



## Assessment of the effects of cage fish-farming on damselfish-associated food chains using stable-isotope analyses



Rong-Quen Jan<sup>a</sup>, Shuh-Ji Kao<sup>b</sup>, Chang-Feng Dai<sup>c</sup>, Cheng-Tze Ho<sup>a,\*</sup>

<sup>a</sup> Biodiversity Research Center, Academia Sinica, Taipei 115, Taiwan

<sup>b</sup> Research Center for Environmental Changes, Academia Sinica, Taipei 115, Taiwan

<sup>c</sup> Institute of Oceanography, National Taiwan University, Taipei 106, Taiwan

### ARTICLE INFO

#### Article history:

Available online 4 August 2014

#### Keywords:

Cage fish farming  
Stable isotope  
Damselfish  
Pelagic food chain  
*Pomacentrus vaiuli*  
*Chromis margaritifer*

### ABSTRACT

To assess the effect of cage fish-farming on the coral reef ecosystem off Xiaoliuchiu Island, southern Taiwan, geographical differences in the food chain of each of two damselfishes, *Pomacentrus vaiuli* and *Chromis margaritifer*, were examined using a stable-isotope approach. For each damselfish, individuals were found to consume similar foods at all sites. However, specimens collected at sites near the cage farm (as the experimental sites) exhibited lower  $\delta^{13}\text{C}$  and higher  $\delta^{15}\text{N}$  signatures compared to those from reference sites. Similar trends also occurred in the zooplankton and detritus, two major food sources for both damselfishes. This finding indicates that particulate organic matter released by the farm may have entered the coral reef ecosystem through the pelagic food chain. Artificial reef emplacement is recommended to provide extra habitats under cage farms to support additional pelagic-feeding fish populations, thereby reducing environmental impacts of cage farming on coral reefs.

© 2014 Elsevier Ltd. All rights reserved.

### 1. Introduction

Cage culture of fish is widely used in rivers, lakes, offshore areas, and sea bays. The operation has extended to coral reefs in the last two decades, leading to a growing concern about its environmental impacts on coral reefs (Heikoop et al., 2000; Beveridge, 2004; Loya et al., 2004, 2005; Rinkevich, 2004). Fish farms release large quantities of excessive feed and fecal material, mostly as dissolved nutrients and organic particles, into the surrounding marine environment.

Previous studies showed that loading of these pollutants may lead to changes in sediment chemistry (Holmer, 1991; Holmer and Kristensen, 1992; Karakassis et al., 2000; Beveridge, 2004; Porrello et al., 2005), algal blooms (Brooks and Mahnken, 2003; Islam, 2005), and reductions in biodiversity of benthic faunas (Beveridge et al., 1994; Hall-Spencer et al., 2006; Fujioka et al., 2007). Suspension feeders such as polychaetes, sponges, ascidians, and bryozoans in close proximity possibly retain the released organic particles and plankton that can flourish as a result of the loading (Lojen et al., 2005; Tsemel et al., 2006). It was also understood that the operational scale, local hydrographical features, and

structure of the local biota were likely to add complexity to the consequences (Iwama, 1991; Loya et al., 2005). However, the trophic pathways of organic waste from cage fish farms are less clear. Since changes in different trophic-level organisms in food webs can be used as a biotic descriptor for environmental impact assessments (Risk and Erdmann, 2000; Vizzini and Mazzola, 2006), a clearer picture of these pathways would be helpful in explaining the ecological influences of cage farming.

A commercial cage fish farm is situated on coral reefs on the northern coast of Xiaoliuchiu Island, off southwestern Taiwan. The farm respectively produced about 650, 450, and 250 t of fish in 2004, 2005, and 2006 (local fish-farmer, personal communication). In the present study, we used two species of resident damselfish as the main bioindicators for monitoring. Fish specimens and their potential food items, including detritus, macroalgae, plankton, and fish feed, were collected from sites near the farm and other reference sites. Stomach contents of the fishes were examined, and stable carbon and nitrogen isotopes were measured in fish muscle tissues and potential food items. With the efficiency of using stable-isotope analyses in food web studies (Polunin et al., 2001; Vizzini and Mazzola, 2004), we first identified the food sources of each fish species, and then tried to delineate the pathway of the introduced organic particles along the food chain by comparing the stable-isotopic signatures in organisms between sites. We also tried to evaluate whether organic particles from the fish farm were a high-quality food for reef fish by measuring

\* Corresponding author. Address: Biodiversity Research Center, Academia Sinica, 128 Academia Road, Sec. 2, Nankang, Taipei 115, Taiwan. Tel.: +886 2 2789 9519; fax: +886 2 2785 8059.

E-mail address: [alexhol@gate.sinica.edu.tw](mailto:alexhol@gate.sinica.edu.tw) (C.-T. Ho).

the C:N ratio of their livers and hepatosomatic index (HSI) as indicators of nutritional condition. The results of this study may thus help elucidate trophic pathways of the organic waste in the damselfishes' food chains. In terms of environmental protection, we hope that knowing which portions of coral reef communities assimilate organic effluents will help elucidate measures for reducing environmental impacts caused by cage fish farming.

## 2. Materials and methods

### 2.1. Study site and cage fish farming at Xiaoliuchiu Island

Xiaoliuchiu Island comprises a coral reef terrain with an area of approximately 6.8 km<sup>2</sup> off southwestern Taiwan. Cage fish farms, which were introduced to this island beginning in 1994, are clustered on the northwestern coast (Fig. 1; 22°20'N, 120°21'E) where the sea bottom is composed mainly of limestone and coral debris and features a steep slope that drops to a depth of 100 m < 1 km from shore. Mean cover of scleractinian corals ranged from 15.5% to 41.0% around several subtidal areas in Xiaoliuchiu Island and major coral colonies are massive and encrusting corals (Dai, 2007). Although the area is relatively protected from typhoons coming from the south, the sea is regularly subject to high current speeds (current velocity measured 29–40 cm s<sup>-1</sup> at 15 m of depth, Huang et al., 2010). Circular cages (12 m in diameter) and square cages (4.5 × 4.5 m) moored 150–250 m offshore at water depths ranging 15–30 m were used to produce commercial fish species (mainly cobia, black porgy, red seabream, and grouper).

Since 2002, cage aquaculture has been dramatically scaled down at this island due to the high incidence of disease outbreaks and losses due to typhoons (Liao et al., 2004). During the study period, only three groups of circular cages (each composed of four sets of circular cages) remained in business in 2005 and two groups of circular cages in 2006.

### 2.2. Sample collection and processing

Fieldwork was carried out at Xiaoliuchiu Island in September 2005 (collection I) and March 2006 (collection II). In collection I, damselfish specimens and their potential food items were collected by scuba diving at 6 fixed subtidal stations: 2 near fish farms (N1 and N2) as experimental sites and 4 others (W1 and W2 on the western coast, S on the southern coast and E on the eastern coast) as reference sites (Fig. 1). In collection II, 2 additional stations near the fish farm were added (N3, N4; Fig. 1). The bottom at all sampling stations was composed mainly of sand, gravel, and coral debris.

#### 2.2.1. Damselfishes

Two species of resident damselfish, *Pomacentrus vaiuli* (Jordan and Seale) and *Chromis margaritifer* (Fowler), were sampled (Table 1). Both fishes inhabited small patches on shallow coral reefs. *P. vaiuli* occurred solitarily and was collected by selective angling (collection I: total  $n = 51$ , 3.4–6.5 cm standard length (SL); collection II: total  $n = 44$ , 4.2–7.3 cm SL). In contrast, *C. margaritifer* often formed small aggregations and was thus collected by handnetting (collection I: total  $n = 42$ , 3.5–5.5 cm SL; collection II: total  $n = 42$ , 2.9–6.1 cm SL).

Specimens were kept in an ice-water slurry in sealed plastic bags and immediately transported to the laboratory, where the SL ( $\pm 1$  mm), body weight ( $\pm 1$  mg) and liver weight ( $\pm 1$  mg) of each specimen were measured using calipers and an electronic balance. The stomach was removed and preserved in a 10% buffered formalin solution for the content analysis, and white dorsal muscle tissue, which tends to be less variable in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values than other tissues and is widely used in food-web studies (Pinnegar and Polunin, 1999), was taken and frozen for later stable-isotope analysis.

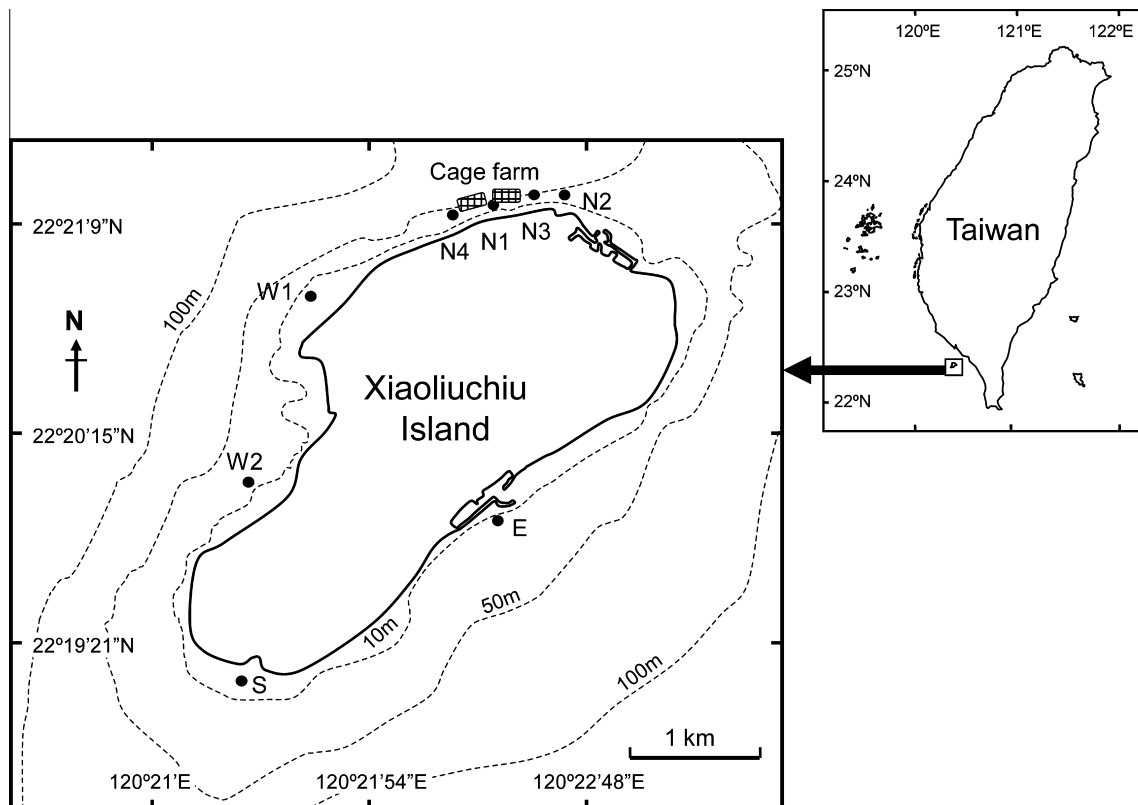


Fig. 1. Map of Xiaoliuchiu Island with isobaths, showing the location of cage fish farms (hatched area) and sampling sites (filled circles).

**Table 1**

Sample sizes (n) of *Pomacentrus vaiuli*, *Chromis margaritifer*, and their potential food items collected at eight sampling sites as W2, W1, N4, N2, N3, N1, E, and S in collection I and collection II.

Organisms	Sampling sites								Total
	W2	W1	N4 <sup>a</sup>	N2	N3 <sup>a</sup>	N1	E	S	
<i>P. vaiuli</i>	I (10); II (4)	I (6); II (6)	II (6)	I (7); II (7)	II (4)	I (9); II (7)	I (11); II (5)	I (8); II (5)	I (51); II (44)
<i>C. margaritifer</i>	I (5); II (3)	I (13); II (6)	II (6)	I (5); II (3)	II (6)	I (11); II (6)	I (3); II (6)	I (5); II (6)	I (42); II (42)
Zooplankton	I (3); II (3)	I (3); II (3)	II (3)	I (3); II (3)	II (3)	I (3); II (3)	I (3); II (3)	I (3); II (3)	I (18); II (21)
Detritus	II (3)	II (3)	II (3)	II (3)	II (3)	II (3)	II (3)	II (3)	II (21)
<i>Algae</i>									
<i>Bryopsis plumosa</i>	I (3); II (3)	I (3); II (3)	II (3)	I (3); II (3)	II (3)	I (3); II (3)	I (3); II (3)	I (3); II (3)	I (18); II (21)
<i>Tricleocarpa fragilis</i>	I (3)	I (3)	II (0)	I (3)	II (0)	I (3)	I (3)	I (3)	I (18)
<i>Hypnea pannosa</i>	II (3)	II (3)	II (3)	II (3)	II (3)	II (3)	II (3)	II (3)	II (18)

<sup>a</sup> N4 and N3 sites were newly added in collection II.

### 2.2.2. Potential food items

Potential food items were mainly sampled from habitats of these two damselfishes (Table 1). The samples were comprised of algae (collected by hand, including *Bryopsis plumosa* [green algae] and *Tricleocarpa fragilis* [red algae] for collection I, and *B. plumosa* and *Hypnea pannosa* [red algae] for collection II), and zooplankton (collected by a 50 µm mesh zooplankton net). Detritus was added to the sample in collection II by collecting detrital and surface aggregates on algal fronds through a pipe using suction (Ho et al., 2007). The sample sizes were presented in Table 1. Before further treatment, zooplankton and detritus samples underwent acid washing to remove carbonates. Acid washing consisted of addition of 1 N HCl until bubbling ceased (Jacob et al., 2005). In addition, three samples of fish feed used in the fish farm were analyzed. The fish feed was mainly composed of trash fish, fishmeal, and other undisclosed ingredients (personal communication).

### 2.3. Stomach content analysis

Stomach contents of the fish were spread on slides, and the components were identified using a stereomicroscope. Food items were categorized into algae (including microalgae and macroalgae), zooplankton, detritus, sand, fish eggs, fish scales, and unidentifiable fragments. The relative volumetric quantity of each food item was estimated using the point frame method (Hyslop, 1980; Jan et al., 1994).

### 2.4. Stable isotope analysis

Muscle tissues of fish specimens and food samples were freeze-dried (at −53 °C for 48 h) and ground up with a mortar and pestle. All samples (1–2 mg) were combusted in an elemental analyzer (Flash EA-1100 NC, Thermo-Finnigan) to produce CO<sub>2</sub> and N<sub>2</sub>, which flowed through a GC column for separation and into a mass spectrometer (Thermo Finnigan Deltaplus Advantage) to separately determine the isotopic compositions. Isotope ratios were expressed as the difference in parts per thousand (‰) from standard reference material:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000;$$

where  $X$  is <sup>13</sup>C or <sup>15</sup>N,  $R$  is the corresponding ratio of <sup>13</sup>C/<sup>12</sup>C or <sup>15</sup>N/<sup>14</sup>N, and  $\delta$  is a measure of the heavy to light isotopes in the sample. The reference materials were the international standards, Pee Dee Belemnite (PDB) for carbon and atmospheric N<sub>2</sub> for nitrogen. USGS 40, which has certified  $\delta^{13}\text{C}$  of −26.24‰ and  $\delta^{15}\text{N}$  of −4.52‰ and acetanilide (Merck) with  $\delta^{13}\text{C}$  of −29.76‰ and  $\delta^{15}\text{N}$  of −1.52‰, were used as working standards. The reproducibility of carbon and nitrogen isotopic measurements is better than 0.15‰.

Since lipids are <sup>13</sup>C depleted relative to proteins (Schmidt et al., 2003; Sweeting et al., 2006), and variations in lipid content among aquatic animals have the potential to introduce considerable bias

into stable isotope analyses that use  $\delta^{13}\text{C}$  (Post et al., 2007), in the present study the lipid-controlled  $\delta^{13}\text{C}$  data (as  $\delta^{13}\text{C}'$ ) of two damselfish species was derived by normalization of  $\delta^{13}\text{C}$  following a Logan et al. (2008) model:

$$\delta^{13}\text{C}' = \delta^{13}\text{C} + \beta_0 + \beta_1 \ln(\text{C} : \text{N}_{\text{bulk}});$$

the model estimate of C:N<sub>lipid-free</sub> is represented by  $e^{(-\beta_0/\beta_1)}$ . This model uses atomic C:N ratios of the bulk, nonextracted sample (C:N<sub>bulk</sub>) to calculate  $\delta^{13}\text{C}'$ . In present study, fish muscle tissue-specific parameters ( $\beta_0 = -4.763$  and  $\beta_1 = 4.334$ , based on Logan et al. (2008)) were used for the best fitting  $\delta^{13}\text{C}'$  estimates.

Besides, the  $\delta^{13}\text{C}'$  of zooplankton was derived following a El-Sabaawi et al. (2009) model:

$$\delta^{13}\text{C}' = \delta^{13}\text{C} - 1.85 + (0.38\text{C} : \text{N}_{\text{bulk}});$$

this model also uses atomic C:N ratios of the bulk, nonextracted sample (C:N<sub>bulk</sub>) to calculate  $\delta^{13}\text{C}'$ .

### 2.5. C:N ratio of liver

In aquatic animals, the C:N ratio is considered to be a suitable proxy for organism condition (Sweeting et al., 2006) and the strong relationships have been found between the C:N ratio and lipid content (Schmidt et al., 2003; Post et al., 2007; Logan et al., 2008). Individuals in better condition (higher lipid content) could be expected to exhibit higher C:N ratios. Herein, a C:N ratio of liver was employed as a surrogate measure of lipid content in damselfish liver.

### 2.6. Hepatosomatic index (HSI)

The HSI was used to estimate the individual nutritive status of a fish. Generally, higher HSI values indicate better nutritional status of fishes for energy conservation.

$$\text{HSI} = [\text{liver weight(g)}/\text{body weight(g)}] \times 100.$$

### 2.7. Data analysis

For *P. vaiuli* and *C. margaritifer* respectively, a two-way multivariate analysis of variance (MANOVA) test was used to test for dietary differences between the two collections and among sampling sites. The volumetric percentage data of stomach contents were square-root transformed prior to the process, and the unidentified portion of the stomach content was excluded from the analyses. In each collection and for each damselfish, differences in  $\delta^{13}\text{C}'$  and  $\delta^{15}\text{N}$  values among sampling sites were analyzed by analysis of covariance (ANCOVA) using fish body length as the covariate. Site effects on the stable isotopes of a potential food category were

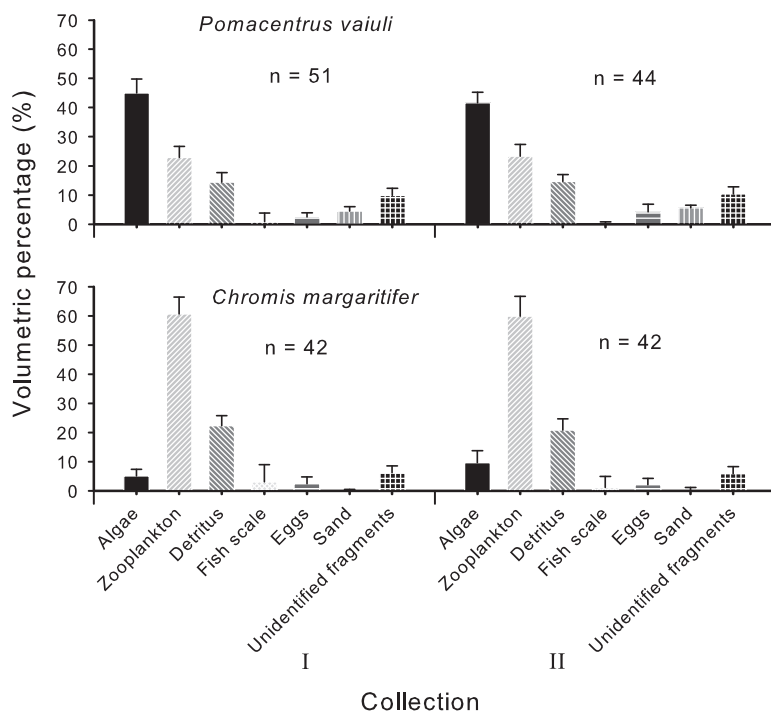


Fig. 2. Stomach content analyses of *Pomacentrus vaiuli* and *Chromis margaritifer* in the two collections. Error bars are standard errors.

tested by one-way analysis of variance (ANOVA). In addition, in each collection, site effects on the C:N ratio of liver and on the HSI of two damselfishes were tested by one-way ANCOVA. Scheffé's post hoc test was used to compare appropriate means in the above ANCOVA and ANOVA ( $p < 0.05$ ).

### 3. Results

#### 3.1. Stomach content analysis

Differences in food uses were found between *P. vaiuli* and *C. margaritifer* (Fig. 2). The major food item of *P. vaiuli* was algae (with mean volumetric percentages of 45.0% in collection I and 41.6% in II), followed by zooplankton (I: 22.9%; II: 23.3%), detritus (I: 14.5%; II: 14.8%), sand (I: 4.5%; II: 5.6%), fish eggs (I: 2.5%; II: 4.2%) and scales (I: 0.9%; II: 0.2%). In contrast, zooplankton (I: 63.3%; II: 60.0%) was the major food item of *C. margaritifer*, followed by detritus (I: 23.6%; II: 20.9%), algae (I: 5.3%; II: 9.7%), fish eggs (I: 2.5% and II: 2.1%), scales (I: 3.1% and II: 1.2%), and sand (I: 0.1%; II: 0.3%).

For each damselfish species, significant differences occurred between collections (*P. vaiuli*: Wilks' lambda = 0.58, d.f. = 6 and

67,  $F = 8.13$ ,  $p < 0.001$ ; *C. margaritifer*: Wilks' lambda = 0.42, d.f. = 6 and 55,  $F = 12.79$ ,  $p < 0.001$ ) and among sampling sites (*P. vaiuli*: Wilks' lambda = 0.34, d.f. = 30 and 270,  $F = 2.79$ ,  $p < 0.001$ ; *C. margaritifer*: Wilks' lambda = 0.32, d.f. = 30 and 222,  $F = 2.44$ ,  $p < 0.001$ ). However, the least squared distance (LSD) showed that site effects occurred mainly in the food categories with smaller volumetric percentages, i.e., sand (d.f. = 5,  $F = 12.34$ ,  $p < 0.001$ ; collection I), fish scales (d.f. = 7,  $F = 6.68$ ,  $p < 0.001$ ; II) and fish eggs (d.f. = 7,  $F = 3.12$ ,  $p = 0.011$ ; II) for *P. vaiuli* and fish scales (d.f. = 5,  $F = 7.02$ ,  $p < 0.001$ , I; d.f. = 7,  $F = 2.35$ ,  $p = 0.045$ , II), fish eggs (d.f. = 7,  $F = 6.67$ ,  $p < 0.001$ , II), and sand (d.f. = 7,  $F = 4.06$ ,  $p = 0.002$ , II) for *C. margaritifer*.

#### 3.2. Stable isotope analyses

##### 3.2.1. Damselfish

Various ranges of stable-isotope values were found in the two damselfish species in different collections (Table 2). With the exception of the  $\delta^{13}\text{C}$  of *C. margaritifer*, where a site effect was not significant, test statistics showed  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for both damselfishes were affected by sampling sites (Table 3).

Table 2  
Sample sizes ( $n$ ), C:N ratio,  $\delta^{13}\text{C}$ , lipid-controlled  $\delta^{13}\text{C}$  (where applicable as  $\delta^{13}\text{C}$ ), and  $\delta^{15}\text{N}$  of *Pomacentrus vaiuli*, *Chromis margaritifer*, and their potential foods collected for two collections.

Collection	Organisms	$n$	C:N ratio			$\delta^{13}\text{C}$ (‰)			$\delta^{13}\text{C}$ (‰)			$\delta^{15}\text{N}$ (‰)		
			Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
I	<i>P. vaiuli</i>	51	2.88–3.09	3.00	0.05	–18.0 to –16.8	–17.5	0.29	–17.8 to –16.9	–17.4	0.26	7.8–11.0	8.7	0.72
	<i>C. margaritifer</i>	42	2.92–3.09	3.00	0.05	–18.5 to –17.8	–18.2	0.16	–18.5 to –17.7	–18.1	0.18	8.8–10.6	9.4	0.41
	Zooplankton	18	4.07–4.62	4.40	0.17	–19.3 to –18.7	–19.1	0.17	–19.6 to –18.8	–19.3	0.21	5.4–6.7	6.1	0.42
	Fish feed	3	3.11–3.17	3.15	0.03	–20.8 to –20.2	–20.6	0.32	–	–	–	8.7–12.4	11.1	2.08
	Bryopsis plumose	18	11.00–13.93	12.42	0.86	–19.3 to –17.7	–18.3	0.42	–	–	–	3.8–4.4	4.1	0.22
	<i>Triclecarpa fragilis</i>	18	7.02–11.54	9.58	1.53	–18.7 to –17.9	–18.3	0.25	–	–	–	3.8–4.5	4.1	0.20
II	<i>P. vaiuli</i>	44	3.13–3.37	3.22	0.04	–18.2 to –17.4	–17.8	0.22	–17.7 to –17.0	–17.3	0.19	7.8–10.2	8.8	0.68
	<i>C. margaritifer</i>	42	3.13–3.30	3.22	0.05	–18.8 to –17.7	–18.4	0.31	–18.5 to –17.3	–18.0	0.32	8.1–10.0	8.7	0.48
	Zooplankton	24	4.51–5.57	5.00	0.33	–19.6 to –18.3	–19.0	0.31	–19.7 to –18.2	–19.0	0.42	5.1–7.2	6.1	0.75
	Bryopsis plumose	24	11.12–15.02	12.68	1.06	–19.8 to –18.0	–18.6	0.46	–	–	–	3.2–4.6	3.9	0.36
	<i>Hypnea pannosa</i>	24	6.56–10.89	8.43	1.25	–18.3 to –16.5	–17.32	0.61	–	–	–	3.1–4.3	3.7	0.30
	Detritus	24	6.44–7.64	7.09	0.37	–19.2 to –17.0	–18.1	0.64	–	–	–	3.3–4.4	3.8	0.32

**Table 3**

F ratio for analysis of covariance (ANCOVA) and analysis of variance (ANOVA) testing the effect among sampling site in each collection on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of *Pomacentrus vaiuli*, *Chromis margaritifer*, zooplankton and on  $\delta^{13}\text{C}$  of detritus and macroalgae. ANCOVA was used for two damselfish and body size (standard length) of the fish was used as covariate.

Organisms	$\delta^{13}\text{C}$ ( $\delta^{13}\text{C}$ )		$\delta^{15}\text{N}$	
	Collection I	Collection II	Collection I	Collection II
<i>P. vaiuli</i>	8.92***	3.60***	39.73***	41.73***
<i>C. margaritifer</i>	0.84	17.70***	11.29***	45.30***
Zooplankton	23.66***	17.16***	34.10***	55.50***
Detritus	NA	15.11***	NA	19.33***
<i>Bryopsis plumosa</i>	3.12	8.30***	6.58**	2.53
<i>Tricleocarpa fragilis</i>	0.26	NA	1.58	NA
<i>Hypnea pannosa</i>	NA	19.74***	NA	8.31***

NA: not available.

\*\*  $p < 0.01$ .

\*\*\*  $p < 0.001$ .

Scheffé's post hoc tests on the site means further indicated a trend of a difference between experimental sites and reference sites (Figs. 3–6). That is, for each collection, the mean  $\delta^{13}\text{C}$  values

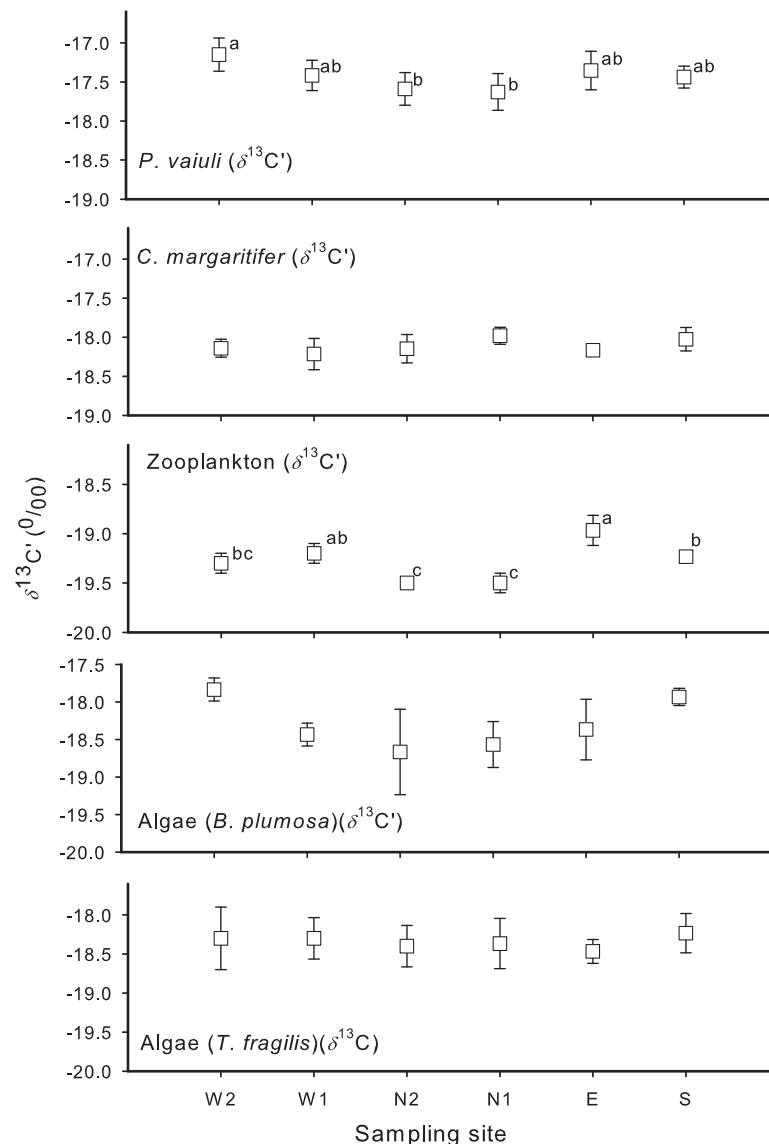
at experimental sites were 0.2–0.4‰ lower (Figs. 3 and 4), and mean  $\delta^{15}\text{N}$  values were 0.7–1.1‰ higher than reference sites (Figs. 5 and 6). The  $\delta^{13}\text{C}$  value of *C. margaritifer* in collection I, which was not affected by site, was an exception.

### 3.2.2. Potential food items

Various ranges in stable-isotope values were also found in potential food items from different collections (Table 2).

Fish feed sampled from the fish farm exhibited  $\delta^{13}\text{C}$  values ranging from  $-20.8$  to  $-20.2$ ‰ and  $\delta^{15}\text{N}$  values of 8.7–12.4‰. In addition, zooplankton in the experimental sites exhibited 0.3‰ and 0.6‰ lower mean  $\delta^{13}\text{C}$  (Figs. 3 and 4) and 0.8‰ and 1.1‰ higher mean  $\delta^{15}\text{N}$  (Figs. 5 and 6) in collections I and II, respectively.

For macroalgae, *B. plumosa* and *T. fragilis*, site differences in mean  $\delta^{13}\text{C}$  values were not significant in collection I. Yet, higher variations occurred at sites N1, N2, and E for *B. plumosa* (Fig. 3). In collection II, except for N4, site differences were not significant in *B. plumosa*. Site differences occurred more widely in *H. pannosa* (Fig. 4) and the differences did not seem to be related to experimental design. In collection I, higher  $\delta^{15}\text{N}$  values occurred in N1 and N2, i.e., the experimental sites, for *B. plumosa*. By contrast, site



**Fig. 3.** Values of  $\delta^{13}\text{C}$  for *Pomacentrus vaiuli*, *Chromis margaritifer*, zooplankton and  $\delta^{13}\text{C}$  for macroalgae at different sampling sites in collection I. Symbols represent the mean of  $\delta^{13}\text{C} \pm 1$  SD. Mean values in the same diagram with different letters significantly differ (Scheffé's post hoc test,  $p < 0.05$ ).

differences were not found in *T. fragilis* (Fig. 5). In collection II, site differences occurred in the  $\delta^{15}\text{N}$  of *B. plumosa* and *H. pannosa*; however, the differences seemed to follow different trends in these two macroalgae (Fig. 6).

The detritus data, which was only available for collection II, showed that lower  $\delta^{13}\text{C}$  and higher  $\delta^{15}\text{N}$  values occurred at the experimental sites as compared with those from reference sites (Table 2; Figs. 4 and 6).

### 3.3. C:N ratio of liver

In *P. vaiuli*, the C:N ratio of liver was in the range of 3.86–6.56 in collection I and 3.87–5.53 in collection II. In *C. margaritifer*, it ranged 4.44–8.90 in collection I and 3.88–7.81 in collection II. Site effects occurred significantly in collection I for both damselfishes (Table 4), each with the highest ratio occurring at N2 (Fig. 7). Meanwhile, site effects were not found in collection II (Table 4).

### 3.4. Hepatosomatic index (HSI)

The HSI of *P. vaiuli* was in the range of 0.5–2.62 in collection I and 0.64–5.28 in collection II. In *C. margaritifer*, it ranged

0.36–1.37 in collection I and 0.31–2.4 in collection II. For both damselfishes, significant site effects occurred in collection I, but not in collection II (Table 4). Coincident to the findings on C:N ratio of liver, the highest HSI occurred at N2 in collection I for both damselfishes (Fig. 8).

## 4. Discussion

### 4.1. Feeding habits of damselfishes

The feeding habits of coral reef fish are generally adaptable. Previous studies on trophic relationships of damselfishes indicated that the feeding habits of *P. vaiuli* varied from herbivory (85% of the stomach content as algae in the Marshall Islands; Hiatt and Strasburg, 1960; Hobson and Chess, 1978) to omnivory (39% as algae and 33% as copepods in Kenting, southern Taiwan; Kuo and Shao, 1991). Stomach contents of *P. vaiuli* in the present study (45.0% as algae in collection I, and 41.6% in II; 22.9% as zooplankton in I and 23.3% in II) were similar to those found at Kenting, indicating a geographical coincidence in the feeding habits of this damselfish. In contrast, the feeding habits of *C. margaritifer* were less variable; it fed mainly on zooplankton (41.7–74.2% in the Marshall

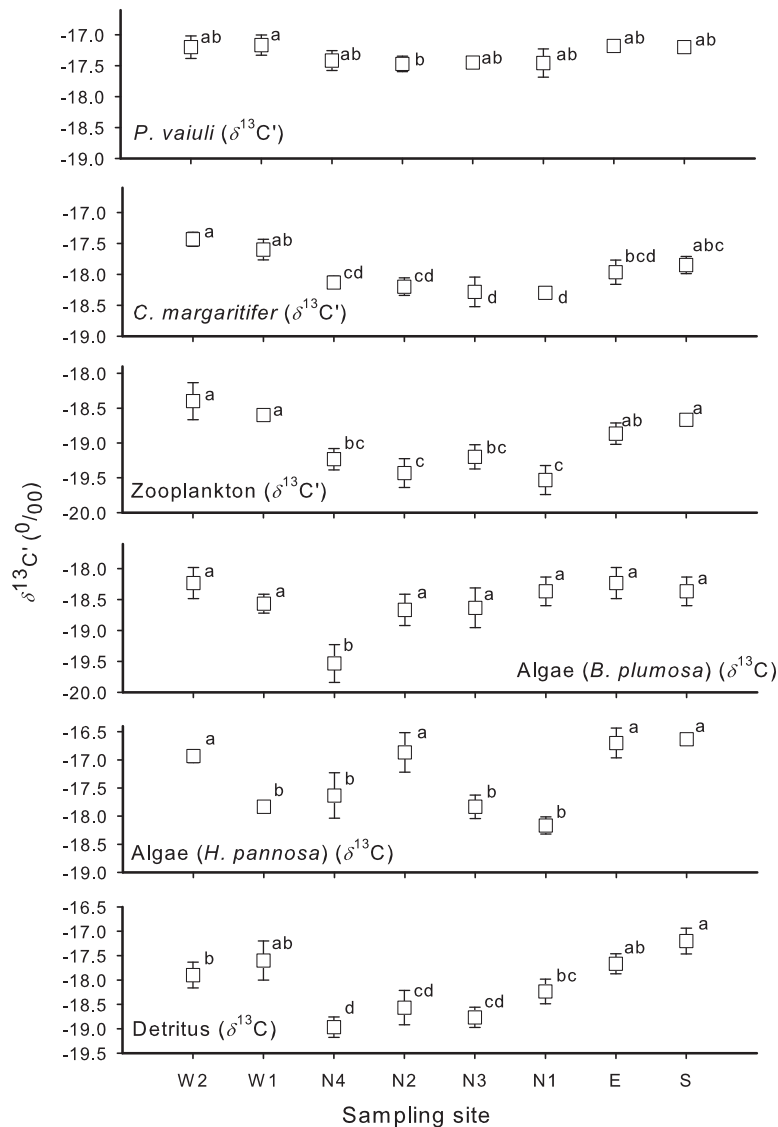


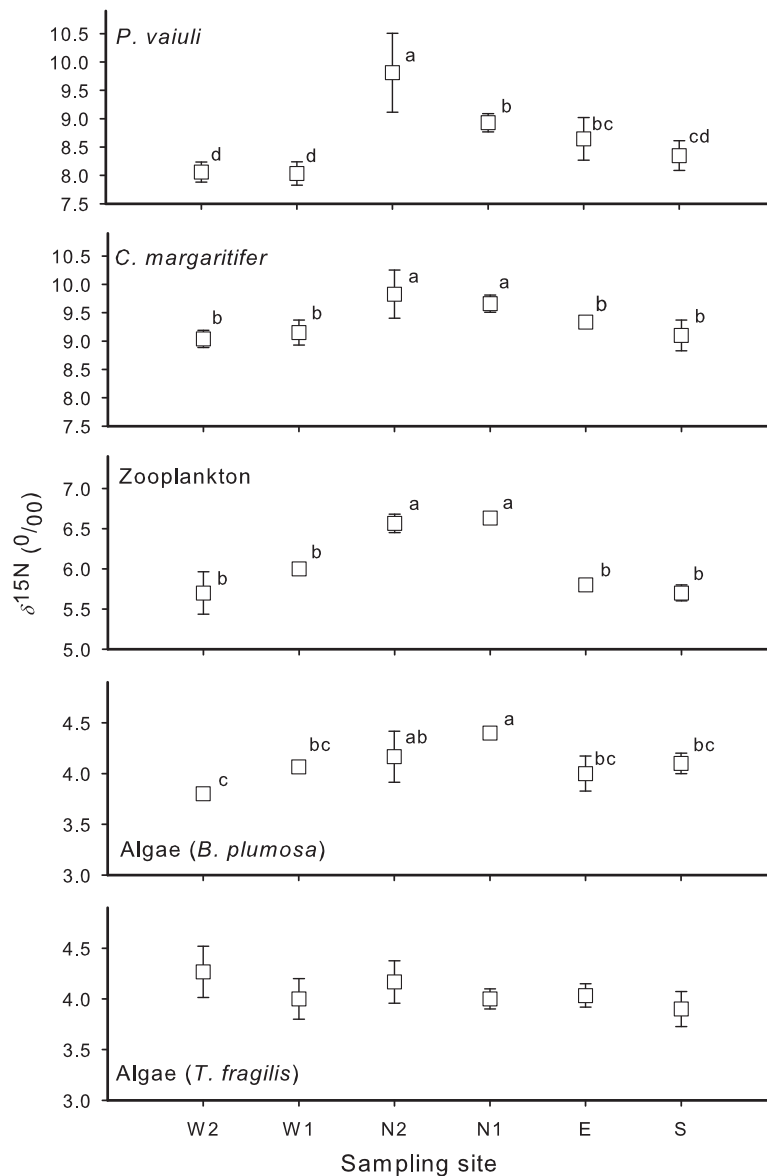
Fig. 4. Values of  $\delta^{13}\text{C}$  for *Pomacentrus vaiuli*, *Chromis margaritifer*, zooplankton and  $\delta^{13}\text{C}$  for macroalgae and detritus at different sampling sites in collection II. Symbols represent the mean of  $\delta^{13}\text{C} \pm 1$  SD. Mean values in the same diagram with different letters significantly differ (Scheffé's post hoc test,  $p < 0.05$ ).

Islands, Hobson and Chess, 1978; 82% in Kenting, Kuo and Shao, 1991; and 63.3% in collection I and 60.0% in II in the present study) and to a lesser extent, detritus (23.6% in I and 20.9% in II in the present study).

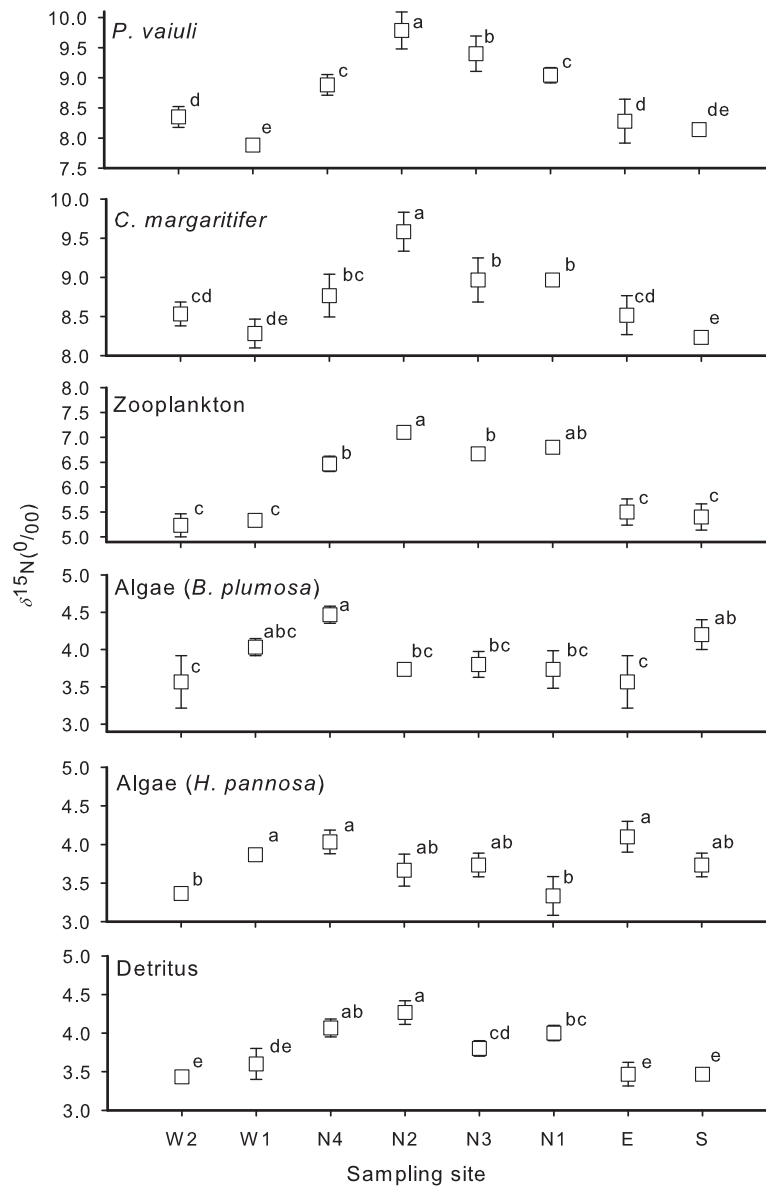
In general, increments in  $\delta^{13}\text{C}$  of 0–2‰ and in  $\delta^{15}\text{N}$  of 3.4‰ ( $\pm 1\%$ , SD) are expected to occur between consumers and their food resources (Minagawa and Wada, 1984; Post, 2002). This relationship can be used to postulate the contribution of a food item to an individual in terms of an energy source. In reference sites, increments in stable-isotopic signatures between *P. vaiuli* and the alga, *B. plumosa* ( $\delta^{13}\text{C}$ : 0.5–1.0‰ in I and 1.0–1.4‰ in II;  $\delta^{15}\text{N}$ : 3.9–4.6‰ in I and 3.9–4.8‰ in II), and zooplankton ( $\delta^{13}\text{C}$ : 0.6–1.1‰ in I and 0.5–0.8‰ in II;  $\delta^{15}\text{N}$ : 2.0–2.8‰ in I and 2.6–3.2‰ in II) were within one trophic level range, thereby matching well with the notion that algae and zooplankton are the major organic sources for *P. vaiuli*. In parallel, differences in stable-isotope signatures between *C. margaritifera* and zooplankton ( $\delta^{13}\text{C}$ : 0.0–0.3‰ in I and 0.0–0.3‰ in II;  $\delta^{15}\text{N}$ : 3.0–3.5‰ in I and

2.8–3.3‰ in II) support findings from the stomach content analysis that zooplankton was the major food item for *C. margaritifera*. In contrast, the difference of 4.7–5.0‰ in  $\delta^{15}\text{N}$  between detritus and *C. margaritifera* (in collection II) was higher than one level difference; thus detritus may be ruled out of a major nutrient source for *C. margaritifera*. It was noted that in most cases, the fish at experimental sites bore lower  $\delta^{13}\text{C}$  and higher  $\delta^{15}\text{N}$  than they did at reference sites (Figs. 3–6). However, because differences in their major potential food items mostly followed similar trends, the above remarks of each damselfish's feeding habits seem likely to be valid.

In a changing environment, fish with a higher feeding plasticity will be less affected by changes in available foods (Dill, 1983; Guzman and Robertson, 1989). They are also more capable of immigrating to newly developed habitat with suitable food substitutions (Ho et al., 2009). Since damselfish species with different extents of feeding plasticity were used in the present study, it is interesting to examine how they were affected by being associated with food chains connected to cage farming.



**Fig. 5.** Values of  $\delta^{15}\text{N}$  for *Pomacentrus vaiuli*, *Chromis margaritifera*, and their potential food items collected at different sampling sites in collection I. Symbols represent the mean of  $\delta^{15}\text{N} \pm 1$  SD. Mean values in the same diagram with different letters significantly differ (Scheffé's post hoc test,  $p < 0.05$ ).



**Fig. 6.** Values of  $\delta^{15}\text{N}$  for *Pomacentrus vaiuli*, *Chromis margaritifer*, and their potential food items collected at different sampling sites in collection II. Symbols represent the mean of  $\delta^{15}\text{N} \pm 1$  SD. Mean values in the same diagram with different letters significantly differ (Scheffé's post hoc test,  $p < 0.05$ ).

**Table 4**

Analysis of variance (ANOVA) testing the effect of site on C:N ratio and hepatosomatic index (HSI) of *Pomacentrus vaiuli* and *Chromis margaritifer*.

Damselfish	Collection	C:N ratio			HSI		
		d.f.	F-ratio	p-Value	d.f.	F-ratio	p-Value
<i>P. vaiuli</i>	I	5	3.34	0.01	5	6.72	<0.001
	II	7	3.13	0.01	7	0.99	0.45
<i>C. margaritifer</i>	I	5	2.39	0.06	5	4.96	<0.01
	II	7	3.18	0.01	7	1.80	0.12

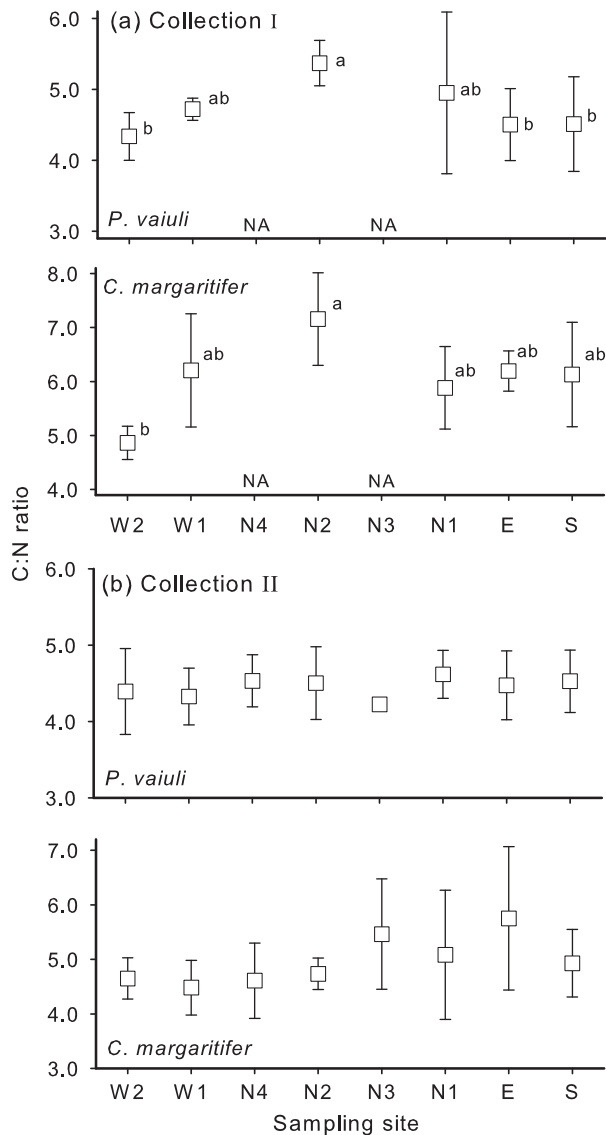
#### 4.2. Released organic waste and its trophic pathways in the food chain

In the present study, the fish feed used in the cage farm was mainly composed of trash fish and fishmeal. Assuming that the feed conversion rate was close to 2.5 (Marte et al., 2000; where, the feed is trash fish; and the targets are grouper, sea bass, and snapper), i.e., 2.5 kg of feed was required to produce 1 kg of fish biomass, the farm which produced 250 t of fish in 2006 would have released 375 t of lost feed (in the form of excess fish feed and feces)

to the environment. In other words, as much as 33.125 t of nitrogen would have been discharged from this cage culture system, according to the nutrient mass balance model proposed by Islam (2005).

The organic waste may occur in different forms. While soluble wastes are expected to have been diluted by the water mass and particulate matter lighter than sea water to have been suspended in it, the denser particulate matter would sink to the sediment, with dispersion possibly occurring over a spatial scale controlled

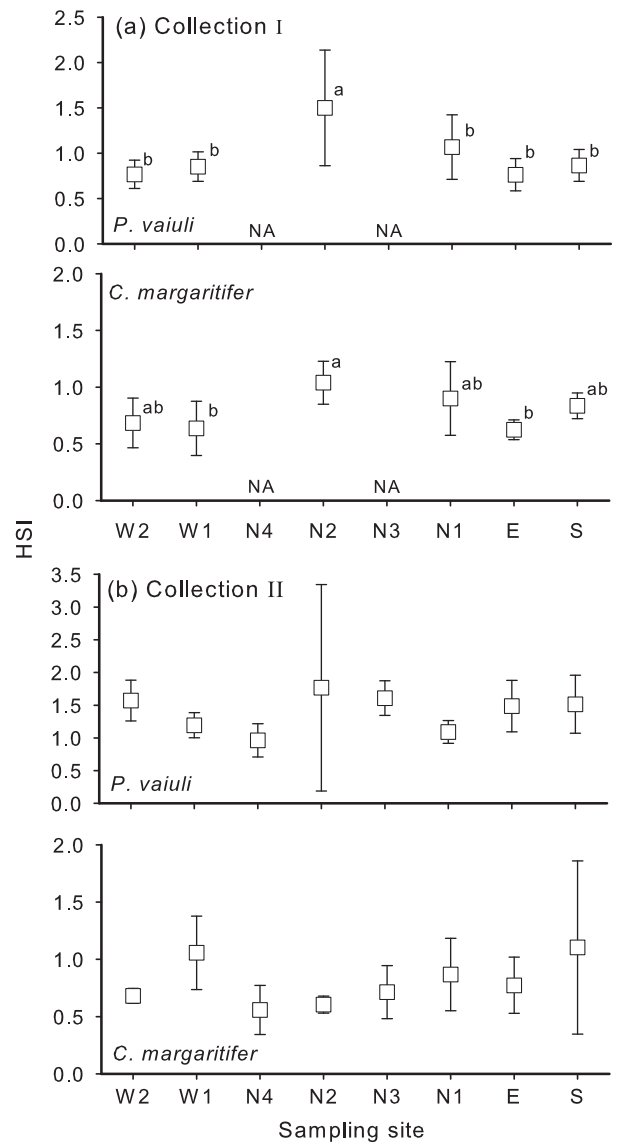




**Fig. 7.** C:N ratios of liver of *Pomacentrus vaiuli* and *Chromis margaritifer* from different sampling sites in collections (a) I and (b) II. NA: data not available. Symbols represent the mean C:N ratio  $\pm$  1 SD. Mean values in the same diagram with different letters significantly differ (Scheffé's post hoc test,  $p < 0.05$ ).

by water depths and current speeds (Sarà et al., 2006; Yokoyama et al., 2006). Accordingly, organic waste could enter the food web of the coral reef ecosystem through one or more food chains. When organic matter is retained in the sediment regime, phytoplankton/algae may assimilate additional sources of nitrogen (fish farm-derived nutrients), and predators, grazers, and deposit-feeding organisms may exploit the developed biofilm and detritus particles in the benthic food chain (Tewfik et al., 2005; Yokoyama and Ishihi, 2007). Otherwise consumers can directly exploit particulate organic matter (i.e., excessive feed) in the water column in the pelagic food chain.

The trophic pathway of these wastes was traced in the present study by comparing (1) stable-isotopic differences between organic waste and other elements in the food chain and (2) the stable-isotopic differences in organisms between experimental and reference sites. The stable isotopic signatures of the fish feed, i.e.,  $\delta^{13}\text{C}$  ranging from  $-20.8$  to  $-20.2\text{‰}$  and  $\delta^{15}\text{N}$  of  $8.7$ – $12.4\text{‰}$  (Table 2), did not deviate from those from fish feed used in fish cage farms in Japan (Yokoyama et al., 2006).



**Fig. 8.** Hepatosomatic indices (HSI) of *Pomacentrus vaiuli* and *Chromis margaritifer* from different sampling sites in collections (a) I and (b) II. NA: data not available. Symbols represent the mean HSI  $\pm$  1 SD. Mean values in the same diagram with different letters significantly differ (Scheffé's post hoc test,  $p < 0.05$ ).

Since the  $\delta^{13}\text{C}$  values of the fish feed were lower and the  $\delta^{15}\text{N}$  values were higher than those found in damselfishes, zooplankton, algae, and detritus at the reference sites (Figs. 3–6), lower  $\delta^{13}\text{C}$  and higher  $\delta^{15}\text{N}$  would be expected to have occurred in organisms at the experimental sites if they fed directly on the fish feed (viz., at the experimental sites). In most instances in the present study, isotopic differences in both zooplankton and damselfishes between the experimental and reference sites (Figs. 3–6) lend support to this prediction, indicating that the excess fish feed from the fish farm may have entered the food web through the pelagic food chain. While both zooplankton and damselfishes exploited this additional food source, damselfishes may have further benefited from preying on the nourished zooplankton.

On the other hand, the trophic pathway of feces was less clear. The isotopic composition of fish feces, which was not collected in the present study, was assumed to be  $3.5\text{‰}$  reduced in  $\delta^{13}\text{C}$  and  $3.3\text{‰}$  reduced in  $\delta^{15}\text{N}$  relative to the feed according to Yokoyama et al. (2006). So, it is unlikely that the damselfishes directly feed on the feces discharged from fish cages in view of the more than

one trophic level difference between feces and damselfishes in isotopic compositions. However, feces would have settled and mixed with natural detritus on the sediment, as implied by the erratic  $\delta^{13}\text{C}$  depletion and/or  $\delta^{15}\text{N}$  enrichment in detritus over the experimental sites (Figs. 4 and 6).

In the present study, the C:N ratio of livers and HSI were both used as indicators of the nutritional condition of sampling fish. Site effects occurred significantly in collection I in both damselfishes (Table 4), each species at N2 having higher C:N ratio and/or HSI. The higher values in damselfish from experimental sites (N2 in particular) in collection I (Figs. 7 and 8) suggest that organic fish farm wastes may have been a high-quality food for these fishes; with it individuals inhabiting close to the fish farm were able to maintain at a higher nutritional condition level. However, this phenomenon was not obvious in collection II. The nutritional status of damselfish varied temporally and seasonally and was highly related with environmental factors and life-history of these two fishes (Post and Parkinson, 2001; Bapary et al., 2012). Thus, further research on the effects of organic wastes on damselfish condition is needed.

When the uneaten waste particles settle down on the sea bottom, they are hydrolyzed to ammonia, thus forming  $^{15}\text{N}$ -enriched nitrates (Tenore, 1988); these nitrogenous nutrients could subsequently be taken up by primary producers such as phytoplankton and benthic algae (Umezawa et al., 2002; Vizzini and Mazzola, 2004; Yokoyama and Ishihi, 2007). In the present study, the relatively high  $\delta^{15}\text{N}$  detected in *B. plumosa* at site N1 in collection I (Fig. 5) may indicate exploitation of nutrients from farm wastes by this algal species at specific sites. Otherwise the direct effects of cage farm wastes on macroalgae were less evident, since unlike damselfishes or zooplankton, no clear patterns were found in differences in isotopic compositions between the experimental and reference sites. Given the semidiurnal tidal movements, strong currents, and steep reef slope in the study area, it is possible that the settled waste particles would be continually re-suspended by water movements, and taken by pelagic feeders (Cromey et al., 2002; Sarà et al., 2004). Also, the nitrogenous nutrients arising from the waste particles would be readily diluted by the large water mass. This may explain why overwhelming changes did not occur in the isotopic composition in macroalgae at sites near the cage farm.

The scale of cage farming at Xiaoliuchiu Island is relatively small (fish production = 250 t yr<sup>-1</sup>), compared to that at the northern tip of the Gulf of Aqaba (3000 t yr<sup>-1</sup>; Tsemel et al., 2006). Overall, at Xiaoliuchiu, the trophic pathway of the farm wastes through primary producers such as macroalgae was rarely evident. Given the scale and the local hydrological characteristics, the quantity of farm waste deposited on the sea bottom did not seem sufficient to be converted into additional dissolved inorganic nutrients for use by macroalgae. Alternatively, it appeared that the loading of farm wastes mainly entered the pelagic food chain linking zooplankton and omnivorous damselfishes.

#### 4.3. Fish act as environment cleaners

While it seems inevitable that cage fish farming will release organic waste into the surrounding marine environment, we are obliged to look for proper measures to reduce its impacts on ecosystems. Several authors emphasized the exploitation of particulate organic matter originating from aquaculture farms by filter feeders such as corals, shellfish, sponges, and polychaetes (Shpigel and Blaylock, 1991; Mazzola and Sarà, 2001; Lojen et al., 2005; Tsemel et al., 2006). Apparently these filter feeders share one characteristic in common: they are either sessile or benthic; they are passive feeders waiting for the food brought by currents. Our finding of farm wastes entering the coral reef ecosystem

through the pelagic food chain provides another thought for environment cleaning. Fish are important components of the pelagic food chain, and they are characterized by diverse feeding habits. Therefore, in a properly designed system, they could act as effective environment cleaners to remove introduced particulate organic matter from the environment. Damselfishes are particularly good candidates since they are among major residents of coral reefs. Damselfishes are diurnal feeders; many of them are omnivores or plankton feeders (like *P. vaiuli* and *C. margaritifera* in the present study) capable of grasping waste particles in the water column. Most damselfish species are inhabitants of shallow waters (<15 m deep); however, with the suspension of particles by water movements, they are able to remove the particulate organic matter released from the cage farm moored in deeper waters, as shown in the present study. In environments where strong currents prevail like the northern coast of Xiaoliuchiu Island, artificial reef emplacement is recommended to provide extra habitats under cage farms to support additional fish populations. It is expected that fish assemblages together with benthic organisms developed around artificial reefs could act as an efficient biological sink for waste organic matter released from fish cages, thereby helping reduce the environmental impacts of cage farming on coral reefs.

#### Acknowledgments

We thank Y.H. Liu for help with the fieldwork and an anonymous reviewer for substantial improvements to this manuscript. This research was funded by the National Science Council of the Republic of China under Grants NSC93-2311-B-001-051, 93-2621-B-001-005, and 96-2621-B-001-009-MY3 and the Biodiversity Research Center, Academia Sinica. The experiments were performed with permission of the IACUC, Academia Sinica, under Protocol No. RfICBDJR2006028.

#### References

- Bapary, M.A.J., Amin, M.N., Takemura, A., 2012. Food availability as a possible determinant for initiation and termination of reproductive activity in the tropical damselfish *Chrysiptera cyanea*. *Marine Biol. Res.* 8, 154–162.
- Beveridge, M.C.M., 2004. *Cage aquaculture*, third ed. Blackwell Pub, Oxford.
- Beveridge, M.C.M., Ross, L.G., Kelly, L.A., 1994. *Aquaculture and biodiversity*. *Ambio* 23, 497–502.
- Brooks, K.M., Mahnken, C.V.W., 2003. Interactions of Atlantic salmon in the Pacific northwest environment II. Organic wastes. *Fish Res.* 62, 255–293.
- Cromey, C.J., Nickell, T.D., Black, K.D., Provost, P.G., Griffiths, C.R., 2002. Validation of a fish farm waste resuspension model by use of a particulate tracer discharged from a point source in a coastal environment. *Estuaries* 25, 916–929.
- Dai, C.F., 2007. Distribution and species diversity of reef coral in Taiwan (III) Southern Taiwan including Xiaoliuchiu. *Fisheries Agent. Council of Agriculture, Taipei*.
- Dill, L.M., 1983. Adaptive flexibility in the foraging behavior of fishes. *Can. J. Fish. Aquat. Sci.* 40, 398–408.
- El-Sabaawi, R., Dower, J.F., Kainz, M., Mazumder, A., 2009. Characterizing dietary variability and trophic positions of coastal calanoid copepods: insight from stable isotopes and fatty acids. *Mar. Biol.* 156, 225–237.
- Fujioka, Y., Shimoda, T., Srithong, C., 2007. Diversity and community structure of macrobenthic fauna in shrimp aquaculture ponds of the gulf of Thailand. *Jarq-Jpn. Agric Res. Q* 41, 163–172.
- Guzman, H.M., Robertson, D.R., 1989. Population and feeding responses of the corallivorous pufferfish *Arothron meleagris* to coral mortality in the Eastern Pacific. *Mar. Ecol.-Prog. Ser.* 55, 121–131.
- Hall-Spencer, J., White, N., Gillespie, E., Gillham, K., Foggo, A., 2006. Impact of fish farms on maerl beds in strongly tidal areas. *Mar. Ecol.-Prog. Ser.* 326, 1–9.
- Heikoop, J.M., Risk, M.J., Lazier, A.V., Edinger, E.N., Jompa, J., Limmon, G.V., Dunn, J.J., Browne, D.R., Schwarcz, H.P., 2000. Nitrogen-15 signals of anthropogenic nutrient loading in reef corals. *Mar. Pollut. Bull.* 40, 628–636.
- Hiatt, R.W., Strasburg, D.W., 1960. Ecological relationships of the Fish Fauna on coral reefs of the Marshall Islands. *Ecol. Monogr.* 30, 66–127.
- Ho, C.T., Kao, S.J., Dai, C.F., Hsieh, H.L., Shiah, F.K., Jan, R.Q., 2007. Dietary separation between two blennies and the Pacific gregory in northern Taiwan: evidence from stomach content and stable isotope analyses. *Mar. Biol.* 151, 729–736.
- Ho, C.T., Fu, Y.C., Sun, C.L., Kao, S.J., Jan, R.Q., 2009. Plasticity of feeding habits of two *Plectroglyphidodon* damselfishes on coral reefs in southern Taiwan: evidence from stomach content and stable isotope analyses. *Zool. Stud.* 48, 649–656.

- Hobson, E.S., Chess, J.R., 1978. Trophic relationships among fishes and plankton in lagoon at Enewetak Atoll, Marshall Islands. *Fish. Bull.* 76, 133–153.
- Holmer, M., 1991. Impacts of aquaculture on surrounding sediments: Generation of organic-rich sediments. In: De Pauw, N., Joyce, J. (Eds.), *Aquaculture and the Environment*. Eur. Aquacult. Soc. Spec. Publ., pp. 155–175.
- Holmer, M., Kristensen, E., 1992. Impact of marine fish cage farming on metabolism and sulfate reduction of underlying sediments. *Mar. Ecol.-Prog. Ser.* 80, 191–201.
- Huang, C.C., Tang, H.J., Wang, B.S., 2010. Numerical modeling for an *in situ* single-point-mooring cage system. *IEEE J. Ocean Eng.* 35, 565–573.
- Hyslop, E.J., 1980. Stomach contents analysis: a review of methods and their application. *J. Fish Biol.* 17, 411–429.
- Islam, M.S., 2005. Nitrogen and phosphorus budget in coastal and marine cage aquaculture and impacts of effluent loading on ecosystem: review and analysis towards model development. *Mar. Pollut. Bull.* 50, 48–61.
- Iwama, G.K., 1991. Interactions between aquaculture and the environment. *Crit. Rev. Environ. Contrl.* 21, 177–216.
- Jacob, U., Mintenbeck, K., Brey, T., Knust, R., Beyer, K., 2005. Stable isotope food web studies: a case for standardized sample treatment. *Mar. Ecol. Prog. Ser.* 287, 251–253.
- Jan, R.Q., Dai, C.F., Chang, K.H., 1994. Monitoring of hard substrate communities. In: Kramer, K.J.M. (Ed.), *Biomonitoring of coastal waters and estuaries*. CRC, Boca Raton, pp. 282–310.
- Karakassis, I., Tsapakis, M., Hatziyanni, E., Papadopoulou, K.N., Plaiti, W., 2000. Impact of cage farming of fish on the seabed in three Mediterranean coastal areas. *ICES J. Mar. Sci.* 57, 1462–1471.
- Kuo, S.R., Shao, K.T., 1991. Feeding habits of damselfishes (Pomacentridae) from the southern part of Taiwan. *J. Fish. Soc. Taiwan* 18, 165–176.
- Liao, I.C., Huang, T.S., Tsai, W.S., Hsueh, C.M., Chang, S.L., Leano, E.M., 2004. Cobia culture in Taiwan: current status and problems. *Aquaculture* 237, 155–165.
- Logan, J.M., Jardine, T.D., Miller, T.J., Bunn, S.E., Cunjak, R.A., Lutcavage, M.E., 2008. Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. *J. Anim. Ecol.* 77, 838–846.
- Lojen, S., Spanier, E., Tsemel, A., Katz, T., Eden, N., Angel, D.L., 2005.  $\delta^{15}\text{N}$  as a natural tracer of particulate nitrogen effluents released from marine aquaculture. *Mar. Biol.* 148, 87–96.
- Loya, Y., Lubinevsky, H., Rosenfeld, M., Kramarsky-Winter, E., 2004. Nutrient enrichment caused by *in situ* fish-farms at Eilat, Red Sea is detrimental to coral reproduction. *Mar. Pollut. Bull.* 49, 344–353.
- Loya, Y., Rosenfeld, M., Kramarsky-Winter, E., 2005. Nutrient enrichment and coral reproduction: empty vessels make the most sound (response to a critique by B. Rinkevich). *Mar. Pollut. Bull.* 50, 114–118.
- Marte, C.L., Cruz, P., Flores, E.E.C., 2000. Recent developments in freshwater and marine cage aquaculture in the Philippines. In: Liao, I.C., Lin, C.K. (Eds.), *Cage Aquaculture in Asia*. In: *Proceedings of the First International Symposium on Cage Aquaculture in Asia*. 2–6 November 1999, Asian Fisheries Society, Manila and World Aquaculture Society-Southeast Asian Chapter, Bangkok, pp. 83–96.
- Mazzola, A., Sarà, G., 2001. The effect of fish farming organic waste on food availability for bivalve molluscs (Gaeta Gulf, Central Tyrrhenian, MED): stable carbon isotopic analysis. *Aquaculture* 192, 361–379.
- Minagawa, M., Wada, E., 1984. Stepwise enrichment of  $^{15}\text{N}$  along food chains: further evidence and the relation between  $\delta^{15}\text{N}$  and animal age. *Geochim. Cosmochim. Acta* 48, 1135–1140.
- Pinnegar, J.K., Polunin, N.V.C., 1999. Differential fractionation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  among fish tissues: implications for the study of trophic interactions. *Funct. Ecol.* 13, 225–231.
- Polunin, N.V.C., Morales-Nin, B., Pawsey, W.E., Cartes, J.E., Pinnegar, J.K., Moranta, J., 2001. Feeding relationships in Mediterranean bathyal assemblages elucidated by stable nitrogen and carbon isotope data. *Mar. Ecol.-Prog. Ser.* 220, 13–23.
- Porrello, S., Tomassetti, P., Manzueto, L., Finoia, M.G., Persia, E., Mercatali, I., Stipa, P., 2005. The influence of marine cages on the sediment chemistry in the Western Mediterranean Sea. *Aquaculture* 249, 145–158.
- Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83, 703–718.
- Post, J.R., Parkinson, E.A., 2001. Energy allocation strategy in young fish: allometry and survival. *Ecology* 82, 1040–1051.
- Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., Montaña, C.G., 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia* 152, 179–189.
- Rinkevich, B., 2004. Nutrient enrichment and coral reproduction: between truth and repose (a critique of Loya et al.). *Mar. Pollut. Bull.* 50, 111–113.
- Risk, M.J., Erdmann, M.V., 2000. Isotopic composition of nitrogen in stomatopod (Crustacea) tissues as an indicator of human sewage impacts on Indonesian coral reefs. *Mar. Pollut. Bull.* 40, 50–58.
- Sarà, G., Scilipoti, D., Mazzola, A., Modica, A., 2004. Effects of fish farming waste to sedimentary and particulate organic matter in a southern Mediterranean area (Gulf of Castellammare, Sicily): a multiple stable isotope study ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ). *Aquaculture* 234, 199–213.
- Sarà, G., Scilipoti, D., Milazzo, M., Modica, A., 2006. Use of stable isotopes to investigate dispersal of waste from fish farms as a function of hydrodynamics. *Mar. Ecol.-Prog. Ser.* 313, 261–270.
- Schmidt, K., Atkinson, A., Stubing, D., McClelland, J.W., Montoya, J.P., Voss, M., 2003. Trophic relationships among Southern Ocean copepods and krill: some uses and limitations of a stable isotope approach. *Limnol. Oceanogr.* 48, 277–289.
- Shpigel, M., Blaylock, R.A., 1991. The Pacific oyster, *Crassostrea gigas*, as a biological filter for a marine fish aquaculture pond. *Aquaculture* 92, 187–197.
- Sweeting, C.J., Polunin, N.V.C., Jennings, S., 2006. Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. *Rapid Commun. Mass Spectrom.* 20, 595–601.
- Tenore, K.R., 1988. Nitrogen in benthic food chains, in: Blackburn, T.H., Sørensen, J. (Eds.), *Nitrogen cycling in coastal marine environment*. John Wiley & Sons, pp. 191–206.
- Tewfik, A., Rasmussen, J.B., McCann, K.S., 2005. Anthropogenic enrichment alters a marine benthic food web. *Ecology* 86, 2726–2736.
- Tsemel, A., Spanier, E., Angel, D.L., 2006. Benthic communities of artificial structures: effects of mariculture in the Gulf of Aqaba (Eilat) on development and bioaccumulation. *Bull. Mar. Sci.* 78, 103–113.
- Umezawa, Y., Miyajima, T., Yamamuro, M., Kayanne, H., Koike, I., 2002. Fine-scale mapping of land-derived nitrogen in coral reefs by  $\delta^{15}\text{N}$  in macroalgae. *Limnol. Oceanogr.* 47, 1405–1416.
- Vizzini, S., Mazzola, A., 2004. Stable isotope evidence for the environmental impact of a land-based fish farm in the western Mediterranean. *Mar. Pollut. Bull.* 49, 61–70.
- Vizzini, S., Mazzola, A., 2006. The effects of anthropogenic organic matter inputs on stable carbon and nitrogen isotopes in organisms from different trophic levels in a southern Mediterranean coastal area. *Sci. Total Environ.* 368, 723–731.
- Yokoyama, H., Ishihi, Y., 2007. Variation in food sources of the macrobenthos along a land-sea transect: a stable isotope study. *Mar. Ecol.-Prog. Ser.* 346, 127–141.
- Yokoyama, H., Abo, K., Ishihi, Y., 2006. Quantifying aquaculture-derived organic matter in the sediment in and around a coastal fish farm using stable carbon and nitrogen isotope ratios. *Aquaculture* 254, 411–425.