds.

In overview, it seems to us that the focus on negative food choices, that is, avoidance of particular secondary metabolites in particular algal species, has been highly productive (e.g. Targett & Arnold 2001), but is unlikely to provide a general framework for diet choice in marine herbivorous fishes. This is because the primary functional role of feeding is nutrition, and it thus follows that a general framework for nutritional ecology must ultimately build on nutrients. Allelochemicals undoubtedly do play a role, but studies of terrestrial herbivores have demonstrated that even this role is better understood in the context of nutrition (Raubenheimer 1992; Simpson & Raubenheimer 2001).

#### (ii) Diet quality and food selection

Since 1998 an increasing number of studies on herbivorous reef fishes have acknowledged the importance of a nutritional approach to diet quality, as advocated by Montgomery & Gerking (1980). In this respect the marine fish literature has lagged well behind that on the algalivorous Galápagos marine iguana, where interpretation of diet choice has consistently focused on nutrient content (e.g. van Marken Lichtenbelt 1992; Wikelski *et al.* 1993; Rubenstein & Wikelski 2003; Vitousek *et al.* 2007). Recent studies on the nutrient content of marine algae are beginning to challenge the assumption that the diets of grazing reef fishes are necessarily of 'low quality' (e.g. Harmelin-Vivien 2002). For example, Rubenstein & Wikelski (2003) suggest that while the nitrogen content of the algal diet of the marine iguana may be low, there is no evidence that it is limiting to growth. Smit *et al.* (2006) indicate that macroalgae have higher nutritional value and are usually more palatable than seagrasses, and that epiphytic algae have higher nutritional value than macroalgae. In both marine iguanas and isopods, variation in algal quality (in terms of nutrient content) has a far greater effect on reproductive success than algal quantity (Hemmi & Jormalainen 2002; Rubenstein & Wikelski 2003).

Marine algae differ greatly from terrestrial vascular plants in both the ratio of cell wall to cell contents and biochemical composition (Montgomery & Gerking 1980; Lahaye 1991).

Furthermore, recent studies have highlighted several reasons why appropriate methodologies are required for analyses of structure and biochemical composition in marine algae.

1. Algae may have high concentrations of non-protein nitrogenous substances (Fleurence 1999; Barbarino & Lourenço 2005), and thus conversion factors for N to protein in algal species vary greatly (Lourenço *et al.* 2002; Barbarino & Lourenço 2005).
2. Simple elemental ratios used in stoichiometric studies can be inadequate also because availability and utilization are influenced by the biochemical composition of the diet (Anderson *et al.* 2004; Raubenheimer *et al.* 2008).
3. Different freezing protocols can affect protein recovery and estimation (Crossman *et al.* 2000), and the efficiency of extraction can be influenced by both the chemical composition and morphological and structural characteristics of algae (Barbarino & Lourenço 2005).
4. Spectrophotometric methods such as Lowry and Bradford can be problematical for protein quantification (Crossman *et al.* 2000; Barbarino & Lourenço 2005). Thus analyses should involve macronutrients, and most authors recommend extracting protein before quantification.
5. There is considerable variation among algal species in the extent to which chlorophyte and rhodophyte starches are hydrolysed by fish amylases (Zemke-White & Clements 1999).

The view that detritus is a key dietary component for many of the grazing fishes on coral reefs is more widely accepted now than in 1998 (Crossman *et al.* 2001; Choat *et al.* 2002; Wilson *et al.* 2003; Crossman *et al.* 2005). This resource can contain higher nutrient levels than turfing algae (Crossman *et al.* 2001), and yet its composition and functional role in coral reef nutrient cycling remains poorly understood. The grazing fishes that appear to focus on detritus often constitute a dominant proportion of overall fish biomass (Wilson *et al.* 2003; Depczynski *et al.* 2007). Consequently, many of the fishes on coral reefs that are commonly categorized as herbivores, in the sense of ingesting large amounts of primary production, are in nutritional terms actually functionally distinct from herbivores. This distinction may underpin important differences between temperate and tropical reefs in both biotic composition and ecosystem processes, and should thus be taken into account in broad scale biogeographic comparisons (cf. Harmelin-Vivien 2002; Floeter *et al.* 2005; Tolentino-Pablico *et al.* 2008).

Work in terrestrial systems has shown that animals frequently ingest mixtures of different foods to achieve adequate nutrient intake. This so-called complementary feeding improves the balance of nutrients over the short term, and allows the animal to subsist on nutritionally imbalanced foods over prolonged periods and then select foods that restore nutritional balance (Raubenheimer & Jones 2006). One recent study suggests that complementary feeding may occur in a temperate girellid species that feeds on a mixture of algae and animal material, perhaps relying on the former for energy and the latter for protein (Raubenheimer *et al.* 2005). This strategy is perhaps driven by the distinctive amino acid

composition of algal proteins (Fleurence 1999; Barbarino & Lourenço 2005). One important implication of complementary feeding is that in some cases it makes little sense to rank foods in terms of their individual 'quality', because the value of a given food to the animal depends on the proportions and nutrient composition of other foods in the diet. Such interactions are best understood using multi-dimensional nutritional analyses (Raubenheimer *et al.* 2005; Raubenheimer & Jones 2006).

## DIGESTION AND ASSIMILATION

### *Digestive mechanisms defined*

Choat & Clements (1998) noted that digestion in marine herbivorous fishes was usually interpreted within the framework of the four non-exclusive 'digestive mechanisms' or gut types developed by Lobel (1981) and refined by Horn (1989). Under this scheme, digestion in different fish species is considered in terms of gut anatomy, stomach pH, pharyngeal morphology, and the presence of a fermentative hindgut microflora. The pervasive influence of this framework is illustrated by the suggestion by Targett & Arnold (1998) that 'the gut characteristics of marine herbivorous fishes are well-defined and categorized'.

The understanding of these four 'mechanisms' has improved considerably since 1998 (e.g. Zemke-White *et al.* 1999; Zemke-White *et al.* 2000; Mountfort *et al.* 2002; German & Horn 2006; Wainwright 2006). Recent authors have suggested that factors other than gut length, gut pH and microbial activity are required to understand digestion and diet choice (e.g. Targett & Arnold 2001). Karasov & Martínez del Río (2007) argued that we know little of the relative importance or phylogenetic distribution of these mechanisms, and even less about their functional relationship with particular algal resources. We agree, and add the caveat that the focus on what are largely morphological categories ignores the complexities of vertebrate digestion. Work to date suggests that alimentary anatomy is frequently not a reliable guide to functional capacity in herbivorous fishes (Choat *et al.* 2004; Horn *et al.* 2006), especially when comparisons involve unrelated species (German & Horn 2006). Furthermore, the focus on these simple categories tends to obscure the importance of parameters that are used to model digestive processes in other animals (see Parra 1978; Van Soest 1994; Sakaguchi 2003; Hummel *et al.* 2006; Clauss *et al.* 2007; Karasov & Martínez del Río 2007). We will explore these modelling parameters in the context of herbivorous fishes below, and in the remainder of this section we will examine some recent developments concerning the mechanical processing of food.

Wainwright, Westneat, Bellwood and colleagues have assembled a detailed body of work on the biomechanics and musculoskeletal functional morphology that underpins prey capture and handling in marine herbivorous fishes (see Wainwright & Bellwood 2002; Wainwright 2006; Westneat 2006). Patterns of approach, strike and recovery phases of the feeding sequence can differ between closely-related species that feed on different food types (Rice & Westneat 2005), and the coordination of motor systems (e.g. skull kinetics, locomotor

behaviour, oculomotor function, sensory input) has been shown to be an important predictor of feeding performance (Rice *et al.* 2008). Comparative studies have revealed an evolutionary wealth of feeding mechanisms in these fishes, for example, secondary lower jaw (intramandibular) joints that reduce the functional trade-off between bite force and the amount of substrate that can be covered during each bite have evolved at least five times (Konow *et al.* 2008).

An important contrast between the terrestrial and marine literatures on mechanical processing is that the former considers the plant diet (e.g. Clissold 2008), while the latter considers mainly the animal itself. There is little consideration in the marine literature of how different processing mechanisms interact with different sorts of herbivorous diets, and especially how different algal morphologies and cellular structures influence post-ingestive mechanical processing and digestion. It is not possible to discuss plant quality without some understanding of how food is initially processed (Clissold *et al.* 2006), and a more comprehensive approach requires information on both plant composition and the 'deeper' post-ingestive processes concerning digestion and assimilation. We agree with Wainwright and Bellwood (2002) that understanding the functional differences in feeding ability between species greatly enhances our ability to predict responses in unstudied systems, and believe that the functional work on prey capture and handling serves as a useful framework in which studies of post-ingestive mechanisms can be developed.

### Digestive enzymes

Since 1998 only a handful of studies conducted on temperate marine herbivorous fishes have examined the activity of endogenous digestive enzymes. Chan *et al.* (2004) and German *et al.* (2004) compared the activity of proteolytic, lipolytic and amylolytic enzymes in carnivorous and herbivorous pricklebacks. Their results suggested an influence of phylogenetic constraint on enzyme activity. Moran & Clements (2002) demonstrated the expected positive correlation between quantity of dietary storage polysaccharides and activity of their corresponding carbohydrases in two red algae-eating species, but not in two brown algae-eating species. In part these results must be seen in the context of the varying roles of the gastrointestinal microflora in these four fish species. More recently, the activities of both endogenous and exogenous carbohydrases were compared along the gut in these and three additional species (Skea *et al.* 2005, 2007). The results of Skea and colleagues suggested that the fish species examined fell on a continuum from maximizing gut throughput and digesting easily hydrolysed substrates via endogenous mechanisms in the foregut to relying more heavily on microbial fermentation in the hindgut. The role of gastrointestinal symbionts will be discussed in more detail in the following section.

### Role of symbionts

In our opinion, the functional and phylogenetic characterization of gastrointestinal microbial communities is among

the most significant development in the nutritional ecology of marine herbivorous fishes since 1998, although this work has concentrated largely on temperate species. Clostridia appear to be very important components in the gastrointestinal communities of most (Mackie *et al.* 2004; Moran *et al.* 2005; Clements *et al.* 2007) but not all (Fidopiastis *et al.* 2006) of the marine species examined, but the sensitivity of these results to variations in methodology (see Forney *et al.* 2004) suggests caution when comparing across different studies. Rates of fermentation in marine herbivorous fishes are only available for three temperate-water species (Mountfort *et al.* 2002), and were surprisingly high given the low ambient temperatures in which these ectotherms are found. In addition, the proportion of production of short-chain fatty acids (SCFA), that is, the end-products of gastrointestinal fermentation by microorganisms (Karasov & Martínez del Rio 2007), that is available for host uptake appeared very high, given that only a small fraction of SCFA is lost through methanogenesis, sulphate reduction and acetate oxidation (Mountfort *et al.* 2002). The contribution of algal structural polysaccharides to fermentation is unclear due to the low apparent activities in most symbiont extracts against these polymers, although carrageenan and agar appear to be important in some fish species (Skea *et al.* 2005, 2007). Gastric pepsin activity and low pH are known to degrade hemicelluloses bound in the cell walls of terrestrial plants, thus making substrates more easily fermentable when entering the hindgut (Parra 1978; Hummel *et al.* 2006). This may be important to the fermentation of algal structural polysaccharides in marine herbivorous fishes that exhibit low gastric pH, as well as the host-mediated (endogenous) hydrolysis of intracellular polymers (see Zemke-White *et al.* 2000). The sugar alcohol mannitol may be an important fermentation substrate in brown algae-eating fish species, especially given that mannitol fermentation by Clostridia is vastly more efficient at high pH conditions (Kazamias & Sperry 1995) such as those found in the hindgut of some of these fishes (Zemke-White *et al.* 1999).

Work on the adaptation of gastrointestinal communities to different diets is required for marine herbivorous fishes. In ruminants, high quality feeds induce high digestibility of both high and low quality substrates, but do not necessarily improve digestibility of very poor substrates (Gordon *et al.* 2002). Mixed diets can also reduce the effects of secondary compounds contained in individual diet components (Gordon *et al.* 2002). Thus diet mixing may also be an important factor in some marine herbivorous fishes, although diet breadth is probably greater in grazing than browsing species (Horn 1989; Choat & Clements 1998; Choat *et al.* 2002), and few grazing species display high levels of gastrointestinal fermentation (Crossman *et al.* 2005). The pathways of carbohydrate fermentation in bacteria such as Clostridia are complex and involve multiple proteins. Some phosphotransferase systems are constitutive, for example, glucose, while others are inducible (e.g. xylose Mitchell *et al.* 1995). This means that *in vitro* experiments only identify current patterns of substrate use, rather than potential patterns, and testing the latter requires an *in vivo*

approach. An interesting example of the latter involves the temperate hindgut fermenter *Hermosilla azurea*, which assimilated carbon, nitrogen and protein as efficiently or more efficiently from three species of non-dietary brown algae as from three species of dietary red algae and green algae (Sturm & Horn 1998).

Recent years have also seen work on gastrointestinal fermentation in other aquatic vertebrates, notably tadpoles (Pryor & Bjorndal 2005) and Galápagos marine iguanas (Mackie *et al.* 2004; Vitousek *et al.* 2007). These studies collectively reinforce the view that symbiotic fermentation can be important in animals that have relatively fast gut throughput times, do not maintain constant elevated body temperatures, and lack highly specialized alimentary structures, as is the case in insects for example (Douglas, 2009). The importance of these symbioses to some fishes with high levels of gastrointestinal fermentation is underscored by their ability to use SCFA in energy metabolism (Willmott *et al.* 2005).

This progress on gastrointestinal fermentation notwithstanding, we agree with Karasov & Martínez del Río (2007) that we still have only a very weak understanding of the contribution that gut symbionts make to the nutrition of their host fishes. Comparative metagenomic investigations of gastrointestinal communities (e.g. Ley *et al.* 2008) hold great potential for elaborating microbiota function in marine fishes, especially since these animals can be sampled from the wild thus retaining the evolutionary relationships between diet and host phylogeny.

#### Assimilation of nutrients

Compared to the previous decade, little work has been done in recent years on assimilation of nutrients in marine herbivorous fishes. Total protein amino acids, carbohydrate and lipid were assimilated at levels similar to their dietary proportions in fourteen algivorous, detritivorous, omnivorous and planktivorous species from the Great Barrier Reef, emphasizing the important differences in diet that exist among nominally 'herbivorous' species (Crossman *et al.* 2005). The importance of the post-ingestive processes of nutrient assimilation and regulation was also recently stressed in the context of understanding trophic pathways in ecosystems. MacArthur & Hyndes (2007) suggest that in temperate fishes seagrass may play a role that is not identified by isotopic analysis of fish tissue. This is isotopic routing (Karasov & Martínez del Río 2007; Wolf *et al.* 2009), where isotopes from a high protein source such as animals are incorporated while carbohydrates from a low protein source such as algae or seagrasses are catabolized directly for energy. Isotopic analysis in isolation may thus fail to identify the importance of plant diets to energy balance in fishes that metabolize carbohydrates directly, leading to an underestimation of the importance of plants in energy pathways. Nutrient regulation in marine herbivorous fishes, especially those ingesting mixed diets, promises to be a rewarding area for future research.

#### Food intake rate and gut retention time

Horn & Messer (1992) used a combination of the reactor theory of Penry & Jumars (1987) and the descriptive framework of digestive mechanisms of Horn (1989) to model digestion in representative species of marine herbivorous fishes. They predicted rates of digestion (reaction rate) as an increasing function of initial nutrient concentration (diet quality) and/or retention time. These models were thus comparable in theoretical terms to those developed for mammals to explore the relationships between gut capacity, body weight and retention time (e.g. Parra 1978; Clauss *et al.* 2007). In practical terms, however, the utility of the herbivorous fish models was limited due to the simplifying assumptions required to generate the input parameters. For example, initial nutrient concentration was estimated as percent of organic material, and thus fails to distinguish between assimilable and non-assimilable dietary organic components. Similarly, retention time was based on the retention time of large particles, which for several reasons may underestimate the time available for reactants to mix in the gut (see Clements & Raubenheimer 2006). Thus, while endorsing their general approach, we feel that considerable empirical progress is required before the models developed by Horn & Messer (1992) can be used to make realistic functional comparisons between species.

An approach that has had heuristic value in terrestrial studies is compartment models. In contrast with the gut reactor models adopted by Horn and Messer (1992), which are based on chemical reactor theory, compartment models describe digestive processes by the rate of flow into and out of a series of compartments (Yearsley *et al.* 2001). Although these modelling approaches are broadly equivalent, reactor models are based on detailed mechanisms of digestion, whereas compartment models have a more empirical formulation which allows a broad range of issues to be investigated without detailed parameterisation (Yearsley *et al.* 2001). A useful form of gut compartment model was introduced by Sibly (1981), and subsequently modified by Raubenheimer & Simpson (1996, 1998) to deal with several nutrients simultaneously.

Using data from a range of terrestrial vertebrate herbivores (mostly mammals), Parra (1978) showed that total gastrointestinal wet weight scales nearly isometrically with body mass ( $BM^{1.08}$ ). Clauss *et al.* (2007) suggested that this model had stood the test of time, and added that in mammals dry matter intake scales with body mass, and gut capacity scales nearly isometrically to body mass. Adaptation to different levels of fibre digestion in mammals meant that mean retention time was basically independent of body mass (Clauss *et al.* 2007). Almost nothing is known of these relationships in marine herbivorous fishes. Although Parra's predicted relationships between gastrointestinal mass and body mass (i.e.  $BM^{1.08}$ ) appear to hold in tadpoles (Pryor & Bjorndal 2005), it is unlikely that this will be the case in herbivorous fishes for a variety of instructive reasons. First, and most importantly, the scaling of skeletal mass to body mass is more variable in fishes compared to terrestrial vertebrates, and

appears to be related to modes of foraging and locomotion in water, whereas in terrestrial vertebrates it is driven by the requirements of resisting gravity (Berrios-Lopez *et al.* 1996). Second, the importance of symbiont-mediated digestion varies far more in marine herbivorous fishes than it does in mammalian hindgut fermenters (Sakaguchi 2003), as many fishes appear to be less reliant on exogenous digestion of refractory polysaccharides (Choat & Clements 1998; Choat *et al.* 2004, Crossman *et al.* 2005). This, in turn, is probably related to the enormous differences in dietary biochemical composition among marine herbivorous fishes compared to terrestrial mammals. The few fish species in which these gut mass relationships have been examined differed greatly in terms of the relationship between volume of the hindgut and body mass (Mountfort *et al.* 2002; Choat *et al.* 2004). Perhaps partly as a result, marine herbivorous fishes also vary greatly in retention time (Horn 1989; Choat & Clements 1998; Clements & Raubenheimer 2006), notwithstanding the limitations of the data available (see above). Variation in mean retention time in hindgut fermenting mammals is partly caused by selective retention mechanisms for fluid and/or large or fine particles, that is, colonic separation mechanisms (Sakaguchi 2003). Almost nothing is known about selective retention of digesta in marine herbivorous fishes. Third, food intake rates (which are obviously related to retention times) appear to vary greatly in fishes, especially since fishes such as some parrotfishes and surgeonfishes process large amounts of inorganic material each day (Wilson *et al.* 2003; Bellwood *et al.* 2004). As said previously, many nominally 'herbivorous' fish species are actually detritivores (Choat & Clements 1998; Crossman *et al.* 2001; Wilson *et al.* 2003), and thus will be subject to a different set of functional constraints than genuine herbivores (e.g. algivores). Addressing these empirical deficiencies in marine herbivorous fishes is a critical area for future research.

#### NUTRITIONAL ECOLOGY FOR HERBIVOROUS FISHES?

As a first step towards applying an integrative nutritional ecology framework to marine herbivorous fishes, Choat & Clements (1998) examined how a sample of nominally herbivorous species of reef fishes could be characterized in terms of gastrointestinal fermentation. It was partly as a result of this exercise, and in particular the pattern that it suggested in terms of food resource use, that we began to categorize species on the basis of nutrient intake. To reflect this we have split this section into two. The first examines how knowledge of gastrointestinal fermentation in marine herbivorous fishes compares to comparable groupings in terrestrial herbivores, and in the second we emphasize the importance of nutrients in generating broader categories for these fishes in terms of diet.

##### *Groupings via SCFA levels*

The size-specific constraints of metabolic rate on digestive strategy and diet choice have been explored in detail for

herbivorous mammals, in which herbivory is underpinned by gastrointestinal fermentation of plant cell walls (Van Soest 1994; Sakaguchi 2003; Karasov & Martínez del Río 2007). Because nutritional requirements scale with metabolic demand, and food intake is limited by gut capacity, small mammalian herbivores must eat highly concentrated diets of nutrients (Sakaguchi 2003). Small mammalian herbivores often get around nitrogen limitation with coprophagy (i.e. a protein gain from digesting hindgut flora), which is not an option for fishes living in a turbulent aquatic medium. Interestingly, many marine herbivorous fishes are carnivorous as juveniles (Horn 1989). The effects of body size on digestive strategy and diet have not been explored for marine herbivorous fishes except in an ontogenetic context, where there appears to be a shift from endogenous digestion to exogenous digestion with growth in some species (Moran & Clements 2002; Moran *et al.* 2005). Short retention times in small mammalian herbivores are believed to be due to the fact that the required fermentation time for digesta of low digestibility is too long to fulfil energy demand. Retention times in hindgut fermenters should be related to fermentation characteristics of ingesta, which are unknown for algal diets.

Some of the 'rules' concerning constraints on hindgut fermentation in small animals may be bent, if not broken, by terrestrial vertebrates (see Foley & Cork 1992). Folivory in the white-tipped plantcutter, a South American bird with a relatively short gut, involves maximizing cell content extraction through physical breakdown of cell walls, rapid gut transit, high activities of digestive enzymes, and selection of a highly nutritious diet (i.e. high protein) throughout the year (Rezende *et al.* 2001; Bucher *et al.* 2003). These traits make highly nutritious cell contents available to the white-tipped plantcutter without the capital and maintenance costs of anatomical structures for bacterial fermentation or slow digesta passage rates of herbivorous birds which rely extensively on fermentation of cell walls (Rezende *et al.* 2001). It is highly likely that many species of marine herbivorous fishes fall into this category, that is, the main substrates for fermentation are not cell wall polymers but refractory storage polysaccharides and poorly assimilated monomers such as mannitol.

Another 'rule' that may be broken in some terrestrial hindgut fermenters is that microbial protein be made available to the host, a phenomenon that is generally thought only to apply in foregut fermenters or hindgut fermenting caecotrophs (Foley & Cork 1992). For this to take place in non-caecotrophic hindgut fermenters requires (i) a mechanism to degrade components of the microflora *in situ*, and (ii) a mechanism whereby microbial amino acids or polypeptides are absorbed by the host across the wall of the hindgut. Achieving the former via the host secreting proteases or lysozymes into the hindgut would compromise the function of the gut flora (Karasov & Martínez del Río 2007). However, another possibility in vertebrate hindgut fermenters is that eukaryotic members of the gastrointestinal community ingest and degrade bacterial symbionts, thus releasing microbial amino acids and polypeptides into the hindgut lumen as excreta. An example of this may be the dense populations of nematodes

that inhabit the fermentation regions of the gut in many herbivorous reptiles (Bjorndal 1997). Little is known of the activities of nematodes in the hindgut communities of marine herbivorous fishes, but a possible analog are the very abundant predatory balantidid ciliates that inhabit the intestines of herbivorous surgeonfishes (Grim 1993). These ciliates appear to feed on the giant bacterial symbionts *Epulopiscium* sp. (Mendell *et al.* 2008), which are selectively digested but are non-selectively ingested along with other components of the gut flora (Grim 2006). Digestion in the ciliates appeared to be inefficient, resulting in the release of both intact and partially digested bacteria into the host lumen (Grim 2006). Hindgut fermenting vertebrates including rats, humans and pigs are known to have the capacity to assimilate amino acids across the wall of the hindgut (Torrallardona *et al.* 2003), and intact proteins have been shown to be absorbed in the posterior intestine of Antarctic fishes (Hernandez-Blazquez & da Silva 1998). The possibility that the gastrointestinal communities of marine herbivorous fishes could have a role in host protein metabolism could have profound impacts on our understanding of nutrient flux on reefs, especially given that components of these communities, for example symbiotic spirochaetes, are known to fix nitrogen in other animals (Lilburn *et al.* 2001). Characterizing the functional role of gastrointestinal communities in the great diversity of marine herbivorous fishes provides a major challenge for the future.

#### Groupings via nutrient levels

Interspecific variation in post-ingestive factors has important ramifications for developing dietary groupings for so-called generalist herbivores on coral reefs. Some clades of parrotfishes and surgeonfishes that have undergone recent diversification show little morphological differentiation in terms of jaw morphology and feeding behaviour (Bellwood 2003). However, these taxa may display species-specific differences in nutrient intake and assimilation which allow the co-existence of closely related species (see Crossman *et al.* 2005). This sort of differentiation has been suggested among terrestrial herbivores, where it has been described in the context of the nutritional niche, that is, the blend and ratio of nutrients that maximize fitness in a given species (Behmer & Joern 2008). To date, only one study has attempted to explain interspecific dietary variation in marine herbivorous fishes in terms of dietary macronutrient composition (Crossman *et al.* 2005).

Support for the importance of variation in post-ingestive factors in fishes is also provided by studies on isotopic and elemental ratios. The incorporation of interspecific data on assimilation efficiency, feeding rates and excretion into isotope ratio models was shown to improve precision in predicting dietary differences among three herbivorous marine species (Mill *et al.* 2007). Similarly, Hood *et al.* (2005) suggested that the use of generalized nutrient assimilation efficiencies may be a source of error for estimates of nutrient recycling both because of the variation between herbivorous species and the variation in food nutrient content. Further, when nutrient excretion by herbivorous fishes is substantial, the

N : P ratio recycled can determine the extent of algal nutrient limitation, and thereby influence algal community composition (Hood *et al.* 2005).

#### Recent paradigms

In this section we consider two areas that have developed since our review was published in 1998. We consider that these are worthy of detailed consideration here, for two reasons. First, they have represented significant and influential areas of activity within the general area of fish nutritional ecology. Secondly, these areas demonstrate how an integrative nutritional ecology perspective (or lack thereof) can influence conclusions in ecological research.

#### ROLE OF TEMPERATURE IN LATITUDINAL DIVERSITY GRADIENTS IN MARINE HERBIVOROUS FISHES

The view that low temperature limits the digestion of algae in marine fishes is now so widespread that it has almost become conventional wisdom (e.g. Harmelin-Vivien 2002; Floeter *et al.* 2005; Pérez-Matus *et al.* 2007; Mora 2008; Tolentino-Pablico *et al.* 2008). It is essentially a hypothesis of temperature constraint on the digestive processes of herbivorous fishes. This idea has underpinned the development of a substantial and diverse literature relating to ecological interactions among reef fishes, biogeographic patterns of diversity and abundance and the evolution of herbivorous fish faunas. A hypothesis of temperature constraint was originally suggested by Gaines & Lubchenco (1982) as one of several to explain what was then considered to be the 'rarity' of herbivorous fishes in temperate and polar waters, that is, (i) insufficient time for cold-adaptation, (ii) a constraint of low temperature on the digestion of algae, (iii) latitudinal differences in algal productivity, (iv) latitudinal differences in algal composition, and (v) a combination of these. Horn (1989) thoughtfully revisited the subject of latitudinal patterns in these fishes, and concluded that there was no evidence for hypotheses (iii) or (iv). However, the hypothesis of temperature constraint has had such an important recent impact on the direction of this field that we will discuss it in some detail here, with the caveat that the greater detail on the phylogenetic and biogeographic aspects will be presented elsewhere.

Two recent studies illustrate how the temperature limitation hypothesis has been used to develop ecological and evolutionary generalizations concerning herbivorous fishes. Harmelin-Vivien (2002) used a modification of the temperature limitation hypothesis to examine geographical distributional patterns in herbivorous fishes and the underlying evolutionary processes. Algae and detritus were identified as low quality food resources. Harmelin-Vivien suggested that digestion of plant material was more difficult than digestion of animal material, and was also limited by temperature. Harmelin-Vivien (2002) considered that 'more energetic food sources' are thus required to overcome the constraints of low temperature on food-processing rates, and that climatic stability was required for progressive adaptation to use low energy food

resources. The latter is equivalent to hypothesis (i) from Gaines & Lubchenco (1982).

Floeter *et al.* (2005) examined the relationships between latitude, diversity and abundance in marine herbivorous fishes using a data set based largely on new world literature records plus some original data. They concluded from their comparative distribution data and their consideration of potential explanatory hypotheses that 'temperature-related feeding and digestive processes are most likely involved in the distribution patterns of herbivorous fishes' (Floeter *et al.* 2005). These two studies illustrate the difficulties in attempting to develop ecological and evolutionary syntheses that capture the important elements of herbivorous fish distributional patterns and the processes that underlie these patterns. In order to develop a perspective that integrates physiological mechanisms and distributional and biogeographic patterns we need (i) a better understanding of digestive processes in herbivorous fishes and the impact of environmental variation on digestive mechanisms and rates, and (ii) a more comprehensive analysis of the distributional patterns and evolutionary relationships of cold water herbivorous species. For example, support for the view that digestion of plant material was limited by temperature (Harmelin-Vivien 2002) was canvassed from Ebeling & Hixon (1991). However, the latter study simply noted that temperature may limit the rate at which refractory plant material was degraded relative to the energetic needs of large and mobile fishes, but contained no details of the digestive processes themselves.

The quantitative analysis of Floeter *et al.* (2005) was based on census data from the depauperate fish fauna of the western Atlantic. However, there is a diverse fauna of herbivorous fishes in temperate waters of the southern hemisphere, and these fishes can achieve high local abundances in some locations (Hickford & Schiel 1995; Kingsford 2002; MacArthur & Hyndes 2007; Pérez-Matus *et al.* 2007; Castellanos-Galindo & Giraldo 2008). Even more striking is that algae are eaten selectively by at least four species of notothenioid fishes in Antarctica (Barrera-Oro & Casaux 1990; Iken *et al.* 1997; Barrera-Oro 2002; Casaux *et al.* 2003). It is thus clear that there are fishes functioning as herbivores at high latitudes. In addition, our understanding as to what constitutes herbivory by fishes in shallow reef environments has been compromised by misclassifications of diets in many species. For example, Floeter *et al.* (2005) included in their analysis of 'herbivores' abundant and diverse tropical taxa (e.g. surgeonfishes and parrotfishes) that are known to be detritivorous, and as a result their analysis includes species in which algal digestion is not the primary source of nutrition (e.g. Ferreira & Gonçalves 2006). In summary, we argue that in addition to a better understanding of digestive processes the analysis of biogeographic patterns in herbivorous fishes requires that we revisit the basic information on diet linked to a more comprehensive sampling of geographic distribution patterns in both hemispheres.

The constraint hypothesis is based largely on the claim that herbivorous ectothermic vertebrates may not be able to process enough food material to meet metabolic demands at cooler temperatures because gut passage rate (and hence

feeding rate) decreases more rapidly than metabolic rate as temperature declines (Floeter *et al.* 2005). Specifically, the critical temperature required for net positive energy balance is thought to be higher for herbivores than for carnivores because 'algae are relatively low-quality foods and harder to assimilate than animal material' (Floeter *et al.* 2005). Data on this are scarce, although the relationship between feeding rate and temperature was examined in two recent studies. Floeter *et al.* (2005) presented data on feeding and (estimated) metabolic rates in a surgeonfish at three different locations, noted that the former decreased with temperature more rapidly than the latter, and concluded that this suggested a temperature-related physiological constraint. Behrens and Lafferty (2007) examined the effects of different temperature and diet regimes using DNA : RNA ratios as a proxy for growth performance in the omnivorous opaleye. Opaleye exhibited greater 'performance' at low temperatures when their algal diet was supplemented with animal material, yet performed equivalently on different diet mixtures at warmer temperatures. Behrens and Lafferty (2007) concluded that the algal diets were inadequate for opaleye to meet nutritional demands at lower temperatures.

In our view, the nutritional implications of relative feeding rate cannot be interpreted without taking into account nutrient intake. Overall nutrient intake may have been relatively equivalent across the sites examined by Floeter *et al.*, despite the differences in feeding rate, due to variation in factors such as algal biomass (hence the amount procured per bite) or nutrient levels in the algae. Both biomass and nutrient content of dietary algae can be negatively correlated with temperature (Vitousek *et al.* 2007), a pattern that could also explain the negative relationship between mean feeding rate and algal productivity reported by Smith (2008). Behrens and Lafferty (2007) lacked a balanced design in terms of nutrient intake: the algal diet was available *ad libitum* across the temperature treatments, but the animal diet was limited to a set intake level. It is likely that proportionately more algae was eaten at warmer temperatures simply because increased intake was required to satisfy elevated metabolic requirements, and opaleye were limited in their intake of animal material. Our alternative interpretation is consistent with Norris (1963), who found that opaleye grew better in lower temperature treatments and failed to reproduce at temperatures warmer than those at which Behrens and Lafferty (2007) claimed a constraint on algal digestion. Reconsidering these two studies from a nutritional perspective thus casts doubt on their claim that a constraint of temperature on the digestion of algae limits the distribution of herbivorous marine fish species. A critical deficiency with these studies is the failure to recognize that these fishes, like other animals, feed to satisfy nutrient demands, and thus the relationships between feeding rate and temperature across species, seasons and locations are confounded by variation in nutrient demand and intake.

We believe that the perceived relationship between gut passage rate and temperature in herbivorous fishes has been unduly influenced by some of the literature on temperature constraint in terrestrial ectotherms, despite important



differences between the terrestrial and marine systems. One difference is that hindgut fermentation is critical to digestion in all herbivorous lizards (Bjorndal 1997), but to a varying extent in marine herbivorous fishes (Mountfort *et al.* 2002; Skea *et al.* 2007). Furthermore, data on the influence of temperature on digestion in terrestrial herbivorous reptiles are somewhat equivocal. Several studies show that body temperature in various herbivorous reptiles greatly affected gut transit time, but had little influence on digestive efficiency (e.g. van Marken Lichtenbelt 1992; Wikelski *et al.* 1993; van Marken Lichtenbelt *et al.* 1997). Schall & Dearing (1994) showed that herbivorous Bonaire Island whiptail lizards did not raise their body temperature after feeding on plants, and had a lower body temperature than carnivorous conspecifics. In contrast, other studies carry the implicit or explicit view that low temperature constrains the hindgut fermentation on which these herbivores depend (e.g. Espinoza *et al.* 2004; Vitt *et al.* 2005; Tracy *et al.* 2005). Most of these studies cite Janzen (1973), a paper on insect ecology but which includes the following statement on herbivorous *Ctenosaura* lizards: 'I assume here that basking is necessary for a poikilothermic vegetarian; it must maintain the internal compost heap at a high temperature to process foliage fast enough to get the energy needed.' Most herbivorous lizards require behavioural shifts to maintain a constant temperature throughout the day (Tracy *et al.* 2005; Vitt *et al.* 2005), and a constraint of temperature may also involve an inability to undergo voluntary hypothermia in periods of food shortage (Tracy *et al.* 2005).

There is thus only weak, indirect evidence for a direct constraint of temperature on plant digestion in herbivorous lizards (reviewed by Bjorndal 1997), and most of that applies to passage rate, not the breakdown of plant material *per se*. Part of the problem is the great difficulty of conducting experiments that disentangle the general effects of temperature on lizard metabolism and activity from the specific effects of temperature on digestive processes. The constraint (if there is one) of temperature on plant digestion may not be absolute, but rather the degree to which temperature varies. Given adequate substrate concentrations, rapid degradation of polysaccharides by consortia of anaerobic microorganisms can occur even at low temperatures (Arnosti *et al.* 1998; Pomeroy & Wiebe 2001; Arnosti 2004). Heterotrophic bacteria, such as gastrointestinal symbionts, can thus adapt to different temperature conditions given time (Pomeroy & Wiebe 2001), but obviously herbivorous reptiles are subject to diurnal and probably seasonal fluctuations in temperature (e.g. Schall & Dearing 1994) that subtropical marine fishes are not (Mountfort *et al.* 2002).

The functional viability of gastrointestinal communities in the face of temperature change involves the optimization of fermentation processes over both evolutionary and ecological timescales, and its relationship to the metabolic demands of the host herbivore (Bjorndal 1997). Evolutionary change in gastrointestinal communities would involve competitive interactions between microbial species (Nedwell 1999) and selection on variation in enzyme performance within species

(Arnosti *et al.* 1998). Change over ecological timescales can involve acclimation through differential expression of isozymes with different temperature sensitivities (Arnosti & Jørgensen 2003), or seasonal shifts in microbiota composition (Thompson *et al.* 2004). Interestingly, extracellular hydrolysis of polysaccharides by microbial communities is not necessarily the rate-limiting step in breakdown: hydrolysis can be faster than microbial uptake of oligosaccharides (Russell 1985; Arnosti *et al.* 1994; Fields *et al.* 2000). Fermentation rate can be limited by surface area for cell attachment (Fields *et al.* 2000), thus not limited by enzyme kinetics, indicating that temperature may not be absolutely limiting.

Adaptation or acclimation by gastrointestinal communities is likely to be far less of a problem for marine herbivorous fishes than terrestrial herbivorous reptiles for several reasons that are, in our view, central to this debate. First, fish are buffered by the relative thermal stability of marine environments, and thus do not carry the energetic burden of behavioural thermoregulation. Second, most herbivorous lizards are more susceptible to predation by endotherms than their marine vertebrate counterparts, especially when confronted by the requirements of behavioural thermoregulation in cool environments. Third, consistent food availability is more of a problem for herbivorous lizards than marine herbivorous fishes in temperate latitudes, where algal biomass is generally very high throughout the year (Gaines & Lubchenco 1982). Fourth, marine algae are very different in biochemical composition to terrestrial plants (Choat & Clements 1998), and thus the substrates for fermentation differ fundamentally between systems. There are thus many reasons why constraints of temperature on plant digestion by ectothermic herbivores may differ between terrestrial and marine environments.

In summary, the suggestion that a 'threshold temperature' for algal digestion may control the latitudinal distribution of marine herbivorous fishes (e.g. Floeter *et al.* 2005) suggests that generalizations about biogeographic patterns can be derived simply from examining the effects of temperature on consumption and metabolism. This ignores (i) that animals and microorganisms adapt over evolutionary and ecological timescales; (ii) that tests with different temperature treatments cannot segregate the many interacting effects of temperature on physiological and ecological function, and interpreting them as if they do is misleading and an inappropriate use of experimental inference (Bernardo & Reagan-Wallin 2002); (iii) that other factors are involved, for example, larval dispersal, the effects of low temperature on reproduction, susceptibility to predation, etc; and (iv) that worldwide generalizations about patterns and processes should be avoided because of the evolutionary and historical distinctiveness of different hemispheres and ocean basins (Wainwright & Bellwood 2002; Valdovinos *et al.* 2003; Castellanos-Galindo & Giraldo 2008; McDowall 2008). We agree with Floeter *et al.* (2005) that the relationships between consumption and metabolism in marine herbivorous fishes require study, but this should happen before, not after, we come to conclusions about the processes that are driving the distribution patterns we see.

## THE IMPACT OF HERBIVOROUS FISHES ON CORAL REEFS

The two prevailing assumptions concerning the current status of coral reefs are that a functionally viable coral reef must have a certain level of live coral cover, and that this is maintained by a minimum level of grazing by fishes, especially parrotfishes (Hughes *et al.* 2003; Bellwood *et al.* 2004; Mumby 2006). The focus on ecological impacts has had three important consequences for the study of trophic biology in marine herbivorous fishes. First, it has directed attention towards estimating algal removal rather than developing an understanding of the nutritional processes involved. Second, it has encouraged research at the level of functional groups, rather than individual species. Third, it has led to the assertion that all members of grazing functional groups are herbivores. We identify the following problems with this approach.

The focus on algal removal has led to the widespread use of what has been referred to the 'cyclones as herbivores' definition, where 'herbivores are defined by their ability to remove algal biomass, regardless of the actual nutritional target of grazing' (Smith 2008). This contrasts with the functional approach to herbivory adopted by most terrestrial studies (see Karasov & Martínez del Río 2007). In the absence of information on nutritional processes it has proved difficult to evaluate the passage of carbon through reef ecosystems, or to compare rates and patterns of processing of plant biomass between different ecosystems.

Assemblages of grazing fishes are diverse and display a variety of nutritional strategies with species-level differences in diets and foraging patterns. Experimental and comparative approaches have not identified species-level patterns of interaction with the sessile benthic biota. Moreover, the literature has been contradictory with respect to functional groups. Meta-analyses of grazing fishes generally quantify the impact of grazing at the level of functional groups (e.g. Hughes *et al.* 2003; Bellwood *et al.* 2004), while other studies argue for the importance of species-specific differences (e.g. Bellwood *et al.* 2003; Fox & Bellwood 2007). Available information suggests that fish species differ in their quantitative impacts on algal communities (e.g. Fox & Bellwood 2007), and therefore that a species-level approach is required.

Members of the grazing fish fauna are collectively identified as herbivores (e.g. Floeter *et al.* 2005). While some members target, process and assimilate living plant material, others, including the largest and most abundant representatives, scavenge protein from a variety of sources including sessile animals. There is mounting evidence that parrotfishes may not only remove large amounts of living coral (Bellwood *et al.* 2003), but through grazing scars predispose coral to infection by bacteria and ciliates, especially under conditions of environmental stress (Rotjan & Lewis 2006; Rotjan *et al.* 2006).

A cornerstone of the terrestrial literature on herbivory is the role of the nutritional quality of plants in determining feeding impacts and herbivore dynamics. This aspect is generally neglected in coral reef studies. For example, in a

thorough empirical treatment of grazing impacts across a depth gradient, Fox & Bellwood (2007) listed the following hypotheses to explain why grazing pressure by herbivorous species differed among habitats: costs of dealing with wave energy, tidal access, effect of territorial damselfishes, and lack of refuges from predation. The first three of these were dismissed as unlikely, whereupon algal productivity and nutritional quality were mentioned only secondarily. In contrast, a comprehensive review of grazing in seagrass systems (Valentine & Duffy 2006) highlighted the importance of nutritional factors: 'Compared to the well-studied role of terrestrial plant nitrogen content on herbivore food selection, the impact of seagrass leaf nutritional quality in determining food selection in seagrass-dominated ecosystems remains inadequately investigated.' In these systems epiphyte composition interacts with seagrass leaf nitrogen content to influence herbivore foraging choices in a way that can have profound ecological consequences. Most mesograzers prefer to eat epiphytes rather than seagrasses or macroalgae, and thus can have a considerable impact on seagrasses and macroalgae if preferred algal foods are exhausted (Valentine & Duffy 2006). Similarly, the ecological impacts of herbivorous fishes can be influenced by nutritional factors. For example, although assimilation efficiency in the parrotfish *Sparisoma radians* is lower on diets containing extract from the calcareous alga *Halimeda* than on untreated diets, the fish can compensate for the effects of the extract on net energy gain by increasing feeding rate (Targett & Targett 1990). This example shows how a consideration of post-ingestive processes greatly improves the ability to predict potential ecological impacts on different food resources.

The focus on algal removal at the expense of nutritional ecology has limited our understanding of the flow of carbon through reef ecosystems, and has inhibited the development of a currency by which we can compare rates and patterns of the trophodynamic processing of plant material between ecosystems. A functional approach enables herbivory to be modelled and understood, that is, to benefit from the power of deduction, whereas approaches that are non-functional are limited to the inductive process of simulation and forecasting (Raubenheimer & Simpson 2003).

## Conclusions

Much of the work on trophic biology in marine herbivorous fishes since 1998 has focused on the ecological impacts of fishes on reefs, the morphological and mechanical aspects of ingestion, and the deterrent properties of the plants. Moreover, two of the elements necessary for an integrative understanding of nutritional ecology, that is, food composition and the physiological processes involved in nutrient extraction and utilization, have been neglected in the study of herbivory in marine fishes. Collectively, these factors have hindered the development of a nutritional framework for analyzing food resources, feeding patterns and evolutionary trends, an approach that has proved successful in the study of terrestrial vertebrate herbivores, especially mammals and insects (see Karasov & Martínez del Río 2007).

In this context it would seem that our 1998 review had little effect on the field. However, three lines of argument suggest that we were on the right track. First, the progress made since 1998 in the study of herbivory in other systems clearly shows the benefits of an explicitly nutritional approach (e.g. Raubenheimer & Simpson 2003; Hummel *et al.* 2006; Clauss *et al.* 2007; Karasov & Martínez del Río 2007; Clissold 2008). Second, the algal secondary metabolite and reef grazing paradigms, while both extremely productive in their own right, have nonetheless failed to develop the clear predictive framework for diet choice required in broader contexts such as reef management and understanding the evolution of herbivory. Third, the lack of a focus on nutritional factors has led to premature conclusions on the influence of temperature on the digestion of algae, both at the level of digestive processes and the biogeography of marine faunas.

Predictive understanding of coral reef trophodynamics and the biogeographic distribution of marine herbivory must grow from analysis of the nutritional ecology of the dominant grazers, marine herbivorous fishes. This is especially relevant in the face of climate change. Achieving this goal will involve a balance between paying attention to what has worked elsewhere (e.g. with terrestrial herbivores) and identifying the critical differences between marine and terrestrial systems. What we do know is that some marine herbivorous fishes may be bending the 'rules' of herbivory, especially with respect to temperature, gut architecture and substrates for hindgut fermentation, and so the study of nutritional ecology in these animals has great potential to generate novel insights for the field of vertebrate nutrition in general.

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