Food habits of the farmer damselfish *Stegastes nigricans* from stomach contents, stable isotope, and fatty acid composition analyses

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Abstract

The territorial damselfish, *Stegastes nigricans*, maintains algal farms by excluding invading herbivores and weeding unpalatable algae from its territories. In Okinawa, Japan, *S. nigricans* farms are exclusively dominated by *Polysiphonia* sp., a highly digestible filamentous rhodophyte. This study was aimed at determining the diet of *S. nigricans* in Okinawa and its dependency on these almost-monoculture algal farms based on stomach contents and chemical analyses. Stomach content analyses revealed that all available food items in the algal farms (i.e., algae, benthic animal inhabitants, trapped detritus) were contained in fish stomachs, but amorphous organic matter accounted for 68% of the contents. Therefore, carbon and nitrogen stable isotope ratios and fatty acid (FA) compositions were analyzed to trace items actually assimilated in their bodies. Stable isotope analyses showed that benthic animals were an important food source even for this farmer fish. Two essential FAs (EFA), 20:4n6 and 20:5n3, which are produced only by rhodophytes among available food items, were rich in the muscle tissue of *S. nigricans* as well as in algal mats and detritus, suggesting that algal mats contribute EFAs to *S. nigricans* directly and indirectly through the food web. In conclusion, *S. nigricans* ingested algal mats, detritus and benthic animals maintained within its farm. Algae and detritus were original sources of EFAs, and benthic animals, which were much more abundant in the farms than in outside territories, provided a nitrogen-rich dietary source for the fish.

KEY WORDS: Coral reef · Fatty acid composition · Herbivory · Stable isotope analysis · Stomach contents

Introduction

Marine herbivorous fishes have diversified feeding behaviors and digestive and absorptive systems (Horn 1989; Clements et al. 2009); this diversity enables fish species coexistence and enhances efficiency in material cycling. However, most algae have refractory cell walls (Zemke-White et al. 1999; 2000), and their nitrogen content is lower than that in the tissues of herbivores (Menzel 1959; Mattson 1980). On coral reefs, some herbivorous fishes compensate for this algal indigestibility and the shortage of nitrogen by defending territories that consist of abundant filamentous algae. This behavior is called "farming" or "gardening" and is well known in fishes and limpets (Branch et al. 1992). Territorial damselfishes defend their territories against intruding herbivores and manage farms of filamentous algae as their exclusive feeding sites (Ceccarelli et al. 2001; Jones et al. 2006). In farms, territorial damselfishes browse mainly on the upright axes of filamentous algae (Hiatt and Strasburg 1960; Hata and Kato 2002), and lyse the cell membranes of these algae through refractory cell walls in highly acidic stomachs for subsequent digestion of cell contents by fish enzymes (Zemke-White et al. 1999; 2000), and absorb them in the long intestines (Horn 1989; Cleveland and Montgomery 2003). Additionally, some of these damselfishes are known to ingest detritus (Wilson and Bellwood 1997; Wilson et al. 2003) and/or benthic animals living in the farms (Lobel 1980; Robertson and Polunin 1981; Zeller 1988) to increase nitrogen intake.

Stegastes nigricans is a territorial damselfish that maintains small farms of digestible filamentous rhodophytes in the Indo-West Pacific (Hata and Kato 2004; Hoey and Bellwood 2010). In Okinawa, Japan, one species of *Polysiphonia* dominates *S. nigricans* farms and occupies nearly 90% of the farms, although *Polysiphonia* algae

cover around 20% of the farms in other areas (Hata et al. 2010). In Okinawa, this fish manages its farm by weeding out algae other than *Polysiphonia* and vigorously defending the farm against intruding herbivores (Hata and Kato 2004). The intensive management by this fish enables *Polysiphonia* species to flourish only in its farms, because the alga is not competitively strong, is easily overgrown by other algae, and is digestible to herbivores, thus suffering from heavy grazing pressure unless defended (Hata and Kato 2003; 2006; Hata et al. 2010). Therefore, it is challenging to examine the ways in which *S. nigricans* in Okinawa depend on almost monocultures of *Polysiphonia* sp. and to investigate whether these monoculture farms contribute a simple food source to the fish or have other functions in terms of nutritional supply.

Although the stomach contents of *S. nigricans* contain large amounts of filamentous algae, they also include amorphous organic matter (Sano et al. 1984; Kuo and Shao 1991; Wilson and Bellwood 1997; Jones et al. 2006). Stomach content analyses show directly what is ingested, but there are uncertainties in the method; not all ingested material is assimilated, some food items are dissolved in the stomach much more quickly than others, and the method reflects only the feeding during short periods just before capture (Menzel 1959; Feller et al. 1979; Michener and Schell 1994). Amorphous material was found to comprise nearly 50% of the stomach contents of *S. nigricans* (Jones et al. 2006) and >50% of the intestine contents (Wilson and Bellwood 1997). Therefore, in addition to stomach content analysis, biomarkers providing time-integrated information can be useful tools to determine the origins of these amorphous materials and to define dietary relationships with potential food items of this territorial damselfish in Okinawa.

The stable isotope ratios of nitrogen and carbon are useful in food-web analysis

because the nitrogen and carbon pools of animals have regularly enriched $\delta^{15}N$ and $\delta^{13}C$ signatures relative to their food sources (Cabana and Rasmussen 1994). The fatty acid composition of animals reflects that of their diets and has served as a qualitative biomarker in food-web analyses (Saliot et al. 1991; Napolitano et al. 1997; Dalsgaard et al. 2003). In particular, essential fatty acids, which animals cannot synthesize and must necessarily ingest with their diets, can be good biomarkers (Watanabe et al. 1983; Sargent et al. 1999; Southgate and Kavanagh 1999). In this study, therefore, we tried to evaluate material flows in territories of *S. nigricans* maintaining monocultures of *Polysiphonia* sp. in Okinawa, using stable isotope ratios and fatty acid composition, in addition to conducting stomach content analysis.

Materials and methods

Collection of benthic invertebrates inside and outside algal farms maintained by *Stegastes nigricans*

To examine whether territorial farms harbor more benthic animals than do areas outside territories, we investigated benthic community structures inside territories and in adjacent extraterritorial sites. In August, 2000, 32 quadrats (7 × 7 cm) were established in 16 *S. nigricans* territories (9.0–13.0 cm, total length, TL) and in 16 extraterritorial sites on dead coral rocks in a reef flat on Sesoko Island (26°38´N, 127°52´E), Okinawa, Japan. All epilithic algal matrices in each quadrat were scraped and collected with a suction-lift sampler powered by diving air cylinders (Munro 2005). Samples were immediately preserved in 10% neutralized seawater–formalin. All benthic animals and foraminiferans were identified, and the numbers of individuals were counted under a

microscope. Numbers of each taxon were compared between intra- and extraterritorial sites using *t*-tests.

Stomach content analysis

We speared adult 11 *S. nigricans* individuals (9.8–14.4 cm, TL) on the same reef flat around Sesoko Island in October 2001 (outside the fish breeding season; Karino and Nakazono 1993). We immediately placed the fishes in an ice-chilled box for transport to the laboratory, where their stomach contents were removed and preserved in 10% neutralized seawater–formalin. All algae were sorted and identified under a microscope, and the wet weight of each was determined. Benthic animals and foraminiferans were also collected from the stomach contents, classified by taxon, measured by wet weight, and counted.

Collection and preparation of samples for chemical analyses

We studied a 20-m^2 area on the inshore reef flat of Kabira Reef (24º25´N, 124º10´E; Umezawa et al. 2002), Ishigaki Island, Okinawa, Japan, in September 2004. The flora and fauna on both reefs (Sesoko and Kabira) are very similar (Tsuchiya et al. 2004), and a specific alga, *Polysiphonia* sp., occupied more than 80% of the *S. nigricans* territories on both reefs. We caught seven *S. nigricans* (8.4–13.4 cm, TL) using a fish net early in the afternoon when the gut is fullest (Letourneur et al. 1997). A chisel and hammer were used to gently collect epilithic algal matrices from five quadrats (7 × 7 cm) on five *S. nigricans* algal farms randomly selected on the reef flat. Samples were immediately

packed into 120-μm mesh bags. To sample detritus trapped within the mats, we selected seven additional *S. nigricans* territories, used a chisel and hammer to gently remove seven epilithic algal matrices, each covering 10 cm^2 of substratum, and packed them in the water into small plastic containers with lids. Some dominant algal species that are abundant outside damselfish territories on this reef, such as *Hypnea* sp., *Digenea simplex*, *Sargassum cristaefolium*, *Padina australis*, *Turbinaria ornate*, and *Dictyota* sp., were collected from undefended substrata for fatty acid composition analyses.

These samples were held in a cool box and processed in the laboratory within 3 h in the following manner. The dorsal white muscles of fishes were excised with a scalpel and rinsed with filtered seawater. Stomachs of the fishes were removed, and the contents were collected and gently rinsed with filtered seawater. Detritus trapped in the algal mats was gently washed out into a container and then filtered onto a pre-combusted (450°C, 3 h) glass fiber filter (GF/F, 47 mm diameter; Whatman). Visible filamentous algae and other benthos on the filter were picked out under a microscope. Organic and inorganic contaminants in the epilithic algal matrix were removed with filtered seawater, and algal mats (comprising only algae), pure stands of each *Polysiphonia* sp. along with its epiphytic algae, other filamentous red algae, and benthic invertebrates and foraminiferans were sorted and collected under a microscope. Subsamples of each sample (muscle, stomach contents, *Polysiphonia* sp., algal mat, epiphytic algae, detritus, each taxon of benthic animals and foraminiferans) were wrapped in pre-combusted aluminum foil for stable isotope analyses. One to 5 g of each sample were soaked in organic solvent (chloroform:methanol = 1:2, v:v) in 28-ml glass vials for fatty acid analyses. All samples were kept frozen until analysis.

Stable isotope analysis

All samples for C and N contents and stable isotopes were dried in an oven at 60°C, and powdered with a mortar and pestle. The samples were processed with acid to remove inorganic carbon as detailed in Umezawa et al. (2007) for algae and fish samples and in Umezawa et al. (2009) for filter samples. C and N contents and their stable isotopes ratios (‰) were measured using a combination of an elemental analyzer and an isotope-ratio mass spectrometer (FLASH EA-Conflo III-DELTA^{PLUS} XP; Thermo Electron Co.). Instrument precision was checked with phenylalanine every fifth sample [standard deviation (SD) <0.10‰ for $\delta^{15}N$ and SD <0.14‰ for $\delta^{13}C$]. The differences in $\delta^{15}N$ and $\delta^{13}C$ among replicate samples were smaller than 0.15‰ and 0.20‰, respectively. C:N element ratios, $\delta^{15}N$, and $\delta^{13}C$ values were compared among *S. nigricans* muscles and stomach contents, and benthic animals, foraminiferans, algae, and detritus collected inside the fish farms (using the Tukey–Kramer test after Levene's test for homogeneity of variance).

Fatty acid composition

Lipid extraction followed a slightly modified version of the procedure in Bligh and Dyer (1959). Each sample, extracted using chloroform, methanol, and distilled water, was centrifuged at 1700 g for 10 min. The chloroform phase was filtered through a pre-combusted glass fiber filter (GF/F; Whatman) with a rinse of residues using 1 ml of chloroform. The chloroform filtrate was evaporated in a 40°C water bath under a stream of nitrogen, and water was then completely evaporated in a vacuum desiccator over 10

min.

For saponification of lipid extracts, residual samples were enclosed with 2 ml of a hydrogen chloride–methanol solution (Kanto Chemical Co., Inc.) and heated for 3 h at 100°C under a stream of nitrogen gas. Subsequently, solutions were shaken intensively after the addition of 2 ml hexane and 2 ml distilled water. The upper hexane phase containing fatty acid methyl ester (FAME) fractions was removed into test tubes and dried by application of the above-mentioned procedures for chloroform and water evaporations.

The FAME fractions were re-diluted with hexane, separated, and quantified on a gas chromatograph (Hewlett Packard 5890) with a high-polarity capillary column (DB-FFAP; 30 m, 0.25-mm internal diameter, 0.25-μm film thickness). After injection at 160°C, the oven temperature was raised to 240°C at a rate of 4°C min^{-1} and finally held constant for 20 min. The flame ionization was held at 250°C. Most FAME peaks were identified by comparing their retention times with those of known standards (Supelco, Inc.).

Multivariate analyses of fatty acid compositions were conducted with ANOSIM (analysis of similarities) and nMDS (nonmetric multidimensional scaling) ordination procedures in the PRIMER 6 software package (Plymouth Marine Laboratories) using Bray–Curtis similarities among the composition data. Percentages of each fatty acid were compared among *Stegastes nigricans* muscles, *S. nigricans* stomach contents, algal mats inside the algal farms, and detritus trapped inside the mat using the Tukey–Kramer test. Data were arcsine transformed and checked for homogeneity of variance by Levene's test. Bonferroni corrections were applied to alpha levels to minimize the probability of type 1 errors arising from multiple comparisons.

Results

Benthic communities within *Stegastes nigricans* algal farms

All benthic invertebrates (nematodes, polychaetes, gastropods, ostracods, copepods) and foraminiferans were more abundant inside the territories of damselfish than in extraterritorial areas (Fig. 1).

Stomach contents of the fish

The mean stomach contents of *Stegastes nigricans* were 40.0 ± 30.3 mg in wet weight (mean ± SD, n = 11). Amorphous organic matter accounted for 68.0 ± 43.8% of the mass (Fig. 2). Apart from this unidentified material, algae dominated the stomach contents, especially the filamentous rhodophyta *Polysiphonia* sp., which made up 28.7% of all algal biomass. Some benthic animals including gastropods, ostracods, and copepods, and foraminiferans, were present in the stomachs of the fish (Table 1), but made up a small proportion of the contents in wet weight. Hence, all available materials within algal farms were present in different proportions in fish stomachs.

Stable isotope analyses and C:N ratios

There were large variations in the $\delta^{15}N$ and $\delta^{13}C$ values of *Stegastes nigricans* muscles and stomach contents and in those values of the potential food items available in the territories (Fig. 3, Table2). Algal items and benthic invertebrates were separated

in the scatter plot. The $\delta^{15}N$ signature of *S. nigricans* muscles (8.5 ± 0.4‰) was significantly higher than those of the algae (2.3 ± 1.0‰), detritus (2.2 ± 0.4‰), and benthic animals (3.7 ± 1.0‰) available inside the algal farms (Tukey–Kramer test, all $p < 0.05$). However, benthic invertebrates such as polychaeta (5.2 ± 0.2‰) and amphipoda (4.1 ± 1.0‰) had relatively high $\delta^{15}N$ values, which were closest among the available items to that of the *S. nigricans* stomach contents (5.6 ± 1.0‰).

Among the range of $\delta^{13}C$ signatures, the *S. nigricans* stomach content value (-15.4 ± 1.2‰) was similar to those of benthic animals (-15.3 ± 1.8‰) and detritus (-13.9 ± 0.7‰); *S. nigricans* muscle had the highest value (-13.5 ± 0.7‰), and algae had the lowest (-17.8 ± 1.1‰). The C:N ratio of algal mats (14.6 ±1.6, Table 2) was significantly higher than that of fish (3.7 ± 0.1, Tukey–Kramer test, $p < 0.05$), whereas the ratio of benthic animals (5.0 ± 0.3) was similar to that of fish (Tukey–Kramer test, $p > 0.05$). The detrital C:N ratio (10.6 ± 0.7) was intermediate between the values for algae and benthic animals. The C:N ratio of *S. nigricans* stomach contents (8.2 ± 2.8) was similar to those of detritus and benthic animlas (Tukey–Kramer test, both $p > 0.05$).

Fatty acid composition

Fatty acid compositions varied significantly among *Stegastes nigricans* muscle and stomach contents, algal mats in farms, detritus in the farms, and algae collected from outside fish territories (Fig. 5, Table 4, one-way ANOSIM, $R = 0.91$, $p = 0.001$). The fatty acid compositions of *S. nigricans* dorsal muscles and stomach contents were significantly different from each other (paired test, $R = 1.00$, $p = 0.008$), and both were different from those of the other materials (all paired tests, $R \geq 0.82$, $p < 0.015$). On the

other hand, algal mats and detritus were similar in fatty acid composition (Table 4, paired test, $R = 0.36$, $p > 0.05$) and arrayed closely by ordination (Fig. 5). *Polysiphonia* sp., which comprised ≥80% of the algal mats inside *S. nigricans* farms, was included in the same cluster with the algal mats and detritus trapped within them (Fig. 5). Algae dominating undefended substrata were plotted separately by ordination (Fig. 5).

S. nigricans dorsal muscles contained more polyunsaturated fatty acids, including 18:2*n*6, 18:3*n*3, 20:3*n*6, 20:4*n*3, 20:4*n*6, 22:5*n*3, 22:6*n*3, and conversely, less saturated fatty acids (especially 18:0) than did stomach contents, algal mats, and detritus in the fish farms (Table 4).

Discussion

In Okinawa, *Stegastes nigricans* algal farms support thick turfs of algae strongly dominated by one rhodophyte, *Polysiphonia* sp. (Hata and Kato 2006, Hata et al. 2010). Benthic invertebrates are much more abundant within the algal farms than outside damselfish territories. These rich communities of benthic invertebrates develop inside the algal farms due to the turf-forming structure of *Polysiphonia*, which provides rich and heterogeneous habitats (Zeller 1988; Hata and Nishihira 2002).

Direct observations of *S. nigricans* stomach contents revealed a preponderance of amorphous organic matter. *Polysiphonia* made up the largest part, 29% of the identifiable material, but this value is likely an underestimate, because the cell wall of this alga is susceptible to gastric acid, and therefore the cell contents are easily accessed by enzymes such as α-Amylase and protenase that are active in the stomachs (Chakrabarti et al. 1995), and digested rapidly (Zemke-White et al. 2000; Hata and Kato 2002). Conversely, corticated algae, *Gelidiella* sp. and *Hypnea* sp., a calcareous alga,

Jania sp., and a phaeophyte, *Padina* sp., were found in the stomachs, but were barely digested even after passage through the gastrointestinal canal (Hata and Kato 2002). Lower composition of benthic animals in *S. nigricans* stomachs observed in this study has also been reported from a single sampling in La Réunion in the west Indian ocean (Lison de Loma and Ballesteros 2002), the Great Barrier Reef (Wilson and Bellwood 1997), Papua New Guinea (Jones et al. 2006), and line islands in the central Pacific (Lobel 1980). However, a year-round intensive study revealed seasonal variation and a higher contribution of benthic animals (i.e., 10.2%) to the stomach contents of *S. nigricans* in La Réunion (Letourneur et al. 1997).

Unlike a single sampling during one season, our results from two biomarkers (i.e., stable isotopes and fatty acids), which record time-integrated information, also support the considerable contribution of benthic animals to the *S. nigricans* diet. The difference in $\delta^{15}N$ ($\Delta\delta^{15}N$) between a herbivorous damselfish genus *Stegastes* and their food sources is reported to be around 5.2‰ (Mill et al. 2007), although the trophic-step fractionation of $\delta^{15}N$ is known to be 3.4‰ on average (Minagawa and Wada 1984). This high trophic-level fractionation value seems to be explained by differences in the diet quality (in C:N terms) and metabolism between herbivores and carnivores (Mill et al. 2007; Pecquerie et al. 2010). In fact, $\Delta\delta^{15}N$ value of animals increases following the increase of C:N value of their diet (Adams and Sterner 2000). In our study, the difference in $\delta^{15}N$ between *S. nigricans* dorsal muscle and benthic animals ($\Delta\delta^{15}N$ = 4.9‰) suggested that benthic animals were important food sources for this species, in addition to the algal items (6.2‰ lower than the damselfish muscle) and detritus (6.3‰ lower than the fish muscle). The $\delta^{13}C$ value of *S. nigricans* white muscle was 4.3, 0.4, and 1.8‰ higher than those of algal items, detritus, and benthic animals, respectively.

Given that one trophic step fractionation for $\delta^{13}C$ ($\Delta\delta^{13}C$‰) is reported to be 1.5–2.0 for the white muscles of marine fishes (Barnes et al. 2007; Sweeting et al. 2007), our $\Delta\delta^{13}C$ results also indicate that *S. nigricans* feeds on a mix of detritus and/or benthic animals, along with algal items.

Because there were 3 potential food sources (i.e., algal mat, detritus and benthic animals) with different C and N contents and isotopes, the concentration-weighted linear mixing model, IsoConc model (Phillips and Koch 2002), was introduced to assess the contribution of potential food items to the assimilated foods into *S. nigricans*. This model deals with unequal assimilation of C and N, and assumes that for each C and N, the contribution of each source is proportional to the assimilated biomass times C and N concentrations in that source. As a first approximation, expected $\delta^{15}N$ and $\delta^{13}C$ values of assimilated foods by *S. nigricans* were calculated based on trophic-step fractionations discussed above, that is, $\Delta\delta^{15}N = 5.2$‰ and $\Delta\delta^{13}C = 1.75$‰ (1.75 is the midpoint between 1.5 and 2.0 mentioned above) for the point, mixture in Fig. 4, although this fractionation can be changed depending on C:N value of the diet. In IsoConc model, these expected $\delta^{15}N$ and $\delta^{13}C$ values (i.e., 3.3‰ for $\delta^{15}N$, -15.25‰ for $\delta^{13}C$) of assimilated foods were explained as a mixture of the C and N concentration-weighted $\delta^{15}N$ and $\delta^{13}C$ values of potential food items within the territories (Table 2). Fractional contribution of biomass, C, and N are estimated by this model as shown in Table 3. The model showed that benthic animals was the highest contributor to *S. nigricans* food sources, especially as a nitrogen source, followed by detritus and algal mat in order (Fig. 4, Table 3). *S. nigricans* seems to require nitrogen-rich benthic animals (i.e., C:N = 5.0 ± 0.3) as the additional nitrogen source to maintain the low C:N ratio (i.e., C:N = 3.7 ± 0.1) of their bodies. It was also estimated that *S. nigricans* assimilate a high volume of

detritus that contains lower C and N compositions.

The similarity in fatty acid compositions between algal mats and detritus suggests a contribution of algal mats to the composition of detritus, although the distinct $\delta^{13}C$ values between living algae and detritus might be caused by temporal variation in $\delta^{13}C$ values in dissolved inorganic carbon (DIC) and/or decomposition of specific components other than fatty acids. On the other hand, marine fishes have three essential fatty acids, which they are unable to synthesize efficiently and must therefore ingest with their diets (Sargent et al. 1999; Southgate and Kavanagh 1999). Therefore, two essential fatty acids produced by red algae (20:4*n*6 and 20:5*n*3) can be used as biomarkers for rhodophytes in food web analyses (Khotimchenko and Vaskovsky 1990; Khotimchenko et al. 2002; Dalsgaard et al. 2003). We found that 20:4*n*6 and 20:5*n*3 were both abundant in algal mats, together accounting for 13% of all fatty acids and 45% of polyunsaturated fatty acids. Furthermore, considerable amounts of these essential fatty acids were also contained in the dorsal muscles of *S. nigricans* (Table 4). On coral reefs, the lipid contents of *Polysiphonia* algae, detritus, and algal mats that consist mainly of filamentous rhodophytes are reported to be very low: 2.1–3.6% in dry weight for *Polysiphonia* (Montgomery and Gerking 1980), 0.8–1.0% for detritus (Wilson 2002, Crossman et al. 2005), and 0.6–2.1% for algal mat (Montgomery and Gerking 1980, Wilson et al. 2001, Crossman et al. 2005). On the other hand, benthic animals such as copepods contain more lipids, i.e., 1.6–6.6% (Shansudin et al. 1997) or 3.3–13.3% (Toledo et al. 1999), which is 2–10 times as much as those in algal mats. Therefore, pathways via benthic herbivores and detritus feeders may be important for the supply of essential fatty acids to damselfish, irrespective of their limited biomasses in the fish stomachs. However, it is still evident that *Polysiphonia* algae contribute

greatly to *S. nigricans* nutrition directly or indirectly through the food web of the system, because of the two essential fatty acids that are produced only by red algae.

The third essential fatty acid is 22:6*n*3, which is rarely produced by red algae (Vaskovsky et al. 1996; Khotimchenko et al. 2002), but which can be a biomarker for dinoflagellates (Sargent et al. 1987) and copepods (Coull 1999). Hence, copepods inhabiting the algal farms were likely the primary source of this essential fatty acid for damselfish. The significance of benthic animals to the nutrient sources of damselfish is also suggested based on the stable isotope analyses in this study (Table 3). Additional analyses of the fatty acid compositions of these benthic animals and their total lipid contents would quantitatively clarify the nutritional pathways from primary producers to the damselfish in their territories.

Thus, our stable isotope and fatty acid composition analyses indicate that this damselfish utilizes algal mats, detritus, and benthic animals in a complementary manner, as described in an algal feeding fish, *Girella tricuspidata* (Raubenheimer et al. 2005). The analyses of stomach contents based on single sampling in this study derived results (i.e., reduced contribution of benthic animals to food sources) that differed from the findings of the chemical analyses, probably because complementary feeding has temporal heterogeneity in food sources. On the other hand, chemical signatures included time-integrated information, which convincingly indicated the characteristics of complementary feeding.

In conclusion, *S. nigricans*, which maintains monoculture of *Polysiphonia* alga in Okinawa, ingested algal mats, detritus, and benthic animals maintained within its farm. Filamentous red algae are the sole producers of essential fatty acids 20:4*n*6 and 20:5*n*3 in the territories. Benthic animals were much more abundant in the farms than they were

in areas outside the territories and provided a nitrogen-rich dietary source for the fish. Further analyses on large spatial and temporal scales and the use of additional biomarkers are needed to determine the material flow in this food-web structure. Macronutrient assays and analyses of the digestibility of each dietary item will reveal the extent to which the fish species depend on each species of alga, benthic animals, and detritus for food resources. However, we have successfully shown that the farming damselfish, *S. nigricans*, depend on their monoculture farms that have three dietary functions: first, growing filamentous rhodophyta produce essential fatty acids; second, they aggregate benthic animals that provide a rich nitrogen source for the fish; and finally, they trap large amounts of detritus, which also contribute to fish diets.

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Figure Legends

Fig. 1. Numbers of individuals of benthic invertebrates and foraminiferans inside *Stegastes nigricans* algal farms and outside their territories. Others include bivalvia, cumacea, tanaidacea, isopoda and amphipoda. Values are means + SD; n = 16. Pairwise comparisons within taxonomic categories between algal farms and territories were performed with *t*-tests: ∗, $p < 0.05$; ∗∗, $p < 0.01$; ∗∗∗, $p < 0.001$.

Fig. 2. Proportional wet weight representation (% of total) in fish stomach contents (*Stegastes nigricans*) of eight taxonomic categories of organisms and unidentified organic matter. Error bars indicate ± SD. n = 11. R, Rhodophyta; H, Heterokontophyta.

Fig. 3. $\delta^{15}N$ and $\delta^{13}C$ values of *Stegastes nigricans* dorsal muscles (closed square) and stomach contents (closed diamond), and of algal items (open circles), benthic invertebrates and foraminiferans (closed circles), and detritus (closed triangle) collected inside the territories. Numbers in parentheses are sample sizes. Error bars represent standard deviations (SD). Two shaded polygons enclose algal items and benthic animals, respectively.

Fig. 4. Concentration-weighted mixing triangles for *Stegastes nigricans* dietary source, with algal mats (open circle), benthic animals (closed circle), and detritus (closed triangle) inside the territories as sources. Error bars represent standard deviations (SD). Mixture (open square) is diet assimilated by *S. nigricans*, and their values were calculated based on expected trophic-step fractionation (i.e., $\Delta\delta^{15}N = 5.2‰$ and $\Delta\delta^{13}C = 1.75‰$). Two shaded polygons enclose algal items and benthic animals as shown in Fig.

3. Two numbers in pairs on the lines between two sources indicate the percent contribution of the two sources, respectively.

Fig. 5. Nonmetric multidimensional scaling (nMDS) ordination of fatty acid composition in the muscles and stomach contents of *Stegastes nigricans* and that of algae collected from farms and adjacent sites. Stress value for plotting in two-dimensions is 0.099. Dashed lines enclose samples with ≥81% similarities (Bray–Curtis coefficient). Letters in parentheses following algal taxon indicate phyla, R: Rhodophyta; H: Heterokontophyta; C: Chlorophyta.

Table 1. Numbers of individuals and percentage wet weight (±SD) composition of benthic invertebrates and foraminiferans among total stomach contents of *Stegastes nigricans*; $n = 11$.

	Foraminifera	Gastropoda	Copepoda	Ostracoda
No. of individuals	2.82 ± 3.68	0.09 ± 0.30	0.36 ± 0.92	0.09 ± 0.30
Percent composition	0.16 ± 0.17	0.22 ± 0.51	0.04 ± 0.09	0.01 ± 0.04

Table 2. Carbon content ([C]%), nitrogen content ([N]%), C:N ratios, $\delta^{13}C$, and $\delta^{15}N$ of *Stegastes nigricans*, algal mats, detritus, and benthic animals inhabiting within the territories. Values are average ± SD. The * indicates that the value is the average of those of benthic animals excluding gastropoda whose [C] and [N] values are quite low because of their shells.

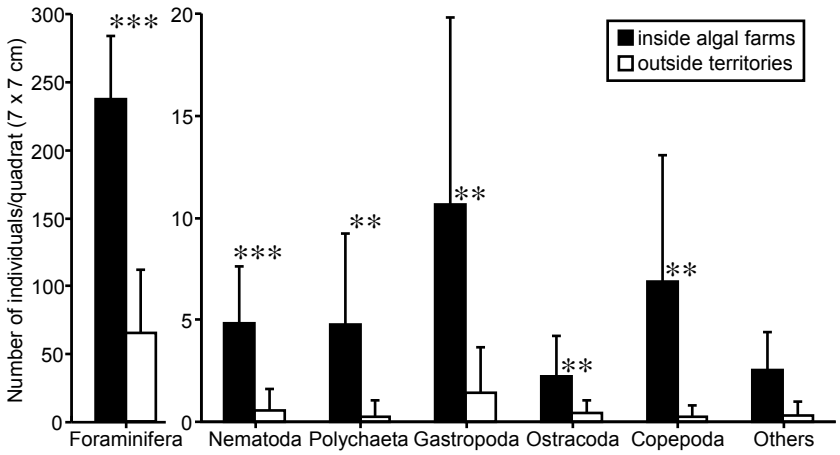
	n	[C]%	[N]%	C:N	$\delta^{13}C$	$\delta^{15}N$
S. nigricans	6	47.7 ± 0.9	15.1 ± 0.4	3.7 ± 0.1	-13.5 ± 0.7	8.5 ± 0.3
Algal mat	4	29.7 ± 5.1	2.4 ± 0.6	14.6 ± 1.6	-18.0 ± 0.3	2.4 ± 0.3
Detritus	5	3.4 ± 1.2	0.4 ± 0.1	10.6 ± 0.7	-14.1 ± 0.6	2.2 ± 0.4
Benthic animals*	13	37.6 ± 9.2	8.9 ± 2.6	5.0 ± 0.3	-15.1 ± 0.5	3.7 ± 0.3
Nematoda	1	47.1	11.7	4.7	-13.9	3.6
Polychaeta	3	46.4 ± 2.4	11.1 ± 0.7	4.9 ± 0.3	-14.5 ± 2.0	5.2 ± 0.3
Gastropoda	2	5.7 ± 0.2	1.4 ± 0.0	4.7 ± 0.1	-14.2 ± 1.0	2.9 ± 0.0
Copepoda	4	37.5 ± 10.4	9.3 ± 2.6	4.7 ± 0.1	-16.5 ± 1.3	2.9 ± 0.7
Amphipoda	3	30.5 ± 2.1	6.7 ± 1.0	5.4 ± 0.4	-17.5 ± 0.9	4.1 ± 1.0
Tanaidacea	2	26.6 ± 5.2	6.0 ± 1.1	5.2 ± 0.1	-13.2 ± 1.4	2.6 ± 0.4

Table 3. Food source partitioning of *Stegastes nigricans* estimated by the concentration-weighted linear mixing model, IsoConc model (Phillips and Koch 2002).

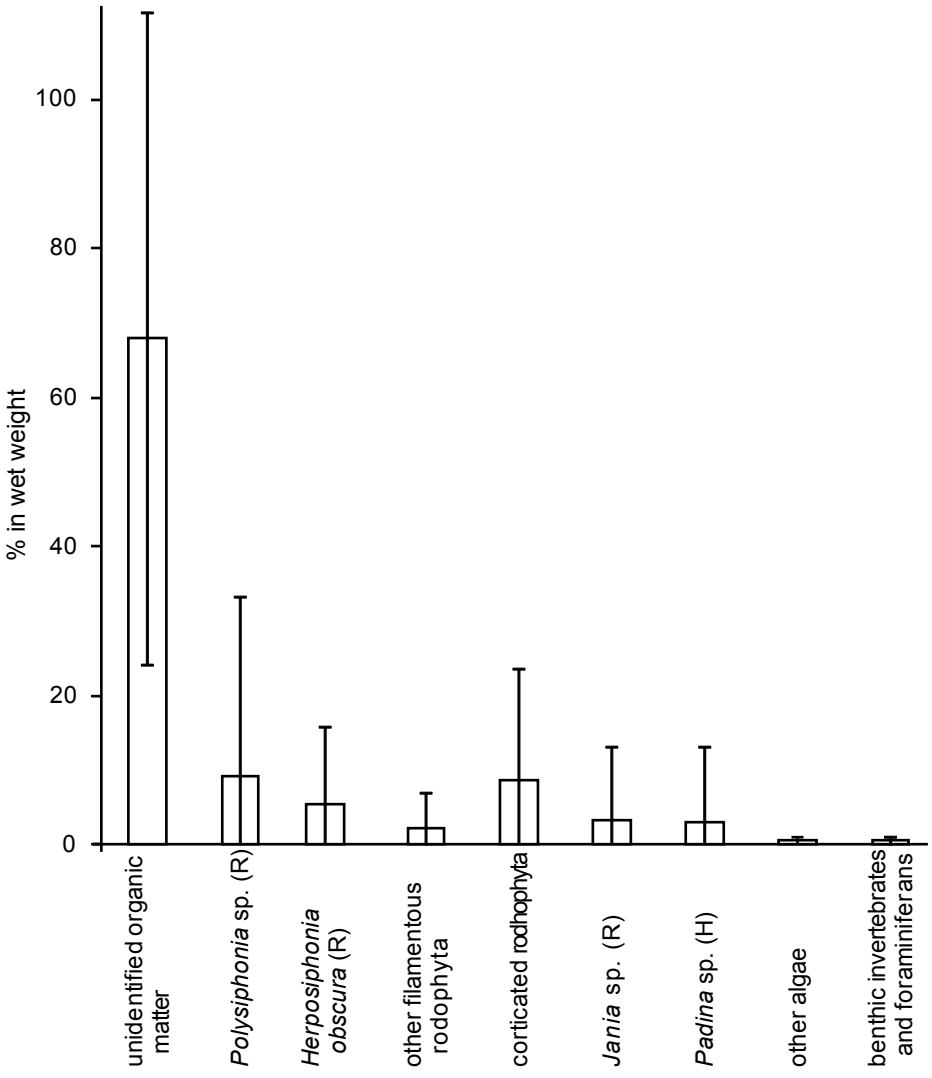
	Fractional contribution		
Dietary items	Biomass	Carbon	Nitrogen
Algal mats	0.05	0.15	0.07
Detritus	0.81	0.29	0.18
Benthic animals	0.14	0.56	0.75

Table 4. Fatty acid composition of *Stegastes nigricans* muscle, the stomach content, algal mats and detritus inside the territories. Values in parentheses are standard deviations. Different letters mean significant differences by Tukey-Kramer test, $p < 0.001$, the alpha level adjusted by Bonferroni correction. SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; * denote essential fatty acids.

Fatty acids	*Stegastes nigricans* (n = 7)		Stomach contents (n = 3)		Algal mats in fish farms (n = 5)		Detritus in fish farms (n = 5)	
14:0	3.3	(1.3)b	2.2	(0.1)b	6.7	(0.6)a	6.1	(1.1)a
iso-15:0	0.1	(0.1)	0.2	(0.1)	0.1	(0.1)	0.4	(0.2)
15:0	0.5	(0.2)	0.3	(0.0)	0.5	(0.2)	0.5	(0.1)
16:0	27.5	(4.1)	27.3	(1.3)	34.0	(2.4)	29.2	(1.5)
16:1*n*7	4.9	(1.6)	1.7	(0.5)	4.6	(1.5)	6.2	(1.2)
16:1*n*5	0.3	(0.1)	0.2	(0.1)	0.2	(0.2)	0.2	(0.1)
iso17:0	0.1	(0.1)	0.2	(0.2)	0.3	(0.0)	0.3	(0.1)
ai-17:0	0.3	(0.1)	0.3	(0.1)	0.1	(0.1)	0.3	(0.1)
16:2*n*4	0.3	(0.1)	0.0	(0.1)b	0.4	(0.3)	0.8	(0.3)a
17:0	0.8	(0.2)	0.9	(0.0)	0.7	(0.0)	0.7	(0.0)
16:3*n*4	0.6	(0.2)	0.0	(0.0)	0.3	(0.2)	1.0	(0.9)
16:4*n*1	0.2	(0.1)	0.0	(0.0)	0.4	(0.4)	0.3	(0.5)
18:0	11.6	(1.0)c	44.0	(1.4)a	27.6	(4.3)b	33.3	(4.6)b
18:1*n*7	2.5	(0.2)a	1.1	(0.1)b	1.3	(0.1)b	1.4	(0.2)b
18:1*n*9	6.7	(0.8)a	1.9	(0.6)b	3.5	(0.9)b	2.5	(0.2)b
18:2*n*6	1.8	(0.2)a	0.7	(0.2)b	1.3	(0.3)	1.0	(0.2)b
18:2*n*6t	0.9	(0.2)	0.5	(0.1)	0.7	(0.1)	0.8	(0.1)
18:3*n*3	1.0	(0.2)a	0.3	(0.1)	0.8	(0.3)	0.4	(0.1)b
18:4*n*3	1.1	(0.3)	0.9	(0.2)	0.9	(0.6)	1.9	(0.9)
20:0	0.3	(0.1)c	1.3	(0.1)a	0.8	(0.1)b	1.1	(0.1)ab
20:1*n*9	0.4	(0.1)	0.1	(0.2)	0.0	(0.0)	0.3	(0.3)
20:1*n*7	0.2	(0.1)a	0.0	(0.0)b	0.0	(0.0)b	0.0	(0.0)b
20:2*n*6	0.2	(0.0)	0.2	(0.3)	0.1	(0.1)	0.1	(0.2)
20:3*n*6	1.0	(0.2)a	0.4	(0.0)b	0.3	(0.2)b	0.1	(0.1)b
20:4*n*6*	10.9	(3.5)a	5.6	(1.6)	4.2	(1.5)b	2.8	(0.7)b
20:3*n*3	0.1	(0.0)	0.0	(0.0)	0.1	(0.1)	0.6	(1.3)
20:4*n*3	0.7	(0.1)a	0.2	(0.1)b	0.0	(0.0)b	0.0	(0.0)b
20:5*n*3*	8.2	(1.6)	3.6	(0.6)	8.7	(3.2)	4.9	(1.5)
22:0	0.3	(0.1)	0.3	(0.1)	0.2	(0.1)	0.2	(0.1)
22:1*n*11	0.4	(0.2)	0.3	(0.3)	0.2	(0.3)	0.1	(0.2)
22:1*n*9	0.1	(0.1)b	0.5	(0.1)a	0.1	(0.1)b	0.3	(0.0)
22:1*n*7	0.2	(0.1)	0.0	(0.0)	0.1	(0.2)	0.0	(0.0)
21:5*n*3	0.6	(0.2)a	0.3	(0.4)	0.0	(0.0)b	0.0	(0.0)b
22:5*n*6	1.4	(0.4)a	0.8	(0.3)	0.1	(0.1)b	0.2	(0.2)b
22:5*n*3	4.8	(1.0)a	1.4	(0.6)b	0.3	(0.1)b	0.6	(0.2)b
22:6*n*3*	5.1	(1.1)a	2.2	(0.6)b	0.3	(0.3)b	1.2	(0.5)b
16:1*n*7/16:0	0.2	(0.0)	0.1	(0.0)b	0.1	(0.1)	0.2	(0.0)a
Σ SFAs	44.9	(4.8)b	76.8	(1.3)a	71.1	(4.2)a	72.1	(5.0)a
Σ MUFAs	15.6	(1.0)a	5.7	(0.8)c	10.0	(0.9)b	11.1	(1.1)b
Σ PUFAs	38.9	(5.5)a	17.2	(1.8)b	18.9	(4.3)b	16.9	(4.2)b
*n*3/*n*6	0.6	(0.1)	0.4	(0.2)	5.3	(3.5)	1.9	(1.8)



Hata & Umezawa, Fig.1



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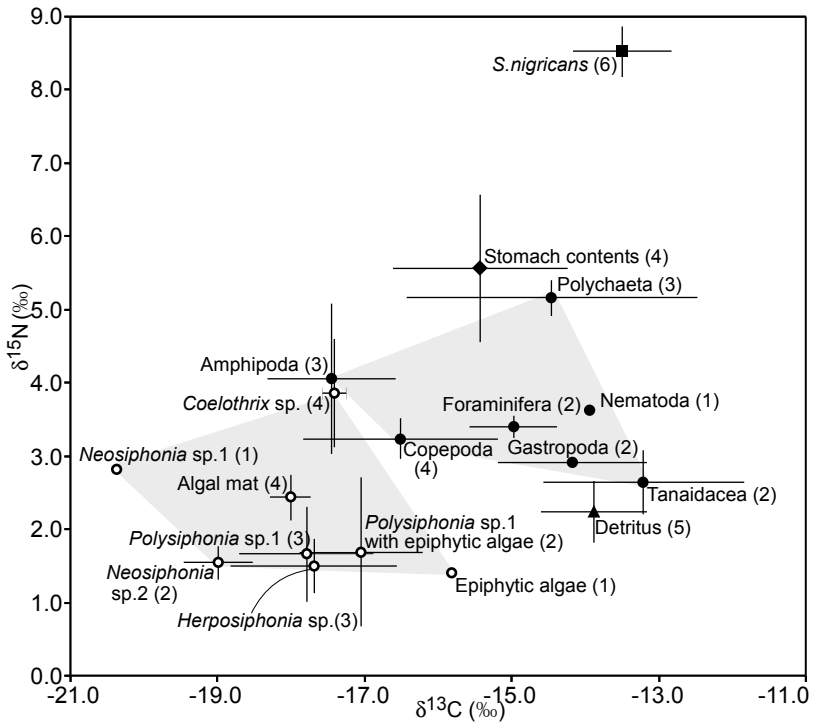

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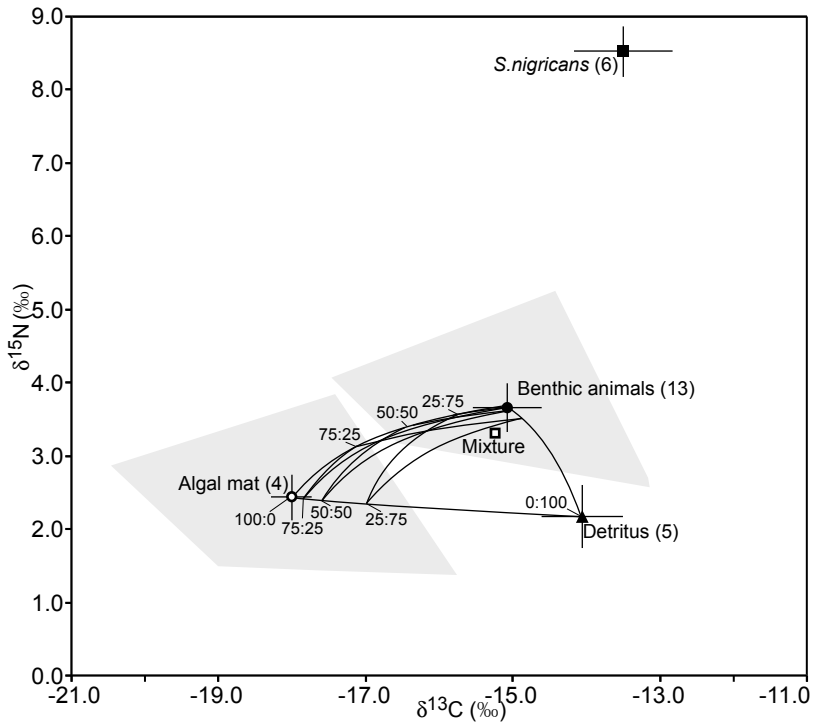

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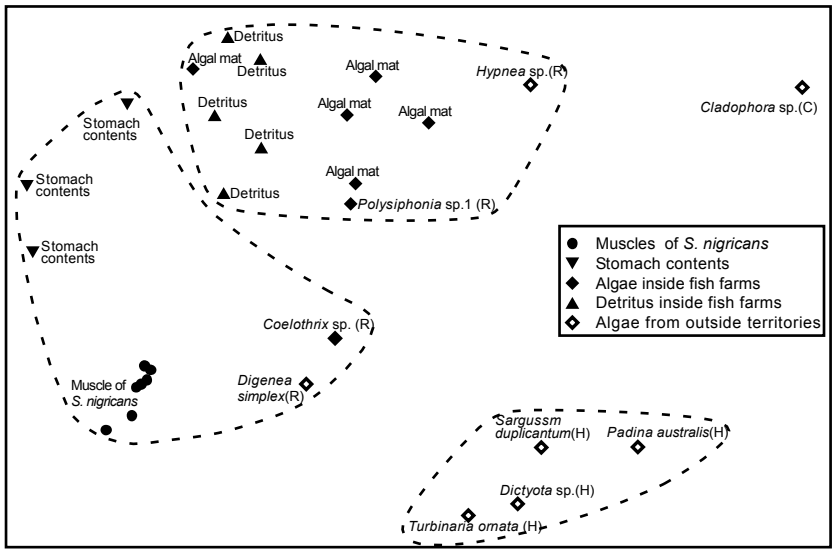

Hata & Umezawa, Fig. 5