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The Use of Stable Isotopes and Stomach Contents to Identify Dietary Components of the Spotted Rose Snapper, *Lutjanus guttatus* (Steindachner, 1869), off the Eastern Coast of the Southern Gulf of California

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Abstract: The food habits of the spotted rose snapper, *Lutjanus guttatus* (Steindachner, 1869), living off of the Eastern coast of the Southern Gulf of California (off the coast of Nayarit) are described based on their stomach contents and isotopic analysis. Fish were collected from the bycatch of shrimp trawling during the 2005-2007 shrimp fishing seasons. Twenty-six taxa were identified in the stomach contents and the geometric importance index suggested xanthid crabs and engraulidae fish are the most important species in the *L. guttatus* diet. Isotopic analysis and mixing models also led to the identification of crustaceans as important species in diets, but fish were considered only as secondary prey in these models. Notably, the diet and trophic level of the spotted rose snapper tend to change as it matures; young fish mainly feed on crustaceans, while larger *L. guttatus* can incorporate fish into their diets. Furthermore, Morisita-Horn index suggests that there are significant differences between the diets of juvenile and adult fish. The estimates of the trophic level for *L. guttatus* from stomach contents (TL_{sc} = 3.7) and isotopic analysis (TL_{iso} = 3.5) are very similar.

Key words: *Lutjanus guttatus*, stable isotopes, stomach contents, feeding habits, Gulf of California

INTRODUCTION

Snapper (Lutjanidae) are an important fish resource in all tropical and subtropical regions and they occupy high trophic levels in coastal ecosystems. They are carnivorous fish. They are mainly piscivorous, but they also consume a variety of benthic organisms including crustaceans and mollusks (Randall, 1967; Duarte and García, 1999). The spotted rose snapper, *Lutjanus guttatus*, is found from the Gulf of California to Peru. It is closely associated with the bottom of the continental shelf, mainly inhabiting rocky reefs, coral reefs and mangroves, although they can be found down to 60 m in depth in sandy areas (Fischer *et al*., 1995). Along the coast of Nayarit, off the eastern coast of the Southern Gulf of California, this species is a target species that is often captured by artisanal fleets that use hook-and-hand lines as fihing gears. Shrimp trawling boats also capture it incidentally. Due to its commercial importance and since it is an important component of the fish community, the study of the trophic relationships of this species is of great interest for those seeking an understanding of how the ecosystems in these areas function (Polis and Winemiller, 1996).

Studies of diets based on the analysis of stomach contents have been widely used to investigate the trophic ecology of organisms (Hyslop, 1980). This type of analysis is very useful due to the

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taxonomic resolution that can be achieved for the prey consumed. However, it only provides information about the recently (short-term) ingested prey and not about food that has already been assimilated. Furthermore, the high degree of decomposition of the tissues in the stomach can present problems for species identification. In addition, the opportunistic behavior of the predator to catch prey that are not usually consumed and the failure of predators to assimilate food that has been eaten, can also influence the identification of the prey found in the stomach contents. These factors often lead to biased estimates of the significance of prey in the diet (Hyslop, 1980; Burns *et al.*, 1998).

An alternative approach for the study of trophic relationships of aquatic organisms that has provided good results is the analysis of stable isotopes in the tissues of both the consumers and their prey (Hobson, 1999). The isotopes used in such studies are those of nitrogen ($\delta^{15}N$: that its relationship between isotopes ^{15}N and ^{14}N) and carbon ($\delta^{13}C$: relationship between isotopes ^{13}C and ^{12}C) because these are derived exclusively from the diet and therefore, provide direct information about the food assimilated (Gearing, 1991). This is because the $\delta^{13}C$ isotope undergoes minimal fractionation when passing from the source of food to the consumer (0 to +1‰, De Niro and Epstein, 1978) and thus, it reflects the origin of the source of carbon into the food web. The $\delta^{15}N$ isotope has a larger isotopic fractionation (3.4‰) and it is used to estimate the trophic level of predators. With this information, it is possible to identify the structure of the food chain (Minagawa and Wada, 1984). On the other hand, isotope data can also be used in mixing models (Phillips and Gregg, 2003) to estimate the contribution of prey species to the predator's diet and, thereby, characterize the structural and functional attributes of the food web. For these models, however, prior knowledge of the potential prey is necessary; so, the combined use of isotope and stomach content analyses could be very useful.

On this basis, the aim of this study is to determine the main dietary components of the spotted rose snapper through the combined analysis of stomach contents and stable isotopes.

MATERIALS AND METHODS

The study area is located on the Eastern coast of the Southern Gulf of California, on the Nayarit state coast, between 21° 54' N, 106° 03' W and 21° 09' N, 106° 02' W (Fig. 1). In this area, sandy bottoms with coarse sand predominate and the finest grain sand is found in shallow waters while muddy-sandy bottoms are found in deeper waters (De la Lanza-Espino, 1991).

Samples of spotted rose snapper were taken from the bycatch of shrimp trawl fishing during the fishing season (early September to March) of the years 2005 to 2007. Samples were taken as part of a large research project aimed to evaluate the impact of the shrimp trawl fishing on the ecosystem on the Eastern coast of the Southern Gulf of California, which is in turn one of the most important shrimp fishing grounds in Mexico. The experimental design for collection of samples consisted of a network of locations where one-hour haul was performed each, we used a trawl shrimp fishing net of 25 m of length made of nylon with a mesh size of 5 cm (denominated buzo type); the average time of tow was about 2-3 h. A sample of 20-50 kg of bycatch was randomly taken from which species were identified, sample proportion, in weight, was recorded; biometric information and samples of tissues of the species were taken, as well as information on trawl duration and geographic position of the hauls. Individuals of *L. guttatus* were separated from the bycatch and their total length was recorded. The stomach contents were collected and preserved with 10% formalin or frozen. For the isotope analysis, a sample of muscle was obtained from the dorsal region and immediately frozen. Additionally, tissue samples from some of the fish and invertebrates that were representative of prey species were also taken. Likewise, when possible, samples of muscle from prey that were found in the stomach contents were also taken for further analysis.

For the stomach content analysis, preys were identified to the lowest possible taxonomic level and the wet weights and numbers were recorded. The identified prey were grouped according to their

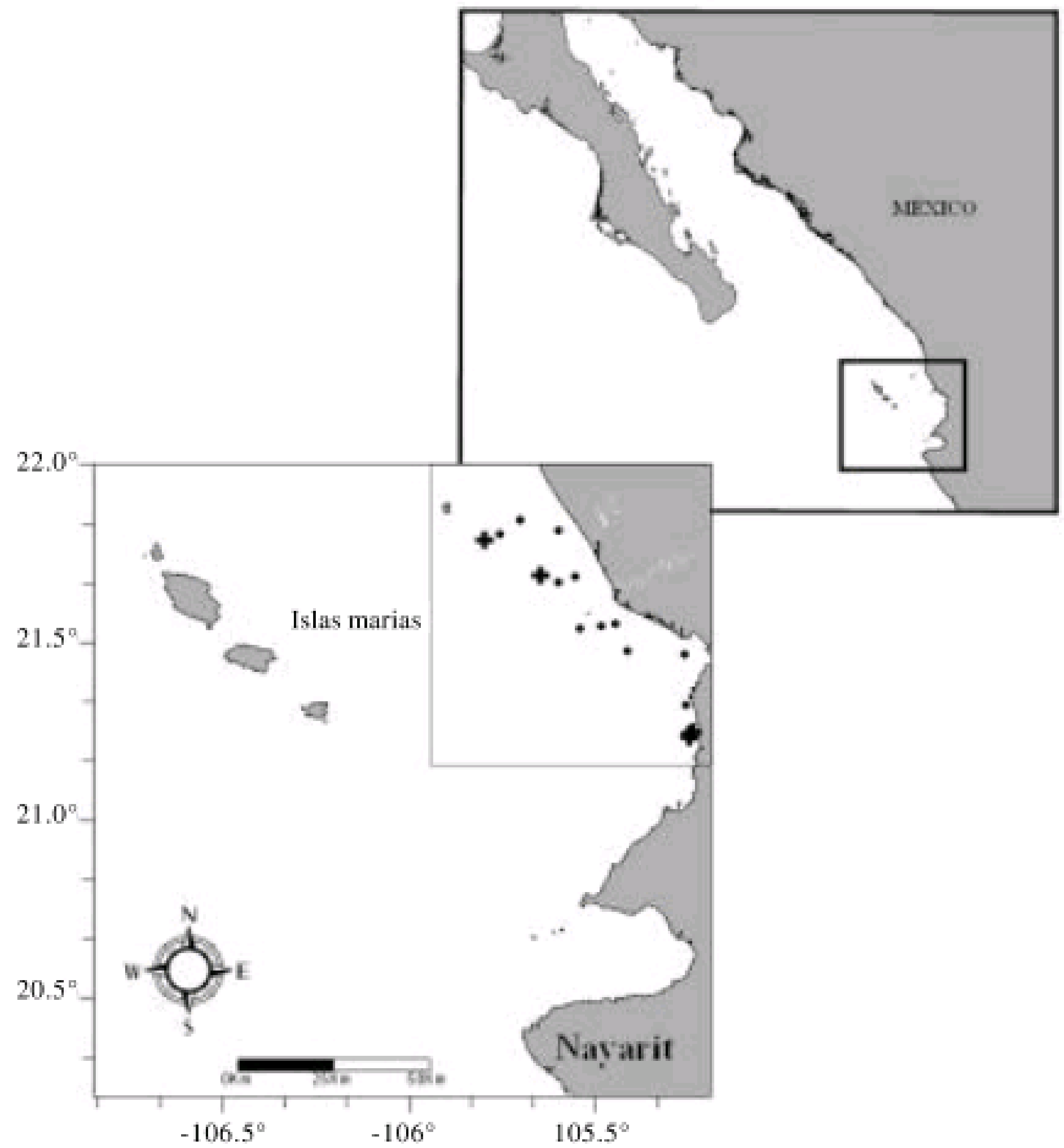

Fig. 1: Study area in the Coast of Nayarit, Mexico and location of sampling sites, (•): Individuals were collected from the (✚) a sites during the fishing seasons from the years 2005 to 2007, the sampling area covers a surface of near 500 km^2

degree of digestion and then, the unidentified prey were generically grouped as other mollusks, crustaceans or fish (Hyslop, 1980). The relative contribution of each individual prey type was estimated using the geometric index of importance (Assis, 1996), using the values of their weight (W), the Frequency of Occurrence (FO) and their number (N), which are all expressed as a percentage (Stevens *et al*., 1982). The computation was as follows:

$$GII_j = \frac{\left(\sum_{i=1}^{n} V_i\right)_j}{\sqrt{n}}$$

where, GII_j is the index value for the jth prey category, V_i is the vector for the ith indexes (W, FO and N) of the jth prey categories and n is the number of indexes used in the analysis (3) (Assis, 1996).

We also computed the trophic level for a predator from the stomach contents (TL_{sc}) using the equation suggested by Odum and Heald (1975):

$$TL_{sc} = 1 + \sum_{j=1}^{n} DC_{ij} \bullet TL_i$$

where, DC_{ij} is the fraction of prey (i) in the diet of predator (j) and TL_i is the trophic level of the prey (i). For those prey about which we did not have data from personal observations, the trophic level was taken from www.fishbase.org.

The analysis of isotopes was carried out at the Universidade da Coruña, Spain (Servicios Xerais de Apoio á Investigación, SXAIN), using an element analyzer FlashEA 1112 (ThermoFinnigan) connected to a mass spectrometer (DELTA plus of Finnigan MAT with a Flo II interface). The relative proportions of isotopes were estimated using the following equations:

$$\delta^{15}N\ ‰ = [(^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{standard} - 1]\times 1000$$

$$\delta^{13}C\ ‰ = [(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{standard} - 1]\times 1000$$

The standards for nitrogen and carbon, respectively, are atmospheric nitrogen and VPDB (Vienna Pee Dee Belemnite). Additionally, because the presence of lipids can enrich ^{12}C values and affect estimates of $\delta^{13}C$, we applied the numerical correction suggested by Post *et al*. (2007):

$$\delta\,^{13}C_{normalized} = \delta\,^{13}C_{wo\_treat} - 3.32 + 0.99\times C{:}N,$$

where, $\delta\,^{13}C_{wo\_treat}$ values came from the mass spectrometer and C:N is the C to N ratio of the sample. As such, the corrected values were the $\delta\,^{13}C_{normalized}$ ($\delta^{13}C$ without the effect of lipids).

The contribution of each prey to the diet of the spotted rose snapper was estimated using the mixing model proposed by Phillips and Gregg (2003), which was designed for situations where n isotopes are estimated from a larger (>n) number of sources that contribute to the mix. For these estimates, we used the software SISUS (http://statacumen.com/sisus/), which uses the isotope values to calculate a range of possible contributions of each source, by using a probabilistic approach (http://statacumen.com/old/sisus/SISUS_Getting_Started_v0_08.pdf). The results of this analysis are reported as the distributions of percentages ranging from 1 to 99%, where the minimum and maximum values are used to determine the importance of food sources or prey to the diet. Prior to this analysis, the isotopic fractionation was corrected for by subtracting the value 2.3‰ from the $\delta^{15}N$ value to estimate the average isotopic signature of the predator (McCutchan *et al*., 2003).

The trophic level for *L. guttatus* was also estimated from the isotope values (TL_{iso}), using the equation proposed by Post (2002):

$$TL_{iso} = \lambda + \left(\frac{\delta^{15}N_{predator} - \delta^{15}N_{base}}{\Delta n}\right)$$

where, λ represents the trophic position of the organism used to estimate $\delta^{15}N_{base}$ and Δn represents the enrichment of $\delta^{15}N$ per trophic level, considering 2.3‰ as the fractionation per trophic level as given by McCutchan *et al.* (2003).

The species selected for the computation of $\delta^{15}N_{base}$ must come from the same habitat as the target species and the isotopic signal from the trophic web must be incorporated over a large enough time scale to minimize the effects of variations that can occur over short time periods.

To test the hypothesis that *L. guttatus* have similar diets within sizes (i.e., juveniles and adults), we used the Morisita-Horn index (Krebs, 1999) that varies within a range of 0 to 1, indicating completely different or equal diets, respectively. According to the interpretation proposed for this index, a value greater than 0.6 indicates a significant diet overlap. This index is expressed by the relationship:

$$C^{\mu} = \frac{2\sum_{i=1}^{n}\left(P_{x,i}.P_{y,i}\right)}{\sum_{i=1}^{n}P_{x,i}^{2} + P_{y,i}^{2}}$$

where, C^{μ} is the Morisita-Horn index, $P_{x,i}$ is the proportion of ith prey with respect to all prey used by predator x; $P_{y,i}$ is the proportion of the ith prey with respect to all prey used by predator y and n is the total number of prey. In the case of the isotopes values with significant differences between developmental stages were tested using the student's t-test.

RESULTS

Stomach Content Analysis

Of the 83 spotted rose snapper stomachs collected, 49 were found with food. The size of the organisms analyzed ranged between 114 and 305 mm in Total Length (LT). According to Cruz-Romero *et al*. (1996), the maturity size for the spotted rose snapper is 170 mm LT, so the samples contained both juveniles and adults. Twenty six taxa were found in the stomach contents (16 crustaceans, 2 mollusks and 6 fish), as along with unidentified organic matter (Table 1).

The geometric importance index GII (Table 1), indicates that the preferred prey for the spotted rose snapper are crabs from the family Xanthidae, as well as some other crustaceans and the fish *Engraulis mordax* (Engraulidae). As a secondary source of prey, we found several crustaceans, such as shrimps and crabs (*Processa* sp., *Solenocera florea* and the families Ogyrididae and Portunidae), some mollusks and fish of the same species as the predator, suggesting some degree of cannibalism (Fig. 2). The rest of the identified preys were considered to be rare or occasional sources of food. The trophic level for *L. guttatus* based on the stomach contents was $TL_{sc} = 3.77$.

Table 1: Prey found in the stomach contents of the spotted rose snapper, *L. guttatus*, off the Eastern coast of the Southern Gulf of California, México

Species present			FO	FO (%)	N	N (%)	G (g)	G (%)	GII	Pref*
Mollusca		Other mollusks	3	6.12	3	2.36	10.34	15.37	13.8	2
	Bivalvia	n.i. Bivalves	2	4.08	3	2.36	0.04	0.06	3.8	3
Crustacea		n.i. shrimps (Natantia)	1	2.04	1	0.79	0.08	0.12	1.7	3
	Ogyrididae	n.i. ogyridids	2	4.08	1	8.66	1.36	2.02	8.5	2
	Penaidae	n.i. penaeids	1	2.04	1	0.79	0.25	0.37	1.8	3
	Portunidae	n.i. portunids	3	6.12	4	3.15	1.73	2.57	6.8	2
		Euphylax robustus	1	2.04	1	0.79	1.17	1.74	2.6	3
	Processidae	*Processa* sp.	3	6.12	12	9.45	2.47	3.67	11.1	2
	Solenoceridae	*Solenocera florea*	2	4.08	14	11.02	0.45	0.67	9.1	2
		Solenocera sp.	2	4.08	4	3.15	0.38	0.56	4.5	3
	Squillidae	n.i squillids	2	4.08	2	1.57	0.43	0.64	3.6	3
		Squilla panamensis	1	2.04	1	0.79	0.65	0.97	2.2	3
		Squilla acueleata	1	2.04	1	0.79	0.64	0.95	2.2	3
		Squillla sp.	1	2.04	1	0.79	0.44	0.65	2.0	3
		Pseudosquillopsis marmorata	1	2.04	1	0.79	0.08	0.12	1.7	3
		n.i. stomatopods	2	4.08	2	1.57	1.17	1.74	4.3	3
	Xanthidae	n.i. xanthids	4	8.16	10	7.87	12.96	19.26	20.4	1
Osteichthes	Engraulidae	n.i. engraulids	5	10.20	10	7.87	13.44	19.98	22.0	1
		Engraulis mordax	2	4.08	8	6.30	11.35	16.87	15.7	1
	Lutjanidae	*Lutjanus guttatus*	2	4.08	3	2.36	3.84	5.71	7.0	2
	Mugilidae	n.i. mugilids	1	2.04	1	0.79	0.48	0.71	2.0	3
	Paralichthydae	n.i. paralichtids	1	2.04	1	0.79	0.06	0.09	1.7	3
		Other fish	2	4.08	2	1.57	0.23	0.34	3.5	3
Other		NIOM	2	4.08	2	1.57	0.08	0.12	3.3	3

*Pref: preference: 1: Preferred prey, 2: Secondary prey, 3: Occasional prey. NIOM: Non-identified organic matter. N: Numbers, G: Volume, FO: Frequency of occurrence, GII: Geometric importance index. All values are also given as percents

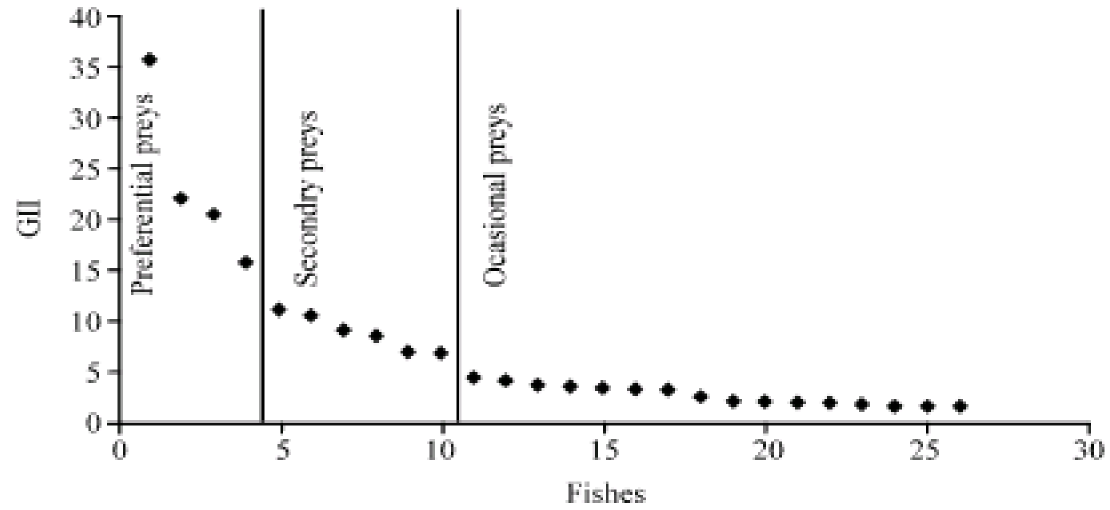

Fig. 2: Values of the geometric importance index, GII, for all fish analyzed. Vertical lines separate preferred prey from the secondary or occasional prey

Table 2: Values of $\delta^{15}N$ and $\delta^{13}C$ and their standard deviation, for the spotted rose snapper, *L. guttatus* and its prey, from off of the Eastern coast of the Southern Gulf of California

Species	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$ s.d.	$\delta^{13}C$ s.d.
L. guttatus	14.66	-15.39	0.89	0.53
Large shrimp (Penaeidae, Sicyonidae and Processidae)	13.82	-17.14	1.19	0.47
Small shrimp (Ogyrididae and Solenoceridae)	13.10	-17.12	0.98	0.45
Stomatopoda *(Squilla acuelata, S. hanckocki, S. bigelowi* and *S. mantoidea*)	13.17	-16.39	0.73	1.55
Portunus sp. (small)	12.63	-15.36	0.61	0.21
Other Portunids (Portunus sp. and *Euphylax robustus*)	13.82	-16.42	1.01	0.31
Xanthidae	12.23	-15.29	0.12	0.53
Anomura	14.04	-16.91	0.60	0.92
Evibacus princeps	12.90	-16.48		
Engraulidae	13.62	-16.81	0.64	0.64

All values expressed as ‰

Isotope Analysis

We analyzed 11 samples of fish ranging between 105 mm LT to 260 mm LT for their isotope ratios. The ratios of nitrogen isotopes were $13.3‰<\delta^{15}N<16.4‰$, with an average of 14.5‰ and the ratios of carbon isotopes were from $-16.3‰ < \delta^{13}C < -14.4‰$, with an average of -15.38‰ (after lipid correction). The values of both the $\delta^{15}N$ and the $\delta^{13}C$ isotopes had a normal distribution (Kolmogorov-Smirnov, $p>0.200$).

To estimate the mixing polygon of isotopes values needed to describe the feeding habits of the spotted rose snapper, the isotope values of the prey were aggregated into nine groups as shown in Table 2.

The polygon of mixed isotope values is shown in Fig. 3. The predator isotopic signature was found to be close to that of some of the main preys that were identified in the stomach contents. One group of species identified as a primary source of food for the spotted rose snapper was the crabs of the Xanthidae family (72-92%), followed by small Portunidae crabs (0-22%); while secondary prey contributed as follows: stomatopods (0-8%) and other portunids (0-8%). The rest of the preys were considered to be of minor importance. Here, the importance of the family Engraulidae contrasts with that determined via the stomach content analysis, in which it appears to be a primary source. The trophic level of the spotted rose snapper from the isotope values was computed following the method described by Post (2002). The trophic level for the Processidae shrimp was taken from www.fishbase.org (TL = 2.2, ISSCAAP) and a value of $\delta^{15}N = 11.8\%$ was used. Based on this information, the estimated trophic level for *L. guttatus* was $TL_{iso} = 3.5$, which is similar to the value obtained from the stomach content analysis.

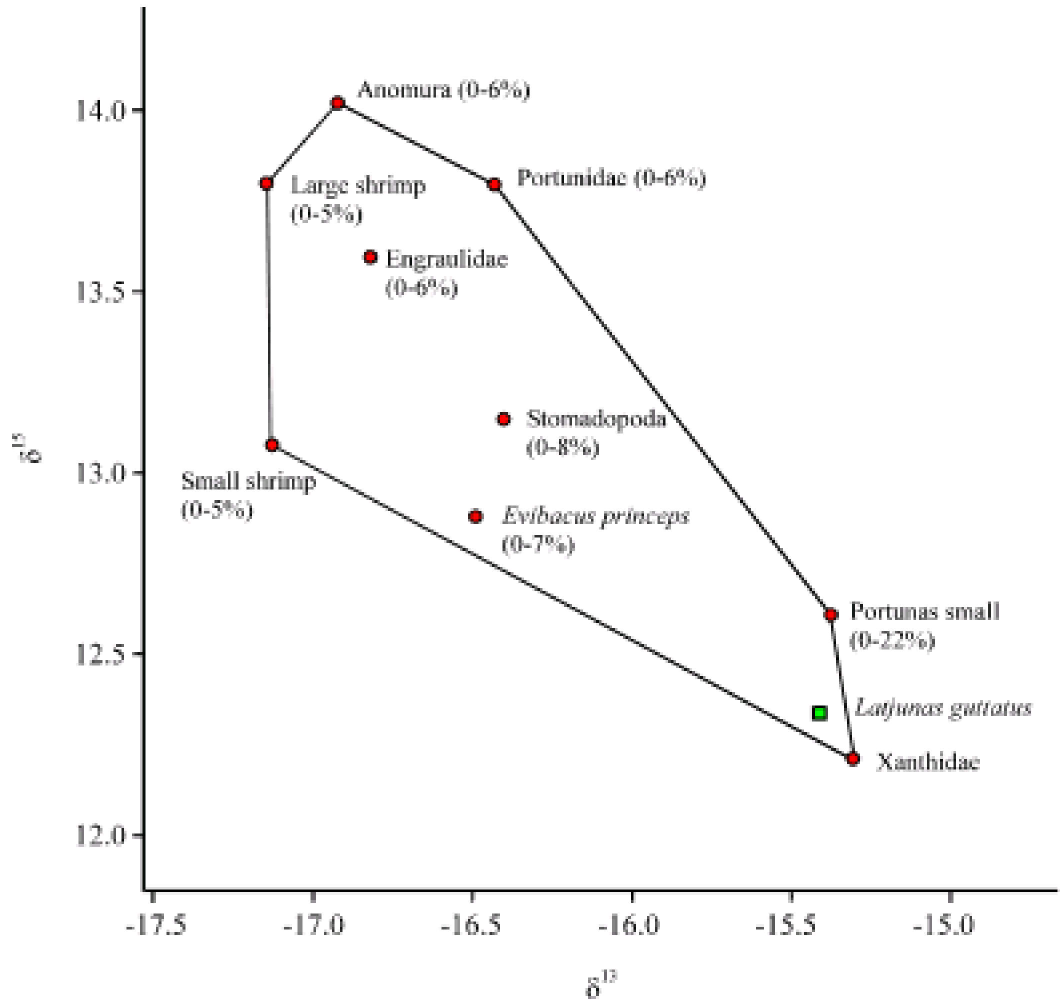

Fig. 3: Polygon of mixed isotope values of $\delta^{13}C$ and $\delta^{15}N$ for the spotted rose snapper and its prey, from off of the Eastern coast of the Southern Gulf of California. Isotopic mixing convex hull, SISUS: Stable Isolate Sourcing using Sampling

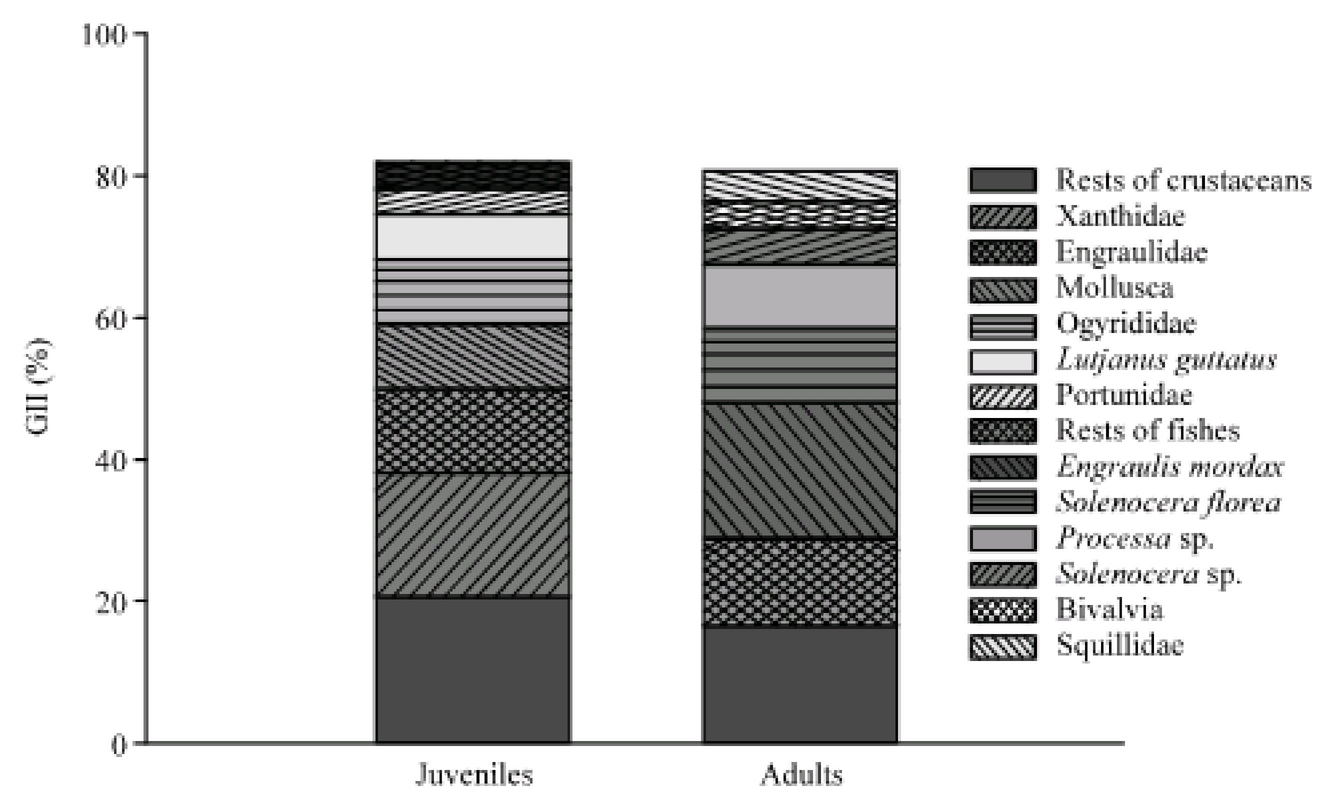

Fig. 4: Dietary comparison for juveniles and adults of the spotted rose snapper off the Eastern coast of the Southern Gulf of California based on values of the GII index

The Morisita-Horn index suggests that there is a difference between the diets of juveniles and adults (C^{μ} = 0.26), which is supported by $\delta^{15}N$ values (t = -3.430, p = 0.009), though the $\delta^{13}C$ were not different (t = -1.084, p= 0.310). Juveniles feed predominantly on crabs, while adults prefer shrimp and some fish (Fig. 4). The lowest value for the difference between the $\delta^{15}N$ of the predator and its prey was 2.33‰. For the $\delta^{13}C$, the minimum difference was -1.74‰. The $\delta^{15}N$ values ranged from

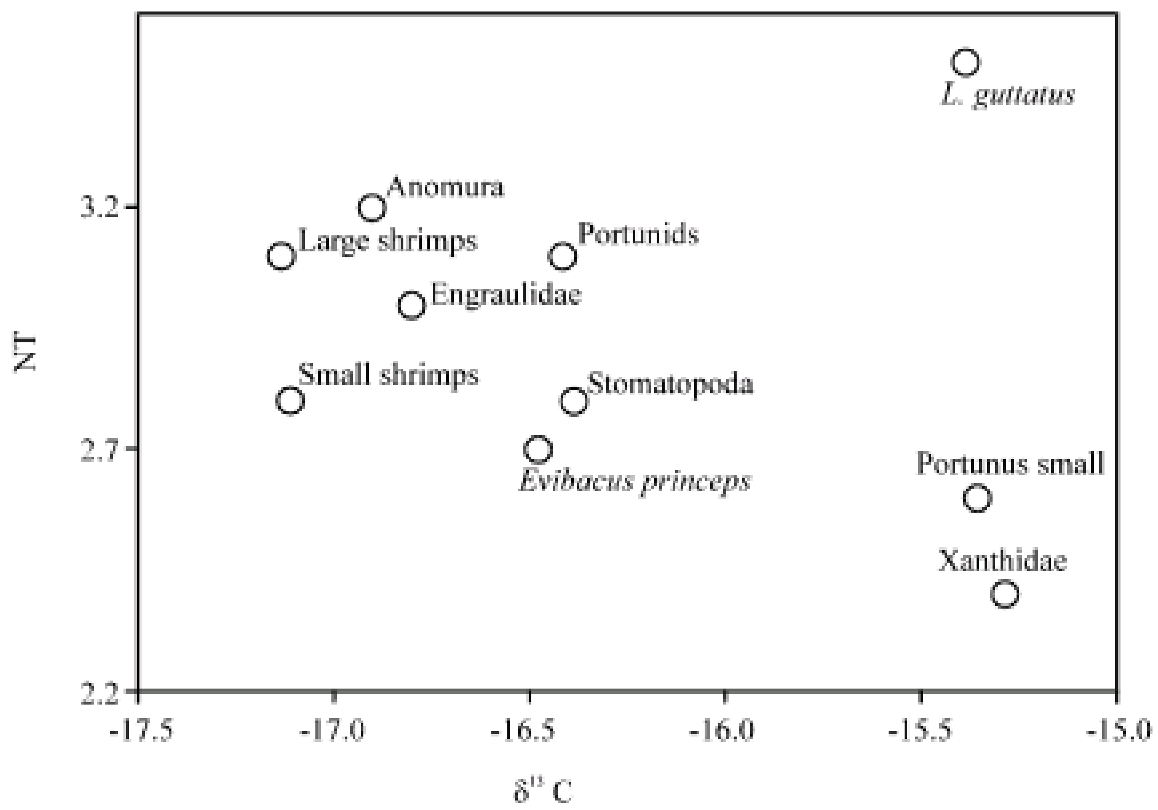

Fig. 5: Trophic levels of spotted rose snapper and its prey *vs.* their $\delta^{13}C$ values

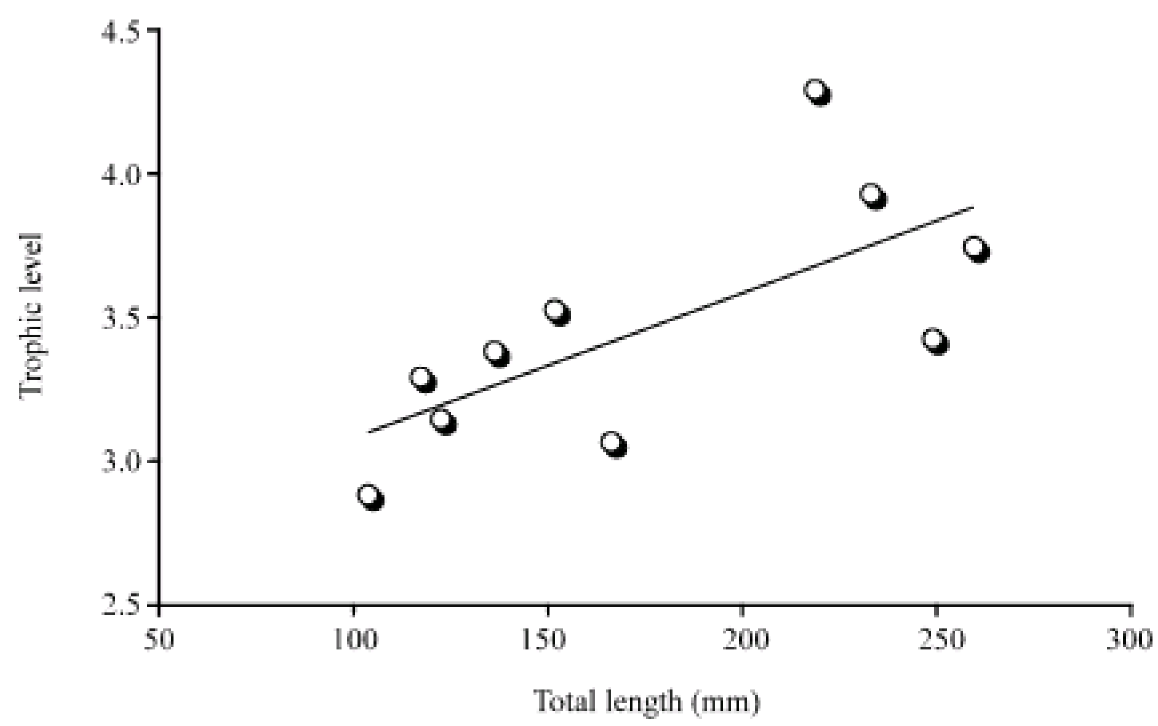

Fig. 6: Change in the trophic level of prey with maturity for the rose spotted snapper obtained off the Eastern coast of the Southern Gulf of California

14.56 to 12.23‰ and for the $\delta^{13}C$ values the range was from -15.29 to -17.14‰. We found that the predator and its preferred prey had more positive values for $\delta^{13}C$. This suggests that predators tend to consume near-shore prey rather than offshore prey, which have more negative $\delta^{13}C$ values (Fig. 5). The trophic level of prey was $2.4<TL_{iso}<3.5$. The higher trophic levels of prey were represented by large Anomura crabs, portunids and shrimps and the lower trophic levels of prey were represented by small xanthid and portunid crabs. We also found that *L. guttatus* increases its TL of prey as it matures, with the biggest fish having higher TLs of prey (Fig. 6).

DISCUSSION

The trophic spectrum found for the spotted rose snapper, *L. guttatus*, in the study area was somewhat narrow (26 taxa found along the sampling period) compared to the diet of other snapper species. Duarte and García (1999) reported 106 prey for *L. synagris*; Rojas (1997b) reported

30 prey for *L. colorado*; Vázquez *et al*. (2008) reported 54 prey for *L. argentiventris*; Santamaría-Miranda *et al*. (2003) reported 51 prey species for *L. peru*. For *L. guttatus* along the coast of Guerrero in the Southern Pacific of Mexico, Rojas-Herrera *et al*. (2004) reported 88 prey. Rojas *et al*. (2004) reported a narrower food spectrum of 15 preys for spotted rose snapper off the coast of El Salvador. The findings of the latter two studies agree with our results in indicating that the preferred preys of *L. guttatus* tend to be fish, crustaceans and mollusks.

The high consumption of Brachyuran crustaceans by spotted rose snapper is consistent with the reports of several authors, suggesting that this species feeds mainly on species associated with ocean bottoms rather than those of pelagic habits, like fish (Rojas *et al*., 2004). This could be because they are opportunistic organisms and although they are able to consume a wide spectrum of prey, they mainly eat prey that possess characteristics, such as color, smell, movement or abundance, that make them attractive to predators (Kaiser *et al*., 1993). In present study area, Brachyuran crustaceans, such as Xanthidae and Portunidae, appear to be more vulnerable to this predator, probably because of their abundance and behavior, while fish and shrimp are consumed in lower proportions. This could also be due to the small number of larger fish in our sample, as larger fish can probably escape from the fishing gear. Regarding their low consumption of mollusks, even when associated with ocean bottoms, they are occasional prey. Rojas (1997a) suggested that this reflects the limitations of the mouth anatomy of *L. guttatus*, which have fine, thin canines in rows and small bands of palatine and vomerin teeth that limit their consumption of this kind of prey.

The results concerning fish consumption appear to be somewhat contradictory since the analysis of stomach contents indicated that engraulid fish are an important component in the diet, while the isotopic analysis suggests that these are only a secondary prey. This, however, seems to indicate an opportunistic behavior for the spotted rose snapper, as it can consume these fish species, but their assimilation reflected by isotopic analysis clearly indicates that engraulids are not a frequent prey source.

Size differences in feeding behavior have been reported for several species: *L. peru* (Santamaría-Miranda *et al*., 2003) *L. apodus* (Rooker, 1995), *L. argentiventris* (Vazquez *et al*., 2008) and *L. guttatus* (Rojas *et al*., 2004). In all of these studies, it has been reported that juveniles mainly consume crustaceans and adults tend to consume more fish and shellfish. In addition, the higher mobility of adults seems to offer a better opportunity for them to capture preys that are more mobile than Brachyuran crustaceans. Size, as a factor influencing the ontogenic differences observed in diets, is likely associated with the maturation process since fish, as a food source, tend to be a higher energy food source than crustaceans (Abitia-Cárdenas *et al*., 1997). This concept is supported by the stomach content analysis and even though the isotopic analysis was not conclusive, adults did have higher values of $\delta^{15}N$ than juveniles. This is also in accord with the observations of Rojas *et al*. (2004), who reported fish consumption by spotted rose snappers larger than 400 mm LT. Here, we found the same trend for fish larger than 300 mm LT (Fig. 6).

Concerning the estimates of the trophic level for *L. guttatus*, both methods (stomach content and isotope analyses) indicate similar results. In our case, we used the nitrogen enrichment value of 2.3‰ per trophic level proposed by McCutchan (2003) instead of that proposed by Post (3.4‰) (2002). This was because the first of these is more appropriate for organisms that primarily feed on invertebrates, which is the case for *L. guttatus*. According to Newsome *et al*. (2007), these estimates, which are highly dependent upon the value used for the trophic level enrichment, can vary from one ecosystem to another. Therefore, it is recommended to not take the estimated value of the trophic level of prey as an absolute value of that trophic level. Instead, this value should be used as an estimate of the relative trophic position of a species according to the nature of the community structure and the food web of the ecosystem.

Present results show that the main dietary components of the spotted rose snappers are Brachyuran crustaceans with a variety of other Decapods crustaceans and even when stomach contents show engraulid fish were consumed by large snappers, isotopes analysis indicates they are not among the main component of the diet, but suggest large snappers have an opportunistic behavior.

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