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## Gut characteristics and assimilation efficiencies in two species of herbivorous damselfishes (Pomacentridae: *Stegastes dorsopunicans* and *S. planifrons*)

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**Abstract** The morphological and physiological mechanisms by which marine herbivores assimilate energy and nutrients from primary producers and transfer them to higher trophic levels of reef ecosystems are poorly understood. Two wide-ranging Caribbean fishes, the dusky damselfish, *Stegastes dorsopunicans*, and the threespot damselfish, *S. planifrons*, defend territories on patch reefs in the Archipelago de San Blas, Republic of Panama. We examined how relative intestine length and retention time influence digestion and absorption of energy and nutrients in these fishes. The dusky damselfish has a relative intestine length (RIL = intestine length/standard length) of 1.2 and a Zihler index { $ZI = \text{intestine length (mm)} / 10[\text{mass(g)}^{1/3}]$ } of 3.4. These values are significantly lower ( $P_{RIL} = P_{ZI} < 0.0001$ ) than those for the threespot damselfish (3.0 and 8.2, respectively). Both RIL and ZI for both species fall well below previously published values for other herbivorous pomacentrids, and may reflect their primary food resource at San Blas (diatoms). Energy-rich diatoms may be easier to digest than refractory macroalgae characteristic of diets of many herbivorous fishes (RIL range: 2–20). Despite differences in RIL and ZI between these two species, gut retention time is the same ( $P > 0.05$ ) for both dusky (6.6 h) and threespot damselfish (6.5 h). Thus, food travels the length of the threespot damselfish intestine ~2.5 times faster than it does in the dusky damselfish intestine. Levels of protein, carbohydrate, and lipid are significantly ( $0.003 < P < 0.030$ ) higher in

the feces of dusky damselfish than in the feces of threespot damselfish, when both species were fed a natural diet of benthic diatoms collected from damselfish territories. This indicates threespot damselfish have a greater nutrient-specific and total assimilation efficiency than do dusky damselfish. Furthermore, when fed an artificial pellet diet, protein absorption efficiency differed significantly ( $P = 0.014$ ) between species; threespot damselfish absorbed 98.3% of dietary protein, whereas dusky damselfish absorbed 96.4% of dietary protein.

### Introduction

As consumers of reef primary production, herbivores occupy a pivotal role in the transfer of energy and nutrients in reef ecosystems (Horn 1989) and contribute significantly to nutrient cycling on reefs (Polunin and Koike 1987). The rates at which primary production is consumed, digested, and absorbed by herbivores are important parameters in understanding energy and nutrient dynamics in these systems. In many reef systems, fishes are the major herbivores (Polunin 1988). Various aspects of the ecology of herbivorous reef fishes have been well documented, e.g. feeding habits (Brawley and Adey 1977; Hixon and Brostoff 1983), social and reproductive behavior (Knapp et al. 1995; Robertson 1996), population structure (Sale 1991), and their effects on the reef community (Choat 1991; Jones et al. 1991). Less attention has been directed at physiological mechanisms underlying digestion and assimilation of primary production (e.g. Benavides et al. 1994a,b; Ojeda and Caceres 1995; Choat and Clements 1998; Sturm and Horn 1998).

Herbivorous fishes are abundant on reefs (Bakus 1969; Ogden and Lobel 1978), and many species assimilate a significant proportion of the nutrients they ingest. The blue angelfish, *Holocanthus bermudensis*, absorbed 72% of the carbon and 85% of the nitrogen from its green algal diet (Menzel 1959). The jewel damselfish,

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*Plectroglyphidodon lacrymatus*, absorbed 56% of ingested carbon and 83% of ingested nitrogen from its diet of benthic algae (Polunin 1988). Two Gulf of California damselfishes, *Microspathodon dorsalis* and *Stegastes rectifraenum*, absorbed 57–67% of protein, 46–56% of lipid, and 37–44% of the carbohydrate from algae eaten in the field (Montgomery and Gerking 1980). The damselfish *Stegastes apicalis* absorbed > 50% of dietary protein from a mixed algal diet on the Great Barrier Reef (Klumpp and Polunin 1989). The temperate herbivore *Cebidichthys violaceus* assimilated 43–81% of protein, 21–44% of lipid, and 31–52% of carbohydrate from a diet of red and green algae (Edwards and Horn 1982).

The proportion of primary production absorbed by herbivores may, in part, be a function of intestine length and retention time. In fishes, intestine length has been correlated with diet (Kramer and Bryant 1995a,b). For example, relative gastrointestinal length (ratio of gastrointestinal length to standard length) typically ranges from 0.5–2.4 for carnivores, 0.8–5.0 for omnivores, and 2–21 for herbivores (Al-Hussaini 1947; Kapoor et al. 1975). Although some herbivorous fishes have relative intestine lengths < 2.0, these fish species usually possess an elongate, rather than deep-bodied, shape (e.g. Gobiidae, Stichaeidae, Hemiramphidae) or they are stomachless (e.g. Odacidae, Scaridae). Increased intestine length accompanies the ontogenetic shifts from carnivory to herbivory [e.g. odacids (Clements 1985), pomacentrids (Lassuy 1984), and stichaeids (Montgomery 1977; Barton 1982)] and may correlate with increased assimilation efficiency (Lassuy 1984; Benavides et al. 1994a,b).

In the present paper we examine relationships among gut morphology, retention time, and assimilation efficiency for the dusky damselfish (*Stegastes dorsopunicans*) and the threespot damselfish (*S. planifrons*). These fishes hold territories on shallow reefs throughout the Caribbean (Randall 1967; Waldner and Robertson 1980; Robertson 1984). In the San Blas Archipelago, Republic of Panama, both species feed almost exclusively on benthic diatoms within their territories (Robertson and Gaines 1986). This diet choice does not appear to be seasonal as fish were reliably seen actively, and exclusively, feeding on diatoms in both wet and dry seasons (Clifton 1995; Cleveland, unpublished data). Specifically, we determined whether intestine lengths differed between the species and how these differences might affect both retention time and assimilation efficiency of ingested nutrients. Because the roles of diatoms, ubiquitous in marine environments, in the diets of herbivorous fishes remain largely unexplored, we also sought to examine the relationships among quality of diet, degree of digestibility, and gut parameters. We examined both the retention time and assimilation efficiency of naturally occurring diatoms from territories of both dusky and threespot damselfish; we also examined these parameters when fish were fed nutrient-rich, easily digested artificial pellets.

## Materials and methods

### Collections

Fish were collected from the shallow reefs of the San Blas Archipelago, Republic of Panama, from February to June 1996. We selected dusky damselfish, *Stegastes dorsopunicans*, that fell between 40 and 60 mm standard length and threespot damselfish, *S. planifrons*, that fell between 50 and 70 mm standard length. Dusky damselfish are approximately 10% smaller than threespot damselfish, and these ranges permitted the greatest degree of size overlap for interspecific comparisons. Fish that met these criteria were captured haphazardly from the reefs. A total of 124 dusky and 128 threespot damselfish were collected for relative intestine length (RIL) and Zihler index (ZI, see below, Zihler 1982) measurements. To determine if differences in RIL and ZI exist between empty and full intestines, 46 dusky and 50 threespot damselfish were collected using microspars and were dissected (see below) within 1 h of capture. Of these fish, 11 of the dusky and 20 of the threespot damselfish were collected at 0600 hours, prior to the onset of feeding (empty intestines), and another 14 dusky and 17 threespot damselfish were collected at 1600 hours (full intestines). The balance of collected fish ( $n=21$  dusky, 13 threespot) were collected haphazardly during the day to establish intestine lengths at various levels of fullness; intestine diameters were also measured in these fish (see below).

To determine retention time and assimilation efficiencies, 47 dusky damselfish and 51 threespot damselfish were captured live and housed in individual aquaria. These fish were assigned to one of three dietary groups (pellet, tile, and string; described below) and were acclimated to that diet for at least 10 days prior to measurements of retention time and assimilation efficiency. An additional 31 dusky and 27 threespot damselfish were captured and maintained in aquaria on diatoms. Neither retention time nor assimilation efficiency was determined for these fishes; only RIL and ZI were determined for these specimens.

### Diets

To examine the influence of diet quality on retention time and assimilation efficiency, we held fish in individual aquaria and fed them one of three diets: artificial pellets (Tetra DoroMarin Sinking Pellets), diatom tiles, or diatom strings. The artificial diet consisted of red and green pellets with an average ash-free dry weight (AFDW) of 45.8% protein, 49.4% carbohydrate, and 4.7% lipid (analytical methods described below). There were no significant differences (ash:  $Z=-0.58$ ,  $P=0.58$ ; AFDW protein:  $Z=-1.15$ ,  $P=0.25$ ; AFDW carbohydrate:  $Z=-1.14$ ,  $P=0.15$ ; AFDW lipid:  $Z=-0.00$ ,  $P=1.00$ ) in specific nutrients between the red and green pellets (Table 1). Their color was retained in the feces and served as a marker to signify a food bolus had moved through the length of the gut.

Diatom tiles were 6 cm×6 cm ceramic tiles, which were placed in individual dusky damselfish territories at least 6 weeks prior to the beginning of the feeding experiments. Diatom strings were 30 cm lengths of cotton string attached to a masonry nail (four strings per nail) at one end and suspended by a small styrofoam pellet at the other end. The nails were embedded in coral rubble within territories. We placed these strings into individual threespot damselfish territories 6 weeks prior to the beginning of the feeding experiments. The tiles and strings were colonized by benthic diatoms naturally occurring in dusky and threespot damselfish diets; microscopic examination indicated the same three diatom genera that were dominant in stomachs (*Asterionella*, *Biddulphia*, *Nitzschia*) were dominant on tiles and strings (a more complete list of diatoms present in dusky and threespot damselfish diets is provided by Robertson and Gaines 1986) and represented over 90% of the samples. We considered tiles and strings ready for use in feeding experiments when territory residents were observed feeding on them.

To establish diet composition and test for equivalence between strings and tiles, we analyzed composition of diatoms removed from those substrata. Ten tiles were removed from dusky

**Table 1** Composition of artificial pellet diet and two natural diatom diets (strings and tile); nutrient content (protein, lipid, carbohydrate) was converted to ash-free dry weight values. Dif-

ferences in ash content and nutrient content (ash-free dry weight) were analyzed using two Mann–Whitney *U*-tests, one each for the pellet types and diatom types; data are means  $\pm$  SE

Diet type	<i>n</i>	Ash (%)	Ash-free dry weight		
			Protein (%)	Lipid (%)	Carbohydrate (%)
Red pellet	10	8.8 $\pm$ 0.5	48.6 $\pm$ 4.3	4.7 $\pm$ 0.5	46.6 $\pm$ 4.2
Green pellet	10	8.4 $\pm$ 0.5	43.0 $\pm$ 1.9	4.8 $\pm$ 0.4	52.2 $\pm$ 2.2
		<i>P</i> = 0.56	<i>P</i> = 0.24	<i>P</i> = 1.0	<i>P</i> = 0.15
Diatom string	6	98.8 $\pm$ 0.1	10.9 $\pm$ 1.3	55.1 $\pm$ 3.9	46.8 $\pm$ 2.6
Diatom tile	8	99.6 $\pm$ 0.1	4.6 $\pm$ 1.2	48.6 $\pm$ 3.3	34.0 $\pm$ 2.9
		<i>P</i> = 0.002	<i>P</i> = 0.01	<i>P</i> = 0.03	<i>P</i> = 0.01

damsel fish territories, brought to the surface, and scraped carefully to remove diatom growth. Ten strings were placed in a 3-l circular cylinder, brought to the surface, placed into a seawater-filled plastic bag, and agitated gently until the diatom filaments were shaken loose from the strings. The strings were then removed from the bag. After the diatoms had settled to the bottom of the bag, the excess water was decanted. Diatoms from both the strings and tiles were then rinsed thoroughly with distilled water, and lyophilized.

#### Measurements of the intestine

The intestine was removed by cutting at the pylorus and anus, uncoiled and measured (mm) without stretching. We measured the diameter (mm) of full intestines (*n* = 21 dusky damselfish, 13 threespot damselfish) at 20%, 40%, 60%, and 80% of the total intestine length. We also calculated the average intestinal circumference, using the diameter measurements, which we then multiplied by intestine length to determine gross surface area. These measurements provided a surface area estimate to detect broad qualitative trends in morphometrics (Montgomery 1977); possible differences in intestinal epithelium were not examined in this study. We estimated intestine volume as the volume of a cylinder with mean intestine diameter and measured intestine length.

We calculated RIL by dividing intestine length (mm) by standard length (mm). Threespot damselfish were significantly heavier (*n* = 100 fish per species, signed rank test, *P* > 0.05) and more robust than dusky damselfish of the same length; Kramer and Bryant (1995a) suggest that mass is a more appropriate measure than length for interspecific comparisons of intestine length allometry. For this reason, we also calculated Zihler's index {Zihler 1982; ZI = intestine length (mm)/10[mass(g)<sup>1/3</sup>]}, which relates intestine length to mass rather than standard length.

#### Gut retention time

Fish were held in individual 50-l aquaria for at least 10 days and fed either diatom strings (*n* = 6 dusky, 8 threespot damselfish), diatom tiles (*n* = 6 dusky, 9 threespot damselfish), or artificial pellets (green pellets only, *n* = 14 dusky, 13 threespot damselfish). Fish were fed to satiation twice daily, and uneaten food was removed; fish that did not eat after 3 days were not included in any of the measurements and are not included in the sample numbers above.

Once acclimated to the diets, fish were fed ten red marker pellets. For fish on the artificial green pellets, red marker pellets were fed at 1000 hours, and fish were checked every 30 min for the presence of the red marker in the feces. Gut retention time for fish fed the artificial diet was defined as the amount of time elapsed between feeding of the red pellets and the appearance of red marker in the feces.

For fish on the diatom diets, red marker pellets, rather than diatom tiles or strings, were given to the fish the evening (1600 hours) prior to the gut retention experiments to insure that food eaten prior to the red pellets had been egested. The red pellets thus served as a marker to delineate diatom meals taken before and after ingestion of the red pellets. These fish were then fed either diatom tiles or strings at 1000 hours the next morning and checked every 30 min for feces containing diatom frustules. Retention time

was defined as elapsed time between ingestion of diatoms and the appearance of frustules in the feces.

Fish captured and dissected early in the morning (~0600 hours) retained some of the food consumed the previous day in the posterior intestine. This was also seen in the aquarium; red pellets consumed at 1600 hours typically did not appear as feces until ~0800 hours the next day. We used this as an indication that all diatom frustules consumed at 1000 hours the previous day had been egested. Pilot studies verified that no diatoms consumed prior to the ingestion of the red pellets were egested subsequent to the egested marker pellets (*n* = 2 fish per species). Because gut retention time can be significantly affected by starvation (Montgomery and Pollak 1988), there was no interruption of the feeding protocol other than feeding the fish the red pellets.

#### Assimilation efficiency

Total and nutrient-specific assimilation efficiencies were estimated by comparing nutrient concentrations in the feces relative to those in the food samples using ash as an assumed non-absorbed marker (Montgomery and Gerking 1980; Horn and Neighbors 1984; Sturm and Horn 1998). Although absolute assimilation efficiencies may be underestimated if ash is absorbed (Galletto and Bellwood 1994), this study was focussed on comparing assimilation between two species, and this error was, thus, acceptable for our purposes. Assimilation efficiencies were calculated following the equations of Montgomery and Gerking (1980).

Assimilation efficiency was measured directly only for fish fed the artificial pellet diet. We compared composition of food and feces relative to assumed non-absorbed ash levels. Ash levels of natural foods (diatoms from strings and tiles) often exceeded values in feces, making them useless for estimates of assimilation efficiency, because their use would lead to negative assimilation values. Rather, we measured nutrient levels remaining in feces as an indirect measure of relative assimilation for the two fish.

We collected feces from individual fish prior to feeding every morning (~0830 hours) and afternoon (~1530 hours) for five consecutive days (Edwards and Horn 1982; Galletto and Bellwood 1994; Sturm and Horn 1998). Destructive sampling of fish for intestinal contents would have interfered with retention time experiments, thus we are unable to assess any leaching of nutrients from fecal material; however both species were held, and samples were taken, under the same conditions. Feces appeared as cohesive, solid strings that were removed from aquaria by suctioning with a disposable Pasteur pipette attached to a thin, flexible tube. Feces were rinsed in distilled water, the water pipetted off, and the feces dried to constant weight at 40°C in a butane cooking oven. At the end of 5 days, samples were frozen (-20°C) and stored in airtight centrifuge tubes until analyzed for ash and specific nutrient content.

#### Chemical analyses

##### Ash

We determined ash content by burning samples of food or feces (~15 mg) in open crucibles in a Thermolyne model 1400 muffle

furnace at 500°C for 1 h. Pilot samples of food and feces ( $n=3$  for each food type and the feces) combusted for a total of 12 h experienced no significant weight loss (repeated measures ANOVA,  $P=0.43$ ) after the first hour. Weights of initial samples and ash were determined to 0.01 mg on a Sartorius R-160-P balance.

#### Protein

Protein was measured by a modified Lowry technique (Markwell et al. 1978) after powdered samples of food or feces (~15 mg) were digested for 12 h in 1 N NaOH. Triplicate aliquots of 0.5 ml were added to sterile glass cuvettes to which 1.5 ml of cupric sulfate in a potassium-tartrate solution was added. The reagent was added to sequential cuvettes at 15 s intervals, and the reaction allowed to proceed for 15 min; 0.15 ml of folin-Ciocalteu phenol reagent:distilled water (1:1, v:v) was then added. After 45 min, protein concentration was estimated on a Milton Roy Spectronic 301 spectrophotometer at 660 nm relative to a bovine serum albumin standard curve.

#### Carbohydrate

We used the phenol-sulfuric acid method (Dawes 1981) to estimate carbohydrate concentration in food and feces. Samples (~15 mg) were placed in borosilicate test tubes and heated in 2.5 ml 5% trichloroacetic acid at 90°C for 3 h. After cooling, triplicate aliquots of 0.2 ml were mixed with 2 ml 2.5% phenol, followed by charring with 5 ml of concentrated sulfuric acid. Absorbance was read at 485 nm relative to a glucose standard curve.

#### Lipid

Total lipid concentration was estimated colorimetrically. Food or fecal samples (~10 mg) were added to 4 ml of 2:1 (v:v) chloroform:methanol in borosilicate tubes and mixed completely; 0.8 ml of 0.7% (w:v) NaCl was then added, and the tubes were mixed using a vortex mixer. After centrifugation (5 min at 2200 rpm), the top layer was pipetted off and discarded. Triplicate aliquots of 0.5 ml were dried under a stream of methane after which 2 ml concentrated sulfuric acid was added. The samples were then heated at 200°C for 15 min. After cooling, 3 ml of distilled water was added to the tubes. The samples were mixed just prior to reading; lipid concentration was estimated at 375 nm relative to a cholesterol standard curve.

#### Statistical analyses

Because variance between dusky damselfish and threespot damselfish was not homogeneous for fish size (weight, standard length) or for gut parameters (relative intestine length, Zihler index, retention time, assimilation efficiency), we used a Mann-Whitney  $U$ -test for all interspecific comparisons (Zar 1996). For fish fed pelleted food in experiments on assimilation efficiency, we used linear regression to examine relationships among intestine length, retention time, and assimilation efficiencies. Two-way ANOVA was performed on arcsine<sup>1/2</sup>-transformed percentages of assimilation efficiency. All data were analyzed using STATISTICA 4.5 (StatSoft, Tulsa, Okla.). Data are expressed as means  $\pm$  SE.

## Results

### Zihler index and relative intestine length

Threespot damselfish (*Stegastes planifrons*) had a significantly higher mean ( $\pm$  SE) Zihler index ( $ZI=8.2\pm$

$0.2$ ,  $Z=-13.41$ ,  $n_1/n_2=124_{\text{dusky}}/128_{\text{threespot}}$ ,  $P<0.00001$ , Table 2) and mean ( $\pm$  SE) RIL ratio ( $RIL=3.0\pm 0.1$ ,  $Z=-13.40$ ,  $n_1/n_2=124_{\text{dusky}}/128_{\text{threespot}}$ ,  $P<0.00001$ ) than did dusky damselfish (*S. dorsopunicans*) ( $ZI=3.4\pm 0.1$ ,  $RIL=1.2\pm 0.04$ ). In addition, the RIL of both threespot damselfish and dusky damselfish fell below reported values of other herbivorous pomacentrids.

Because both mean weight and length for all fish differed significantly between species, we compared ZI, RIL, and total intestine length (IL) between fish of equivalent weight (in 1-g intervals rounded to the nearest gram; Table 2). In all intervals, even though weight and standard length were not significantly different between species, ZI and RIL values for threespot damselfish were significantly higher than those for dusky damselfish (Table 2).

Fish of both species caught in the morning (~0600 hours), prior to the onset of feeding, had significantly lower ZI ( $Z_{\text{dusky}}=-2.7$ ,  $n_1/n_2=11_{\text{am}}/14_{\text{pm}}$ ,  $P_{\text{dusky}}=0.006$ ;  $Z_{\text{threespot}}=-2.8$ ,  $n_1/n_2=20_{\text{am}}/17_{\text{pm}}$ ,  $P_{\text{threespot}}=0.006$ ) and RIL ( $Z_{\text{dusky}}=-2.9$ ,  $n_1/n_2=11_{\text{am}}/14_{\text{pm}}$ ,  $P_{\text{dusky}}=0.004$ ;  $Z_{\text{threespot}}=-1.9$ ,  $n_1/n_2=20_{\text{am}}/17_{\text{pm}}$ ,  $P_{\text{threespot}}=0.05$ ) than did fish captured at 1600 hours. Dusky damselfish captured in the morning with empty guts had  $ZI=4.2\pm 0.3$  and  $RIL=1.5\pm 0.1$ , in contrast to those captured in the late afternoon with full guts, which exhibited  $ZI=5.3\pm 0.2$  and  $RIL=1.9\pm 0.1$ . For threespot damselfish, morning values for ZI and RIL were  $9.7\pm 0.3$  and  $3.6\pm 0.1$ , respectively, compared to  $ZI=11.0\pm 0.3$  and  $RIL=4.0\pm 0.1$  in the late afternoon.

Of the 14 dusky and 17 threespot damselfish captured at 1600 hours with full intestines, 6 of each species fell into the 60–70 mm SL; of these, dusky damselfish had significantly larger average ( $\pm$  SE) intestine diameters ( $2.1\pm 0.1$  mm,  $Z=-2.6$ ,  $n_1/n_2=6_{\text{dusky}}/6_{\text{threespot}}$ ,  $P=0.01$ ) than did threespot damselfish ( $1.0\pm 0.02$  mm). Comparisons of intestine diameters at 20%, 40%, 60%, and 80% of intestine length indicated that intestine diameter was equivalent only in the anterior portion (20%) of the intestine ( $Z=-0.16$ ,  $n_1/n_2=6_{\text{dusky}}/6_{\text{threespot}}$ ,  $P=0.11$ ; dusky:  $2.2\pm 0.1$  mm; threespot:  $1.8\pm 0.1$  mm). The range in intestinal diameter at 40–80% of total intestine length spanned 2.08–2.12 mm for dusky damselfish and 1.50–1.58 mm for threespot damselfish. Despite this difference in intestinal diameter, the gross surface area estimate for threespot damselfish averaged fivefold ( $1718.3\pm 173.1$  mm<sup>2</sup>) that of dusky damselfish ( $378.0\pm 16.9$  mm<sup>2</sup>).

### Gut retention time

Despite significant differences in both ZI and RIL between species, there was no significant difference in gut retention time between the two species (Table 3). Mean retention times over all diets ranged 6.3–7.3 h for dusky damselfish and 6.4–6.6 h for threespot damselfish. Diet quality did not appear to affect retention time for either

**Table 2** *Stegastes dorsopunicans*, *S. planifrons*. Comparisons of body parameters between dusky damselfish and threespot damselfish; first between all fish, and then within 1-g size intervals (e.g. 7 g encompasses 6.51–7.49 g) (*SL* standard length; *IL* intestine length; *ZI* Zihler index; *RIL* relative intestine length). Interspecific

comparisons were made using a Mann–Whitney *U*-test (Zar 1996). These data (means  $\pm$  SE) represent fish in different stages of feeding (e.g. empty intestine vs. full intestine) and fed different diets (wild-caught, diatom tile, diatom string, artificial diet)

Weight interval	Species	<i>n</i>	Weight (g)	SL (mm)	IL (mm)	ZI	RIL
All fish	Dusky	124 <sup>a</sup>	8.94 $\pm$ 0.25	58.89 $\pm$ 0.55	70.77 $\pm$ 2.13	3.42 $\pm$ 0.09	1.20 $\pm$ 0.03
	Threespot	128 <sup>a</sup>	13.81 $\pm$ 0.44	64.90 $\pm$ 0.58	197.09 $\pm$ 5.31	8.24 $\pm$ 0.18	3.01 $\pm$ 0.07
7 g	Dusky	15	6.89 $\pm$ 0.08	54.67 $\pm$ 0.46	53.60 $\pm$ 3.17	2.82 $\pm$ 0.14	0.98 $\pm$ 0.06
	Threespot	4	6.90 $\pm$ 0.17	53.50 $\pm$ 0.65	136.75 $\pm$ 17.42	7.18 $\pm$ 0.92	2.55 $\pm$ 0.32
8 g	Dusky	22	7.97 $\pm$ 0.06	57.73 $\pm$ 0.66	62.55 $\pm$ 3.14	3.13 $\pm$ 0.16	1.08 $\pm$ 0.05
	Threespot	4	8.00 $\pm$ 0.13	57.00 $\pm$ 0.92	124.75 $\pm$ 9.29	6.24 $\pm$ 0.46	2.20 $\pm$ 0.18
9 g	Dusky	24	9.04 $\pm$ 0.06	59.63 $\pm$ 0.51	68.71 $\pm$ 3.88	3.30 $\pm$ 0.19	1.16 $\pm$ 0.07
	Threespot	8	8.97 $\pm$ 0.11	58.00 $\pm$ 0.65	174.88 $\pm$ 18.80	8.40 $\pm$ 0.88	3.03 $\pm$ 0.35
10 g	Dusky	16	10.05 $\pm$ 0.05	61.25 $\pm$ 0.62	79.44 $\pm$ 6.24	3.68 $\pm$ 0.29	1.30 $\pm$ 0.11
	Threespot	11	9.88 $\pm$ 0.08	61.18 $\pm$ 0.84	172.27 $\pm$ 8.24	8.03 $\pm$ 0.39	2.84 $\pm$ 0.17
11 g	Dusky	9	10.87 $\pm$ 0.12	63.00 $\pm$ 1.01	89.33 $\pm$ 10.07	4.04 $\pm$ 0.46	1.44 $\pm$ 0.18
	Threespot	12	11.02 $\pm$ 0.07	63.08 $\pm$ 0.43	170.58 $\pm$ 10.07	7.67 $\pm$ 0.46	2.71 $\pm$ 0.17
12 g	Dusky	9	11.75 $\pm$ 0.09	64.22 $\pm$ 0.28	89.22 $\pm$ 9.18	3.93 $\pm$ 0.41	1.39 $\pm$ 0.14
	Threespot	11	11.84 $\pm$ 0.09	63.45 $\pm$ 0.55	188.18 $\pm$ 16.14	8.26 $\pm$ 0.70	2.96 $\pm$ 0.24
13 g	Dusky	3	13.15 $\pm$ 0.25	65.33 $\pm$ 1.33	95.33 $\pm$ 18.41	4.03 $\pm$ 0.76	1.46 $\pm$ 0.28
	Threespot	10	13.09 $\pm$ 0.09	64.90 $\pm$ 0.55	194.20 $\pm$ 12.37	8.24 $\pm$ 0.53	3.00 $\pm$ 0.20
14 g	Dusky	3	14.12 $\pm$ 0.17	67.67 $\pm$ 1.45	93.00 $\pm$ 13.65	3.85 $\pm$ 0.57	1.38 $\pm$ 0.235
	Threespot	10	13.86 $\pm$ 0.09	64.90 $\pm$ 0.69	201.90 $\pm$ 9.01	8.41 $\pm$ 0.38	3.12 $\pm$ 0.16
15 g	Dusky	2	14.76 $\pm$ 0.09	69.00 $\pm$ 0.09	113.50 $\pm$ 26.5	4.62 $\pm$ 1.07	1.66 $\pm$ 0.43
	Threespot	11	15.17 $\pm$ 0.09	67.55 $\pm$ 0.56	221.91 $\pm$ 19.35	8.97 $\pm$ 0.78	3.29 $\pm$ 0.29

<sup>a</sup>Not all fish examined fell into the 7.0–15.0 g size range and thus are not represented in the balance of the table

**Table 3** *Stegastes dorsopunicans*, *S. planifrons*. Mean ( $\pm$  SE) retention time (h) for three diet types. Intraspecific differences between dusky damselfish and threespot damselfish in mean retention time as a function of diet type were analyzed using a

Kruskal–Wallis ANOVA; interspecific differences in mean retention time for a single diet type were analyzed using a Mann–Whitney *U*-test (Zar 1996); data are means  $\pm$  SE

Species	Pellet (h)	String (h)	Tile (h)	Pellet $\times$ String $\times$ Tile
Dusky	6.25 $\pm$ 0.33 <i>n</i> = 14	6.75 $\pm$ 0.11 <i>n</i> = 6	7.25 $\pm$ 0.69 <i>n</i> = 6	<i>P</i> = 0.31 <i>H</i> = 2.35, <i>df</i> = 2,26
Threespot	6.56 $\pm$ 0.43 <i>n</i> = 13	6.63 $\pm$ 0.08 <i>n</i> = 8	6.39 $\pm$ 0.37 <i>n</i> = 9	<i>P</i> = 0.92 <i>H</i> = 0.16, <i>df</i> = 2,33
Dusky $\times$ Threespot	<i>P</i> = 0.74 <i>Z</i> = -0.34	<i>P</i> = 0.35 <i>Z</i> = -0.93	<i>P</i> = 0.67 <i>Z</i> = -0.42	

dusky (*H* = 2.35, *P* = 0.31) or threespot damselfish (*H* = 0.16, *P* = 0.92).

Retention time correlated significantly with both relative intestine length (*n* = 51, RIL:  $r_{\text{threespot}} = 0.39$ ,  $P_{\text{threespot}} = 0.03$ ) and total intestine length ( $r_{\text{threespot}} = 0.36$ ,  $P_{\text{threespot}} = 0.04$ ) in the threespot damselfish. There were no significant correlations between retention time and either RIL or IL (*n* = 47, RIL:  $r_{\text{dusky}} = -0.38$ ,  $P_{\text{dusky}} = 0.08$ ; IL:  $r_{\text{dusky}} = -0.10$ ,  $P_{\text{dusky}} = 0.64$ ) in the dusky damselfish. The coefficients and probabilities for correlations between retention time and ZI were identical to those for correlations between retention time and RIL for both species.

Natural foods and fecal nutrient levels

We were unable to calculate assimilation efficiencies of dusky and threespot damselfish fed on diatom tiles or diatom strings, because the ash content of the diet samples exceeded that of the feces, yielding negative assimilation estimates for nutrients. Fecal ash contents suggested that both species may be selective in their feeding bites, avoiding ingestion of large amounts of sediment or winnowing sediments from their feeding bites. McCormick (1998) demonstrated that *Cheilodactylus spectabilis*, which feeds on invertebrates within algal turfs, was capable of winnowing all sediment from

feeding bites. For this reason, we report protein, carbohydrate, and lipid levels in feces as an indirect measure of assimilation efficiency of diatom diets.

There were higher levels of protein, carbohydrate, and lipid in the feces of dusky damselfish than in the feces of threespot damselfish, with the exception of indistinguishable fecal lipid levels in fish fed diatom strings (Fig. 1). As both fishes were fed the same diet, differences in fecal nutrient levels indicate that threespot damselfish assimilated more of the ingested nutrients than did dusky damselfish. Ash content was significantly higher in the feces of threespot damselfish for both diatom tiles and diatom strings. Fecal ash content for fish fed diatom tiles was  $99.2 \pm 0.1\%$  for dusky damselfish and  $99.6 \pm 0.04\%$  for threespot damselfish ( $Z = -2.5$ ,  $n_1/n_2 = 6_{\text{dusky}}/7_{\text{threespot}}$ ,  $P = 0.01$ ). Fecal ash content for diatom strings was  $99.1 \pm 0.1\%$  and  $99.4 \pm 0.03\%$  for

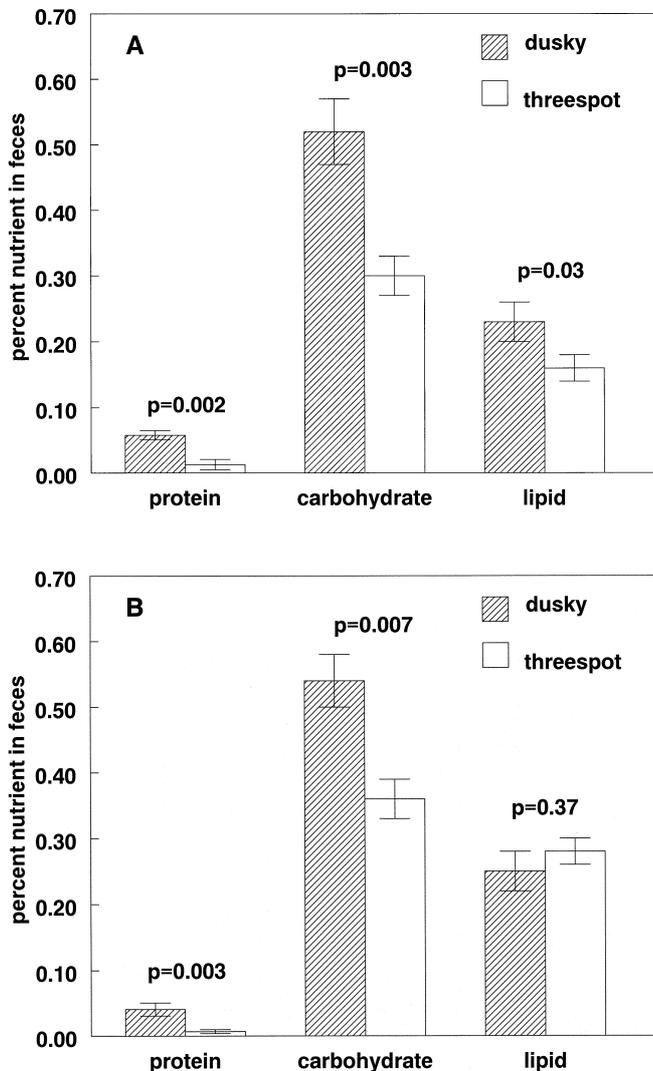
dusky and threespot damselfish, respectively ( $Z = -2.34$ ,  $n_1/n_2 = 6_{\text{dusky}}/7_{\text{threespot}}$ ,  $P = 0.02$ ).

Dusky damselfish and threespot damselfish fed the artificial diet differed only in protein assimilation efficiency (Fig. 2); however, two-factor ANOVA (species  $\times$  nutrients) indicated both significant species ( $F = 6.4$ ,  $P = 0.01$ ) and nutrient ( $F = 51.2$ ,  $P < 0.001$ ) effects. Threespot damselfish absorbed significantly more protein ( $98.3 \pm 0.3\%$ ,  $Z = -2.5$ ,  $n_1/n_2 = 14_{\text{dusky}}/13_{\text{threespot}}$ ,  $P = 0.01$ ) from the artificial diet than did dusky damselfish ( $96.4 \pm 0.8\%$ ). The range of protein assimilation efficiency in threespot damselfish was very small ( $95.4\text{--}99.7\%$ ) compared to that of dusky damselfish ( $88.4\text{--}99.9\%$ ). Assimilation efficiencies for carbohydrate ( $Z = -1.5$ ,  $n_1/n_2 = 14_{\text{dusky}}/13_{\text{threespot}}$ ,  $P = 0.14$ ) and lipid ( $Z = -0.4$ ,  $n_1/n_2 = 14_{\text{dusky}}/13_{\text{threespot}}$ ,  $P = 0.67$ ) were indistinguishable between the two species.

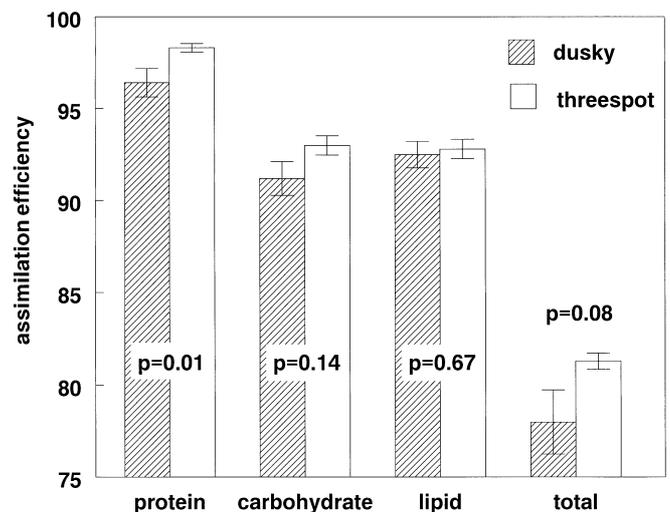
Protein assimilation efficiency was correlated with total intestine length only in threespot damselfish ( $r = 0.63$ ,  $P = 0.03$ ); dusky damselfish did not show this relationship ( $r = 0.27$ ,  $P = 0.36$ ). Neither lipid assimilation efficiency ( $r_{\text{dusky}} = 0.20$ ,  $P_{\text{dusky}} = 0.49$ ;  $r_{\text{threespot}} = 0.36$ ,  $P_{\text{threespot}} = 0.26$ ) nor carbohydrate assimilation efficiency ( $r_{\text{dusky}} = 0.08$ ,  $P_{\text{dusky}} = 0.78$ ;  $r_{\text{threespot}} = -0.05$ ,  $P_{\text{threespot}} = 0.88$ ) correlated significantly with total intestine length in either species.

#### Food quality

Based on the nutrient values of the diatom tiles and strings grown in dusky and threespot territories (Table 1), food quality appeared to be lower in dusky damselfish territories. The diatom tiles had an ash content  $\sim 10\%$  higher than did the diatom strings, likely reflecting accumulation of sediment on the diatom tiles similar to that expected on the flat feeding surfaces of



**Fig. 1A, B** *Stegastes dorsopunicans*, *S. planifrons*. Fecal nutrient levels for dusky damselfish and threespot damselfish fed on natural diatom diets: **A** diatom tiles, **B** diatom strings. Interspecific comparisons were made using a Mann-Whitney  $U$ -test; data are means  $\pm$  SE



**Fig. 2** *Stegastes dorsopunicans*, *S. planifrons*. Nutrient-specific and total assimilation efficiencies for dusky damselfish and threespot damselfish fed an artificial pellet diet. Interspecific comparisons were made using a Mann-Whitney  $U$ -test; data are means  $\pm$  SE

dusky damselfish territories. More importantly, nutrient content of ash-free samples (based on ash-free dry weight calculations) was significantly higher in the diatom strings than in the diatom tiles (Table 1). Protein content of diatom strings was more than twice that of protein content of diatom tiles; lipid content was > 10% higher in diatom strings than in diatom tiles, and carbohydrate content was 40% greater in diatom strings than in diatom tiles. Both of the diatom diets were significantly lower in nutrients than the artificial diet.

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## Discussion

### Gut morphology and diet

The RIL and ZI of dusky and threespot damselfish (*Stegastes dorsopunicans*, *S. planifrons*) were much lower than would be expected for pomacentrids based on reports describing other herbivores. Studies correlating RIL with diet suggest herbivorous fishes generally have longer intestines than omnivorous or carnivorous fishes (Horn 1989). Relative intestine lengths summarized by Al-Hussaini (1947) and Kapoor et al. [1975; note: their data include both relative gut lengths (including stomach) and relative intestine lengths] suggest that ratios for many herbivorous fishes fall between 2 and 21. The damselfishes in our study fed on an unusual food – diatoms – yet were unquestionably herbivores. Despite this, dusky damselfish had a RIL of ~1.0, much lower than that measured for other herbivores with similar body forms. In addition, the RIL of the threespot damselfish (~3) was also lower than that reported for other herbivorous pomacentrids (4.4–10.0; Horn 1989), at the low end of values suggested for other herbivores, and in the mid-range of values suggested for omnivores. In other instances of herbivorous fishes possessing relative intestine lengths < 2, factors such as body form may impose morphological constraints on length or degree of coiling of the intestine (Montgomery 1977). Thus, the short relative intestine lengths of dusky and threespot damselfish are better illustrated by comparisons with other herbivores of similar shape.

Classification of trophic status using the ZI also did not fit our damselfishes. According to Kramer and Bryant's (1995a) classification, for fishes weighing 3.0–30.0 g, a ZI of 12–100 should indicate a herbivore, 3.5–10.0 an omnivore, and 2.4–3.5 a carnivore. Dusky damselfish ( $\bar{x}$  = 8.9 g) had a ZI of 3.4 and threespot damselfish ( $\bar{x}$  = 13.8 g) a value of 8.2. Both species would be categorized as omnivores on the basis of their Zihler indices.

Such indices (e.g. RIL, ZI) are admittedly crude and potentially unreliable indicators of physiologically relevant gut structure, e.g. they do not take true absorptive surface area, or relative gut fullness and other potential sources of processing error into account. This type of error is of particular concern when comparisons are attempted among studies as well as among species, or

where differences are subtle. However, when these metrics fall beyond or near the limits of ranges of previously reported values, particularly when the reports include data from taxa closely related to the subjects, they may suggest unusual biological phenomena worthy of closer examination. So it is with dusky and threespot damselfish.

At least on the patch reefs of San Blas (we are unaware of similar data from elsewhere), the diets of these two damselfishes consist primarily of benthic diatoms which make up ~70–90% of stomach contents (Robertson 1984; Cleveland, unpublished data). Diatoms are unique among algae in possessing a siliceous frustule perforated by small punctae instead of a cell wall of carbohydrate microfibrils and mucilages, and in storing relatively large quantities of lipid rather than starch (Lee 1989; Sze 1993); they are also unicellular in nature and, even when growing in sheets or filaments, expose a large surface area for digestion relative to volume. These qualities may make diatoms both more digestible and richer in available energy than the diets of other herbivorous reef fishes. The intestines of carnivores, and to a lesser extent omnivores, have evolved for processing a highly digestible, nutrient-dense diet that is high in protein and low in carbohydrate (Buddington et al. 1997). In contrast, herbivorous fishes generally feed on items laden with refractory polysaccharides, such as algae and marine plants, as well as fruits, seeds, and leaves (e.g. Horn 1989; Kramer and Bryant 1995b). The failure of dusky and threespot damselfishes to fit into either the RIL or ZI trophic classifications may reflect a response to a readily digested diatom diet.

Dusky damselfish had a slightly larger intestine diameter and thus a slightly larger gross surface area per length of intestine than threespot damselfish. There is a practical limit to increasing intestine diameter as a means of increasing surface area, however. If we view the intestine as an elongate cylinder (Lassuy 1984), food in the center of a wide cylinder may not have sufficient proximity to the intestinal mucosa to allow either immersion in digestive enzyme solutions or contact of digesta with the mucosa. While peristalsis or other muscular activity of the gut may partially mix gut fluids with food materials, at least two factors suggest this may be inefficient in herbivores. First, many herbivores lack the strong muscular layer common to the intestines of carnivorous fishes (Clements and Rees 1998). Second, multicellular algal foods of herbivores often occur as interlocking mats of filamentous thalli or tightly folded leafy forms, suggesting that effective mixing and contact of digestive enzymes with all surfaces may not occur.

### Relative intestine length and retention time

Retention time need not correlate tightly with the speed at which the bolus moves along the length of the intestine. Retention time was equivalent for both damselfishes (~6.6 h), but, because of the large difference in

RIL between species, food moves through the gut of threespot damselfish three times faster than for dusky damselfish. Comparisons of intestine transit rates (ITR=intestine length/retention time) may be more appropriate than retention time among fishes with differing gut characteristics. Intestine transit rate is the relative speed of a bolus of food as it moves through the length of the intestine.

Although interspecific comparisons of retention time and intestine transit rate have not been made, examination of how these parameters change ontogenetically can be instructive. Examination of Lassuy's (1984) data on the ontogeny of intestinal morphology and assimilation efficiency in the damselfish *S. lividus* reveals that the retention time of a bolus of food in juvenile fish is approximately one-half that in adults (4.5 h for 20 mm fish vs. ~10 h for 80 mm fish). Calculations from these data yield a gut transit rate of 0.4 mm h<sup>-1</sup> for a 20 mm fish and 0.48 mm h<sup>-1</sup> for an 80 mm fish. Thus, *S. lividus* experienced both an increase in assimilation efficiency with retention time and an increase in assimilation efficiency with an increase in gut transit rate. In our study, the threespot damselfish exhibited higher protein assimilation with a higher gut transit rate. Buddington et al. (1997) propose that distributing absorptive tissue in a long, thin intestine may be adaptive for herbivores, as it enhances diffusion and absorption. Digesta encounter more gut surface and spend less time at each position in the gut. This may maintain a higher diffusion gradient between nutrients in the bolus and those being absorbed across the mucosa, or insure that membrane-bound transport molecules operate at maximal rates when their target molecules are available in high concentration.

#### Assimilation efficiency and intestine length

The assimilation efficiencies measured for both dusky and threespot damselfish fed artificial diets were higher than those reported for other pomacentrids feeding on algal foods (Lassuy 1984; Polunin 1988; Galetto and Bellwood 1994); this was clearly the result of an artificial diet formulated specifically for easy digestion. Nonetheless, on this diet threespot damselfish assimilated a larger fraction of the available protein than did dusky damselfish. Total assimilation efficiency between the species was not significantly different at the 5% level ( $P=0.08$ ) and likely reflects the high variation within individuals of each species. Total assimilation efficiency was lower than nutrient-specific assimilation efficiency in all cases, as total assimilation efficiency is calculated based on ash content and certain organic compounds (e.g. free amino acids, fatty acids, refractory material) may not be assimilated by the fish. We were unable to obtain nutrient-specific and total assimilation efficiencies for natural diatom diets, as fecal ash content was lower than diatom ash content, yet fecal nutrient levels inferred that threespot damselfish had higher assimilation efficiencies for the natural diatom diet as well.

Although the differences in protein assimilation efficiencies between threespot ( $\bar{x}=98.38\%$ , range: 97.0–99.5%) and dusky damselfish (96.4%, 92.0–99.3%) fed on a highly digestible diet are small, two factors suggest they probably reflect a biologically meaningful pattern. First, the upper limit of 100% was functionally attained by some specimens of both species, but only the threespot damselfish consistently digested protein at this level; the dusky damselfish exhibited a lower mean and broader (and consequently lower) minimum efficiency. Given equivalent retention times, protein digestion and absorption rates appear to be consistently high only in threespot damselfish, suggesting that if more protein were available, or apparent maximum assimilation efficiencies reduced (as with natural foods), threespot damselfish would acquire more protein than dusky damselfish from equivalent foods. Second, assimilation efficiencies are functionally short-term (~6–7 h for these damselfish) rates describing the fraction of a nutrient digested and absorbed in the time required to process a unit of food. If this rate is then used to estimate the amount of a nutrient (in this case protein, an admittedly critical nutrient for herbivores) assimilated from large quantities of food over an extended period (e.g. season or lifetime), small, short-term differences generate large differences in assimilated nutrients.

Lassuy (1984) suggests that the ratio of intestine length to intestine diameter should be a good predictor of assimilation efficiency, with assimilation efficiency increasing as this ratio increases. In his study, the blunt snout gregory, *S. lividus*, showed an ontogenetic increase in assimilation efficiency as the intestine length:intestine diameter (IL:ID) ratio increased; these fish also showed an ontogenetic increase in retention time, which may have enhanced assimilation efficiency. Threespot damselfish have a higher IL:ID ratio and a higher assimilation efficiency than dusky damselfish; this increase in assimilation efficiency may be related to the greater total surface area of the threespot damselfish intestine.

The fact that assimilation efficiency was significantly correlated with total intestine length in threespot but not in dusky damselfish is puzzling, but may be due to higher variation in both protein assimilation efficiency and gut length for the dusky damselfish. Similar lack of correlation has been seen elsewhere. For example, there was no correlation between relative intestine length and assimilation efficiency in the South African herbivore *Sarpa salpa* (Gerking 1984).

#### Food quality and gut characteristics

Morphological parameters such as relative intestine length are not fixed within individual fish or species. Ontogenetic changes occur in relative intestine length and other characteristics (Montgomery 1977; Lassuy 1984; Kramer and Bryant 1995a). Morphological changes in intestinal or surface area may be short-term, phenotypic responses to the nature and quantity of food

resources (Nakagawa et al. 1999). For example, hatchery-reared black sea bream, *Acanthopagrus schlegeli*, have significantly shorter relative intestine lengths than wild fish of the same size (Yamashita et al. 1997); once the hatchery-reared fish are released into the wild, their relative intestine length increases until they are indistinguishable from wild fish. Relative intestine lengths in hatchery-raised ayu, *Plecoglossus altivelis*, are greatest in individuals fed only 60% of the ration of other experimental fish (Nakagawa et al. 1999).

The fact that the dusky and threespot damselfish in our study had relative intestine lengths much lower than those found in other herbivorous fishes, particularly other herbivorous pomacentrids, suggests that a diet dominated by diatoms may influence morphological parameters of the digestive tract. Diatoms appear to be more readily digestible than more refractory algal species and contain large lipid stores, which, at least seasonally in other locations, may contribute significantly to energy intake (Montgomery and Galzin 1993). Furthermore, the relative intestine length of dusky and threespot damselfish may be plastic, as in other fishes, varying in response to fullness and diet quality.

### Ecological implications

Threespot damselfish are the aggressive dominants of the species pair (Robertson 1984; Cleveland 1999) and as a result are able to occupy high-quality territories on the patch reefs of San Blas. Specifically, threespot damselfish are found in slightly deeper water (2–5 m) than dusky damselfish (0–2 m), where our results showed diatom growth to be faster and of higher quality. This competitive exclusion of dusky damselfish from high-quality territories by threespot damselfish has been demonstrated among congeners of other diverse taxa of fish as well. Hixon (1980) demonstrated that the surfperch *Embiotoca lateralis* competitively excluded *E. jacksoni* from shallow, food-rich substrates; Larson (1980) demonstrated that the territorial rockfish *Sebastes chrysomelus* was socially dominant and successfully excluded *Sebastes carnatus* from territories of both high food and high shelter quality. Competitive exclusion from preferred nest site habitats has been demonstrated in estuarine sticklebacks; the larger more aggressive *Gasterosteus aculeatus* prefers nest site habitat under the green alga *Enteromorpha* and successfully excludes *G. wheatlandi* from these areas (Cleveland 1994).

The role that gut morphology and physiology may play in initiating and maintaining competitive exclusion is intriguing and deserves further study. Clearly, threespot damselfish are generally larger and more aggressive than dusky damselfish and thus generally win in interspecific contests (Cleveland, unpublished data). Perhaps greater assimilation efficiency induces a faster growth rate for threespot damselfish, which then translates in their greater ability to establish and maintain territories in superior habitat.

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