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Using Stable Carbon and Nitrogen Isotopes to Evaluate Parrotfish Diet

Maria Elisa B. Gerona[1] and Florence C. Evacitas[2]

[1]Division of Natural Sciences and Mathematics, UPV Tacloban College, Tacloban City, Philippines
[2]Natural Sciences and Mathematics Division UP Cebu College, Cebu City, Philippines

ABSTRACT

With limited inconclusive data provided by gut content analysis, stable isotope analysis has recently emerged to validate trophic position and dietary intake. In this study, a dual isotope approach was used to reveal parrotfish feeding. Comparisons of $\delta^{13}C$ and $\delta^{15}N$ values of muscle and liver among yellowbarred (*Scarus dimidiatus),* rosy cheek *(S. psittacus),* and blue-barred *(S. ghobban)* parrotfishes from Canigao Island, Matalom, Leyte were made to track dietary shifting and to compare dietary carbon intake. Trophic assignment was based on the assumption that consumers are enriched by a factor of 3-4‰ for $\delta^{15}N$, relative to their diet. The $\delta^{13}C$ values of muscle tissues of the three species of parrotfish were significantly higher (p=0.001) than those of their liver suggesting dietary shifting. The $\delta^{13}C$ values of both muscle and liver tissues of *S. dimidiatus* were significantly (p<0.001) higher than those of *S. psittacus* and *S. ghobban,* but $\delta^{13}C$ values of muscle and liver of *S. psittacus* and *S. ghobban* did not vary significantly. These mean that *S. dimidiatus* have different long term and recent dietary carbon intake compared to the other two species, while *S. psittacus* and *S. ghobban* have relatively the same dietary carbon intake. Considering the 1‰ $\delta^{13}C$ trophic enrichment of consumers relative to their diet, possible dietary carbon sources of the sampled parrotfish include *Dendronephthya* spp., *Ulva reticulata*, *Sargassum oligocystum*, *Dictyota* sp., *Digenea* sp., *Chlorodesmis* sp., and *Sargassum muticum* suggesting that parrotfishes are generalist consumers. Mean stable isotope nitrogen ratios of *S. dimidiatus* (5.9‰), *S. psittacus* (6.9‰) and *S. ghobban* (6.7‰) together with their carbon isotope ratios confirmed that all sampled parrotfish species are generalist primary consumers.

Keywords: $\delta^{13}C$, $\delta^{15}N$, Canigao Island, diet, trophic position

Correspondence: M.E. B. Gerona. *Address:* Division of Natural Sciences and Mathematics University of the Philippines Visayas Tacloban College, Tacloban City, Philippines *E-mail*: me_gerona@yahoo.com.

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INTRODUCTION

Parrotfishes are important economic species and are popular to locals because of the potential food source they offer. Field studies have shown their ecological importance in marine food webs (Nemeth and Appeldoorn, 2009; Wainwright *et al.*, 2002). The complexity of foraging studies by parrotfish is probably associated by their mobility and with the difficulty in making direct observations in the field. Moreover, due to their nature of food (algae associated with reef matrix) gut content studies in the laboratory are difficult, laborious, and inconclusive (de la Morinière *et al*., 2003).

Parrotfishes are one of the many herbivorous coral reef fishes that limit the growth of filamentous alga from growing on living corals in shallow zones of coral reef (Wainwright *et al.*, 2002). Scleractinian corals are the major architects of tropical reefs, providing the structural framework for a highly diverse assemblage of marine organisms (Rotjan and Lewis, 2005). Parrotfishes use their fused beak-like jaws to graze epilithic and endolithic alga from dead carbonate substrates, yet some parrotfishes also consume live coral. In previous work on the back reef habitat of Carrie Bow Cay, Belize, Rotjan and Lewis (2005) observed that about 2% of all *Sparisoma viride* bites are taken on live *Porites astreoides* corals, while no live coral consumption was observed for any other parrotfish.

Trophic relationships in marine ecosystems can be studied through stable isotope analysis or gut content analysis. The latter is traditionally employed, however, it is not ideal for parrotfish because of its mill in the pharyngeal region where food is finely grounded and the absence of a true stomach (Alfaro *et al.*, 2009). Furthermore, it has been criticized for the "snapshot" nature of sampling (Pinnegar, 2000; Pinnegar *et al*., 2001), the need for high degree of taxonomic precision, prevalence of unrecognized dietary items, bias from regurgitation during capture, and the difficulty of obtaining sufficient sampling frequency to draw significant conclusions. (Vander Zanden and Hulshof, 1998; Pinnegar *et al*., 2001; MacNeil *et al*., 2005). It is most important to note that stable isotope techniques are most effective when used as a complementary tool with other techniques to ensure the most conclusive studies (Peterson, 1999; de la Morinière *et al*., 2003; McCutchan *et al*., 2003).

Laboratory and field studies report a consistent stepwise increase in the heavy nitrogen isotope ($\delta^{15}N$) of 3-4 ‰ per trophic level increment

(Corbisier *et al.*, 2006; Layman *et al*. 2007). Thus, the $\delta^{15}N$ of an organism can be used to indicate trophic position of consumers (Vander Zanden and Hulshof, 1998). Often, the $\delta^{15}N$ values are presented with $\delta^{13}C$ ratios (^{13}C:^{12}C), because they are generally conserved at each trophic step in an ecosystem, thus, serve as indicators of dietary carbon source (Post *et al*., 2007). Although $\delta^{13}C$ signatures of consumers are similar to those of their food, studies have shown that they generally differ by less than 1‰ (DeNiro and Epstein, 1978; Fry and Sherr, 1984). If two or more species have distinct $\delta^{13}C$ values, this implies multiple sources of dietary carbon within an ecosystem (MacNeil *et al.*, 2005).

In the light of the potential importance of defining parrotfish trophic position, natural diet, and the limitations of stomach content analysis, this study used the stable isotope approach to determine dietary shifts among yellowbarred (*S. dimidiatus),* rosy cheek (*S. psittacus),* and blue-barred (*S. ghobban)* by comparing $\delta^{15}N$ and $\delta^{13}C$ values of muscle and liver tissues, compare the dietary carbon intake among the three species of parrotfish, determine the possible dietary carbon sources of three species of parrotfish, and identify the trophic position of the three species of parrotfish.

MATERIALS AND METHODS

Site and Field Sampling

Samples of parrotfish were collected once from the reef off Canigao Island in Matalom, Leyte on November 2009. Three species of parrotfish (i.e. yellowbarred, rosy cheek, and blue-barred) were obtained from the surrounding areas of Canigao. Samples of possible diet sources including macroalgae, seagrass, and corals were collected from the surrounding areas of the island approximately 5-8 m depth. All samples were packed in ice and were utilized as reference samples for taxonomic determination. Identifications were made with the aid of Calumpong and Meñez (1997), Trono (1997), Gerry (1997) and Carpenter and Neim (1998).

*Preparation of S*amples

Fork length (FL) of each fish was measured to the nearest 0.01 cm from the tip of the upper jaw to the end of the middle caudal rays (Bruggemann *et al.*, 1994). Fish tissue samples were collected by excising approximately

20 g of muscle (along the spine) and 10 g of liver (anterior part of either lobe) from individual samples of parrotfish. Samples of possible diet sources were rinsed with distilled water and all visible encrustation were removed from its surface by thorough scraping (Fourgourean *et al*., 2005).

Stable Isotope Analyses

All samples of approximately 20 g, except liver, were dried at 60°C for six hours in a convection oven (SIBATA) and ground into powder with mortar and pestle, and food mill. Liver samples were dried in a vacuum oven at 25°C at the Department of Science and Technology Laboratory, Lahug, Cebu City. Lipid extraction was not done as suggested by Post *et al.* (2007). Dried samples (5 g) were stored in sealed vials and kept in refrigerator until shipment.

Stable carbon and nitrogen isotope measurements were performed by the Stable Isotope Ratio Facility for Environmental Research (SIRFER) of the Biology Department, University of Utah, USA using continuous flow isotope ratio mass spectrometer.

Data Analyses

Data on the stable carbon isotope ratio between tissue types and among species were tested for significant difference using ANOVA. A Tukey multiple comparison test was performed at 0.05 significance level to determine specific pairwise differences. Data analysis was performed using WINKS SDA Software.

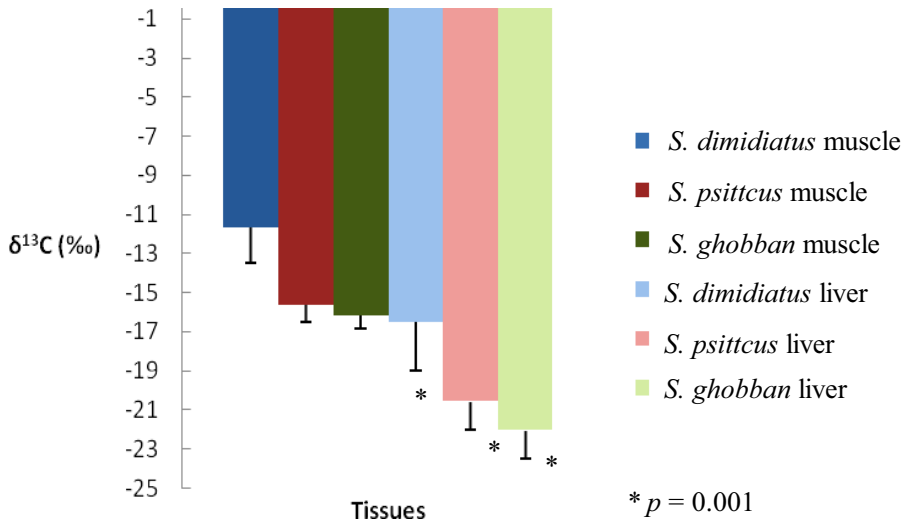
RESULTS AND DISCUSSION

Dietary Pattern of Parrotfish

Intraspecies tissue $\delta^{13}C$ differences were observed in muscle and liver tissues of the three species of parrotfish. *S. dimidiatus, S. psittacus, and S. ghobban* muscles were enriched significantly by 5-6‰ (*p=0.01*) in $\delta^{13}C$ over their liver tissues (Fig. 1). At 0.05 significance level, the mean $\delta^{13}C$ value of the *S. dimidiatus* muscle (-11.66±1.76‰) was significantly higher than that of its liver (-16.50 ±2.50‰). Similarly, mean $\delta^{13}C$ values of muscles of *S. psittacus* (-15.70 ± 0.82‰) and *S. ghobban* (-16.16 ± 0.65‰) were significantly higher than their liver tissues (-20.57 ± 1.41‰ and -22.1

± 1.14‰, respectively). These results imply that all three species of parrotfish exhibit dietary shifting, with their long term diet sources more enriched in $\delta^{13}C$ than their recent diet sources. The similarity of $\delta^{13}C$ values of the *S. dimidiatus* liver with those of the muscles of *S. psittacus* and *S. ghobban* may indicate similarity in the recent diet sources of the former species with long term diet sources of two latter species (Fig. 1).

Fig.1. Mean (±SD) $\delta^{13}C$ values of muscle and liver tissues of *S. dimidiatus, S. psittacus,* and *S. ghobban*



Recent studies have discussed the potential of exploiting metabolic differences among tissues to explain differences in stable isotope values, suggesting that tissues with high metabolic rates, such as liver, more rapidly reflect changes in stable isotope values than less metabolically active tissues, like muscles (Macneil *et al*., 2005). Macneil *et al.* (2005) found that inter-tissue differences in the stable isotope levels in captive stingrays were relative to the metabolic turnover rates of each tissue, demonstrating that liver $\delta^{15}N$ turned over twice as fast as in muscles and that cartilage turnover was slowest among tissues. Subsequently, MacNeil *et al.* (2005) found that relative isotope compositions of shark tissues can elucidate the known seasonal feeding dynamics of inshore shortfin makos and their bluefish prey and support the suspected trophic roles of blue sharks and common threshers as year-round generalist predators in the northwest Atlantic. Therefore, stable isotopes of each metabolically

distinct tissue represent a different period of feeding that decreases with increasing metabolic turnover.

The potential for inter-tissue comparison of $\delta^{13}C$ and $\delta^{15}N$ had been suggested by (MacNeil *et al.* 2005). Wild marine fishes with a wide range of seasonal diet choices exhibit diet switching (MacNeil *et al.* 2005), and this was also supported by the results of this study. The significant difference between muscle and liver $\delta^{13}C$ signatures in *S. dimidiatus, S. psittacus,* and *S. ghobban* indicates difference between dietary $\delta^{13}C$ over the different feeding periods represented by each tissue (Table 1). The results suggest that differences in stable isotope values among parrotfish tissues from Canigao Island, Matalom, Leyte waters reflect a dietary shift for *S.dimidiatus, S. psittacus,* and *S. ghobban*, as reflected in their $\delta^{13}C$ muscle and tissue values. This suggests that even in tropical waters, dietary shifting is possible in species of the same genus (Lobel and Ogden, 1981; Chow, 2005).

Table 1. *S. dimidiatus, S. psittacus and S. ghobban*. Mean (± SD) muscle and liver tissue values of $\delta^{13}C$ and $\delta^{15}N$ for parrotfish sampled from Canigao Island, Matalom, Leyte in November, 2009. FL=fork length (cm)

Tissue	*S. dimidiatus* (N=7)	*S. psittacus* (N=13)	*S. ghobban* (N=14)
Fork length (range)	19 (16-24)	22 (19-25)	24 (21-27)
Carbon			
Muscle	-11.66 ± 1.76	-15.70 ± 0.82	-16.16 ± 0.65
Liver	-16.50 ± 2.50	-20.57 ± 1.41	-22.1 ± 1.14
Nitrogen			
Muscle	5.9 ± 0.84	6.92 ± 0.43	6.70 ± 0.26
Liver	4.4 ± 0.57	5.49 ± 0.38	5.8 ± 0.36

Dietary carbon intake of parrotfish

All sampled parrotfishes have different dietary carbon sources, as reflected in their $\delta^{13}C$ muscle and liver tissue values (Figs.2 and 3). The mean $\delta^{13}C$ value of *S. dimidiatus* muscle varied significantly ($p<0.05$) from *S. ghobban* and *S. psittacus,* but the mean $\delta^{13}C$ value of the *S. ghobban* liver and muscle did not vary significantly from that of *S. psittacus*.

S. ghobban and *S. psittacus* $\delta^{13}C$ liver values are 5.6‰ and 4.1‰ greater than $\delta^{13}C$ liver signature of *S. dimidiatus*. The mean $\delta^{13}C$ liver signature of *S. dimidiatus* varied significantly ($p<0.05$) from that of *S. ghobban* and *S.*

psittacus. However, the means of *S. ghobban* and *S. psittacus* $\delta^{13}C$ liver values did not vary significantly.

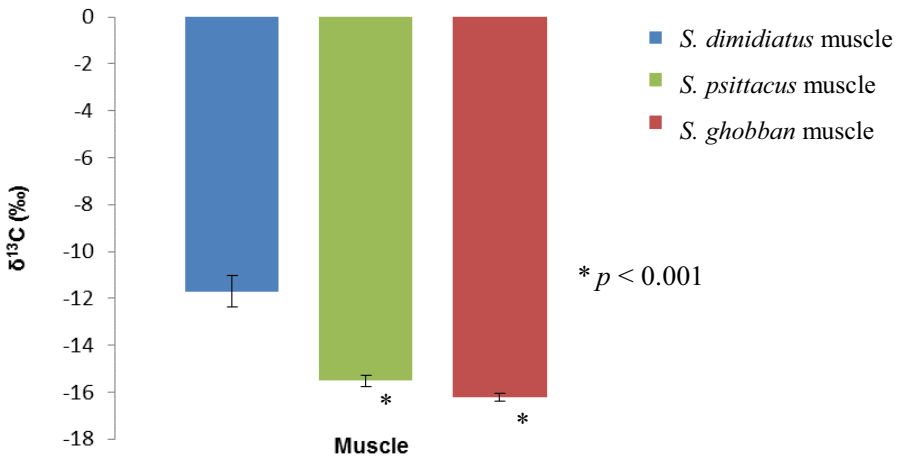


Fig. 2. Mean (±SD) $\delta^{13}C$ of muscle tissues of *S. dimidiatus, S. psittacus,* and *S. ghobban*.

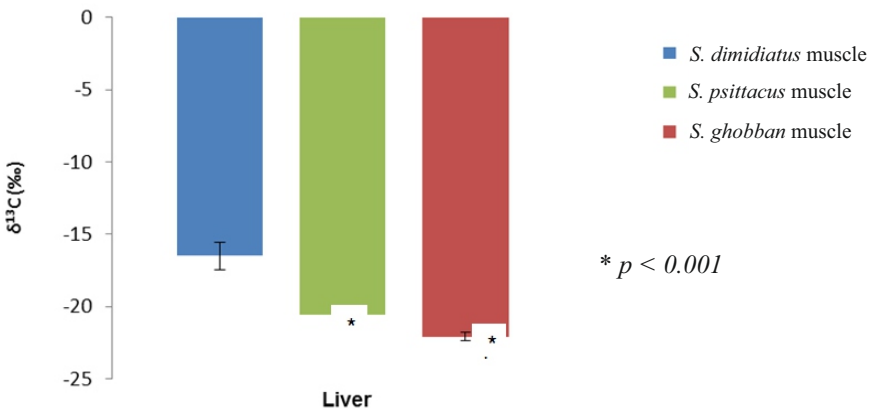


Fig. 3. Mean (±SD) $\delta^{13}C$ of liver tissues of *S. dimidiatus, S. psittacus,* and *S. ghobban*.

Studies of feeding selectivity in herbivorous fishes have provided insight into the factors that affect food choice (Mantyka and Bellwood, 2007). The foraging selectivity of Indo-Pacific parrotfishes have been relatively well-studied (Bellwood and Choat, 1990) and corallivory has been found to be restricted to a few large excavating species that possess oral jaws and pharyngeal teeth capable of exerting large forces on the cutting edge. In the Caribbean, the most important coral grazing fish appears to be the stoplight parrotfish *Sparisoma viride* because of its large adult size (Sanchez *et al.,* 2004).

Horn and Neighbors (1984) showed that food selection by herbivorous fishes is largely dependent on energy value of the plant, assimilation efficiency of the fish for the plant, nutrient content (in particular N), and most importantly, on the relative availability of the plant for the fish. The difference in $\delta^{13}C$ values of the muscle and liver tissues of *S. dimidiatus, S. psittacus,* and *S. ghobban* (Figs. 2 and 3) revealed that the three species have different carbon intake. This suggests that one species of food source is not enough for the nutrient requirement of the fish resulting to a wide variety of food preferences.

Possible Dietary Carbon Sources

The $\delta^{13}C$ and $\delta^{15}N$ composition of possible diet sources of *S. dimidiatus*, *S. ghobban*, and *S. psittacus* is reported in Table 2. Different species of possible diet sources exhibited different $\delta^{13}C$ and $\delta^{15}N$ values. Even species of the same genus have entirely different $\delta^{13}C$ and $\delta^{15}N$ values, as shown by the two species of *Dendronephthya, Sargassum, and Halimeda.*

Table 3 summarizes the possible long-term and recent dietary carbon sources of the sampled parrotfish which were identified based on 0-1‰ theoretical enrichment in the $\delta^{13}C$ values in the fish tissues compared to the sampled macroalgae, seagrass, and corals. Long-term and recent dietary carbon sources of *S. dimidiatus* are the same, except for the two species of *Dendronephthya*. On the other hand, *S. psittacus* had completely different long-term and recent dietary carbon sources. For *S. ghobban*, long-term dietary carbon source includes *Dendronephthya* spp., while its recent dietary carbon sources are composed of the same species of *Dendronephtya*, *Digenea simplex*, *Sargassum muticum*, and *Sargassum oligocystum*.

In principle, consumers are slightly enriched by a factor of 3-4‰ and <1‰ for N and C, respectively, relative to its diet (Peterson, 1999).

Table 2. $\delta^{13}C$ and $\delta^{15}N$ values of possible dietary carbon sources of parrotfish.

Group	Species	$\delta^{13}C$	$\delta^{15}N$
Macroalgae			
	Actinotrichia sp.	-9.7	3.1
	Bornetella sp.	-9.0	2.9
	Caulerpa sp.	-9.7	3.5
	Chlorodesmis sp.	-13.1	2.2
	Cladostephus sp.	-10.1	4.1
	Dictyota sp.	-11.9	3.4
	Digenea sp.	-13.8	3.5
	Halimeda fragilis	-2.2	2.3
	Halimeda incrassata	-8.6	2.5
	Halimeda macrolaba	-5.4	0.9
	Hydroclathrus clathratus	-7.1	3.2
	Padina minor	-6.9	2.7
	Sargassum muticum	-13.1	3.0
	Sargassum oligocystum	-13.1	3.3
	Turbinaria conoides	-8.5	3.2
	Ulva reticulata	-13.6	3.6
	Unknown species	-18.9	3.6
Seagrass			
	Cymodocea rotundata	-8.1	1.7
	Halophila ovalis	-7.3	2.3
	Thalassia hemprichii	-7.4	1.6
Corals			
	Dendronephthya sp1.	-11.5	4.3
	Dendronephthya sp2.	-14.8	3.7
	Diploria sp.	-6.2	4.5
	Isis sp.	-9.6	5.1
	Melithea sp.	-7.1	5.7
	Seriatopora sp.	-6.4	2.7
	Sarcophyton elegans	-7.7	3.6

The present study has provided the possible dietary carbon sources of yellowbarred, rosy cheek, and blue-barred parrotfish. Since all sampled species were identified previously to exhibit dietary shifting, long-term and recent possible dietary carbon sources were identified (Table 3). The possible dietary carbon sources are all within the range $\delta^{13}C$ muscle and

Table 3. Summary of long-term and recent dietary carbon sources of parrotfish.

Parrotfish	*C. hildebrandti i*	*Dendronepthy a* spp.	*Dendronepthy a* spp.	*D. dichotom a*	*D. simple x*	*S. muticu m*	*S. oligocystu m*	*U. reticul ata*	Unknown species
S. dimidiatus	++ +	++	+	++	++ +	++ +	++ +	++ +	-
S. psittacus	-	-	++	-	-	-	-	-	+
S. ghobban	-	-	++ +	-	+	+	+	+	-

++ Long-term dietary carbon source
+ Recent dietary carbon source
- Not found

liver signatures. Possible dietary carbon sources of the sampled parrotfish include *Dendronephthya* spp., *Chlorodesmi* sp., *Dictyota* sp., *Sargassum muticum*, *Sargassum oligocystum*, *Thalassia hemprichii*, and *Ulva reticulata*. Although *Dendronephthya* spp. have been attributed to the presence of terpenes as their chemical defense against predation, these species were reported to not interfere with the physiological processes of *Gambusia affinis* (Coll *et al.* 1982). It is possible that *Scarus* prey on *Dendronephthya* due either to the ability of these fish to convert terpenes to a non-toxic compound, as in the case of *Ovula ovum* (Coll *et al*., 1983) or to the relative concentrations at which they are present in the coral tissue, neither of which is presently known for the parrotfish tested in this study. Most parrotfish were reported to feed on *Thalassia testudinum* and *Halimeda incrassata* (Lobel and Ogden, 1981; Chow, 2005), but this may not be the case for the parrotfish sampled from this study as supported by their $\delta^{13}C$ muscle and liver values. These results imply that *S. dimidiatus, S. psittacus,* and *S. ghobban* are generalist type of consumers.

Trophic Position of Parrotfish

Isotopic N ratios of parrotfish muscle and their corresponding possible sources of diet collected from Canigao Island are shown in Fig. 5. Parrotfish muscles are enriched in $\delta^{15}N$ over their possible dietary carbon sources. Possible sources of diet for *S. dimidiatus*, *S. psittacus*, and *S. ghoban* had $\delta^{15}N$ values of 3.3, 3.7, and 3.7‰, respectively. *S. dimidiatus* is enriched in $\delta^{15}N$ by 2.6‰ over its diet, while *S. psittacus* and *S. ghobban* are enriched by 3.4 and 3.3‰, respectively.

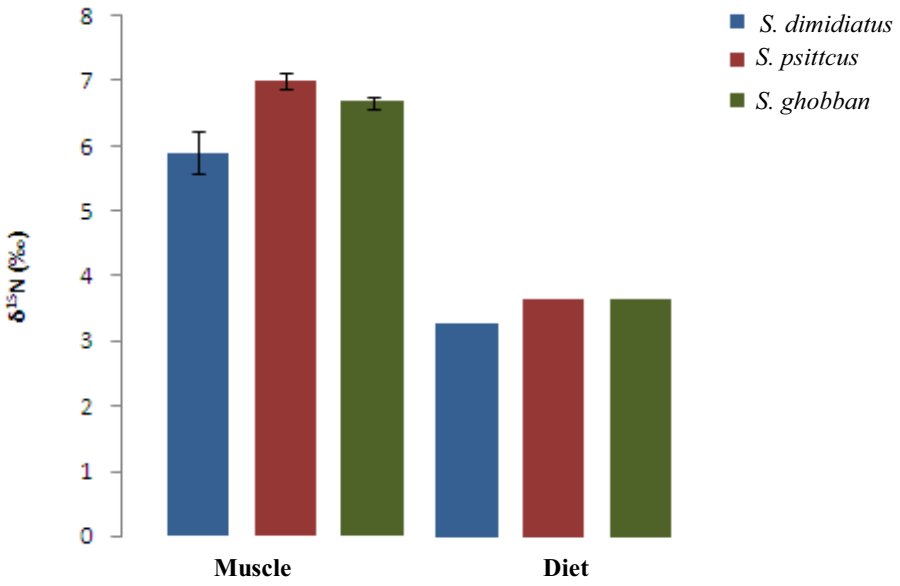


Fig. 4. $\delta^{15}N$ values *Scarus dimidiatus, S. psittacus,* and *S. ghobban* muscle and their diet.

Grazing fish are classified as herbivores and are assumed to derive the significant component of their nutrition through consumption, digestion, and assimilation of living algae (Hixon, 1997). This paradigm is largely based on the field observation by Horn (1989) that only filamentous and coralline algae are being able to tolerate the constant grazing pressure. Parrotfishes have been widely recognized as a major component of the herbivorous fish community and can be expected to play an important role in the transfer of food materials and energy from primary producers to the remaining members of the food chain.

Considering a trophic enrichment of 3-4% by consumers relative to their diet, *S. psittacus* and *S. ghobban* did rely substantially on the identified possible sources of diet. Hence, the trophic classification of these two species would be primary consumers. Possible diet sources of *S. dimidiatus* had $\delta^{15}N$ mean value of 3.3%:, it is lower than the reported average value of 3-4‰ (Vander Zanden and Hulshof, 1998; Corbisier *et al.* 2006). Moreover, stable isotope measurements revealed that sampled parrotfish are not strictly herbivorous because two species of soft corals (*Dendronephthya* spp.) were identified to be parrotfish's source of dietary carbon.

CONCLUSION

This study showed that stable isotope analysis of muscle and liver tissues can be exploited to reveal parrotfish feeding habits. Specifically, this study revealed the dietary shift of *Scarus dimidiatus, S. psittacus* and *S. ghobban,* as reflected in the statistically significant differences between relative isotope compositions of muscle and liver tissues. The differences in $\delta^{13}C$ values of muscle and liver tissues revealed that the *S. dimidiatus* have different dietary carbon intake compared to the other two species, but *S. psittacus* and *S. ghobban* have relatively the same dietary carbon requirements. *S. dimidiatus* has wider range of long-term and recent dietary carbon sources than the other two species suggesting that *S. dimidiatus* is a generalist consumer. Nitrogen enrichment in *S. dimidiatus* is lower than the reported average value of 3-4‰. Stable nitrogen values of muscle suggest that *S. dimidiatus, S. psittacus,* and *S. ghobban* are primary consumers. The stable isotope analyses of muscle and liver tissues of parrotfish provided information on its dietary pattern, carbon intake, possible dietary carbon sources, and trophic position, implying that metabolic differences among tissues can be exploited to characterize fishes. Despite the use of muscle and liver tissues of parrotfsh for stable isotope analysis, multiple tissue sampling is encouraged to evaluate the appropriateness of its trophic position. DNA sequencing of gut contents is also recommended to complement stable isotope analyses in showing species-specific trophic relations under field conditions. Wider sampling of its possible dietary carbon sources is required considering high levels of spatial and temporal variation in feeding.

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