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$\delta^{13}C$ and $\delta^{15}N$ in Dominant Demersal Fish Species in The Southern Gulf of Mexico

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ABSTRACT

Stable carbon and nitrogen isotope ratios of demersal fish species were studied in two areas of the continental shelf in the Gulf of Mexico in the summer of 2004. Samples were obtained from the shrimp bycatch while boarding the commercial fleets in the Veracruz and Campeche fishing area. Stable carbon and nitrogen isotope ratios were determined in a total of 26 demersal fish species. High variations in the isotopic composition were observed, with a general trend towards depleted carbon values in the Veracruz area and enriched nitrogen values in the Campeche area. Detritus seems to constitute the main food sources for primary fish consumers in the continental shelf of Campeche, while seagrass, epiphytes and macroalgae seem to support the structure of the Veracruz trophic web. A benthic-pelagic coupling seems to occur, with some fish of higher trophic levels feeding both on benthic and pelagic prey items. No linear relation was observed between the nitrogen isotope ratios and the trophic level as suggested in the literature. We discussed that fish species can obtain a new muscle isotopic signature relatively fast in response to changes in the isotopic composition of their diet and/or diet shifts regulated by the particular hidrodynamic process in both of the continental shelves.

Key words: Veracruz continental shelf, Campeche sound, isotopic signatures, trophic level

INTRODUCTION

Within the study of ecological ecosystems, there has been an increase in the use of stable isotopes, in order to explore the behavioral patterns during consumer feeding and/or the habitat's utilization in time and space (Sweeting *et al.*, 2005). Frequently, the isotopic signatures have been used to identify the source of organic carbon for marine consumers (Bode *et al.*, 2006), migration patterns (Augley *et al.*, 2007; Bodin *et al.*, 2007), food interrelations (Rau *et al.*, 1983; Sholtodouglas *et al.*, 1991; Wainwright *et al.*, 1993; Persson and Hansson, 1999), trophic position in the trophic web (Fry and Sherr, 1984; Minagawa and Wada, 1984; Owens, 1987; Fredriksen, 2003), variations in the availability of food within different time scales (Vizzini and Mazzola, 2003; Salomon *et al.*, 2008).

The commonly utilized isotopes for the study of trophic aspects are the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes which accumulate in an organism when the tissues of the consumed preys are assimilated (Fry and Sherr, 1984; Owens, 1987). This is the manner by which the isotopes are transferred from one trophic level to another through a process of enrichment known as isotopic fractioning (Deniro and Epstein, 1981; Fry, 1988). Among the consumers in a marine ecosystem, a fractionation of 3‰ has been detected in the $\delta^{15}N$ for each trophic level (Owens, 1987; Post, 2002). By contrast, the $\delta^{13}C$ presents an average value of fractionation of 1‰ (Rau *et al.*, 1983; Fry, 1988). Due to the fact that the $\delta^{15}N$ isotopes present a higher isotopic fractionation, they are generally used to indicate a trophic position of the organisms; while the $\delta^{13}C$ isotopes, for their minimum or null fractionation, reflect the source of autotrophic feeding (Deniro and Epstein, 1981).

On the other hand, the consumers are typically opportunistic organisms that frequently modify their diets with time and based on food availability in the current conditions of the environment (Bearhop *et al.*, 2004) or for example species show differences in the trophic position among ecosystems. Additionally, dietary changes are not immediately expressed in the signatures of these tissues, because it depends on the formation of new tissue with a new feeding signature and the signature renovation in old tissues for other signatures during the reparation of the new tissue (Tieszen *et al.*, 1983; Fry and Arnold, 1982; Fry and Sherr, 1984; Sweeting *et al.*, 2005). In this study, the isotopic proportions were determined to be able to determine the trophic position of the demersal fish within two contrasting areas of the continental shelf of the south of Mexico during the same climate season (Summer): Veracruz with a strong estuary influence and Campeche with typically marine characteristics (Chazaro-Olvera *et al.*, 2007). In this context, stable isotopes are useful because all the animal tissues and/or chemical fractions possess an isotopic memory influenced by environmental conditions; which results in variations in carbon and nitrogen fixation in the ocean (Tieszen *et al.*, 1983).

MATERIALS AND METHODS

The samples were obtained during two commercial trips on board of shrimp commercial boats, both taken place in the Gulf of Mexico in the summer of 2004 during the beginning of fishing season. The first sampling took place in the Campeche Sound during September 5-10th and the latter on the continental shelf of Veracruz, Mexico during September 15-20th (Fig. 1). The Campeche sound is characterized by the influence upon it by the processes associated with the Lazo current, as well as by the Yucatan coastal current which determines that these waters present characteristics that are properly marine waters, such as high transparency (55 to 99%), sandy bottoms of calcareous origin and the presence of sea grass (Yanez-Arancibia and Sanchez-Gil, 1986). By contrast, the Veracruz continental shelf is found to be influenced by the unloading of fresh water originating from both the Papaloapán and Coatzacoalcos rivers (connected by the lagoon system of Alvarado) and it is characterized by concentrations, high in organic matter and fine sediment (sludge and sand) (Chazaro-Olvera *et al.*, 2000; Vazquez-Lopez and Alvarez, 2007).

The organisms employed for the analysis form a part of the accompanying fauna of shrimp and were captured with shrimp hauling nets and the selected species have been characterized as dominant within their respective ecosystems based upon their abundance and occurrence frequency (Yanez-Arancibia and Sanchez-Gil, 1986). In Table 1, the species utilized in each zone are displayed. In order to be comparative the sampling effort was focus on adults specimens based on sizes reported in FishBase (www.fishbase.org).

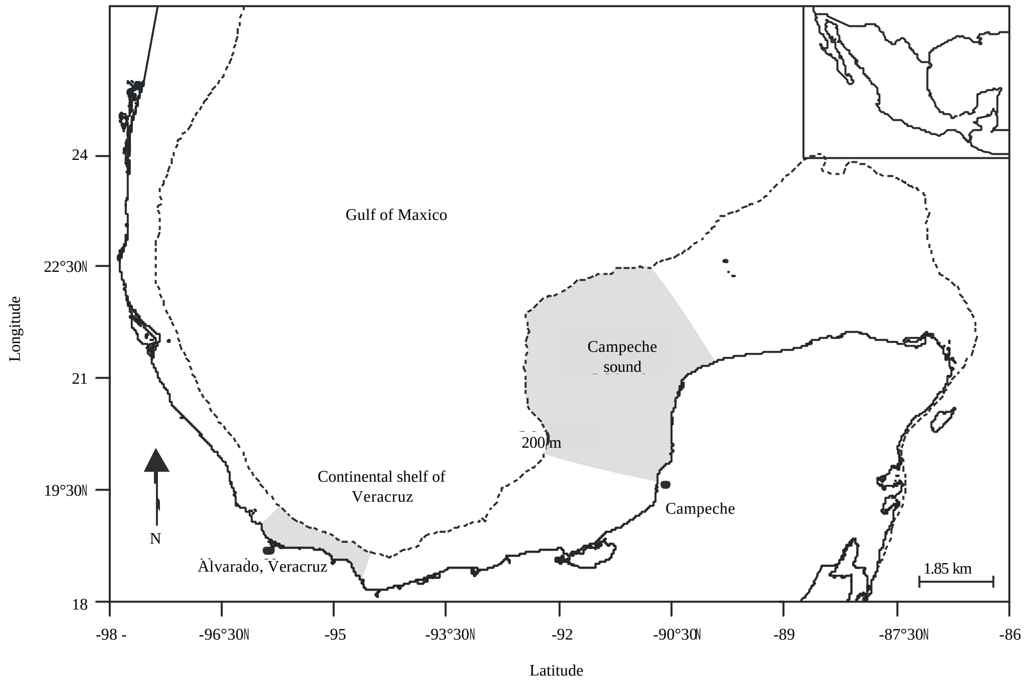

Fig. 1: Area of study, where the continental shelf of Veracruz and the Campeche sound are shown

Table 1: Dominant demersal fish species in the continental shelf of Veracruz and the Campeche sound (According to Yanez-Arancibia and Sanchez-Gil, 1986)

Veracruz	Campeche sound
Acanthostracion quadricornis	*Archosargus rhomboidalis*
Aetobatus narinari	*Bagre marinus*
*Balistes capriscus**	*Balistes capriscus**
*Calamus nodosus**	*Calamus nodosus**
*Citharichthys spilopterus**	*Citharichthys spilopterus**
*Cynoscion arenarius**	*Cynoscion arenarius**
*Decapterus punctatus**	*Decapterus punctatus**
Gymnothorax nigromarginatus	*Etrumeus teres*
Haemulon plumierii	*Lagodon rhomboides*
Heteropriacanthus cruentatus	*Lutjanus campechanus**
Lagocephalus laevigatus	*Lutjanus synagris*
*Lutjanus campechanus**	*Nicholsina usta*
*Prionotus roseus**	*Prionotus roseus**
Scomberomorus cavalla	*Pristipomoides aquilonaris*
Squatina dumeril	*Scorpaena russula*
*Synodus foetens**	*Synodus foetens**
Trichiurus lepturus	*Upeneus parvus**
*Upeneus parvus**	

*Species are common among areas

Analysis of the carbon and nitrogen isotopes: Muscle samples of the mid-dorsal section of the organisms were collected by triplicate for further isotopic analysis. These were placed in vials and frozen for later analysis. Prior to quantifying the stable carbon and nitrogen isotopes, the samples were dehydrated in a lyophilizer at a temperature of -40°C and at a pressure of 55 mbar during a 24 h period. Once the humidity was extracted, the lipid extraction was realized due to the fact that it has been demonstrated that the lipids are reduced in $\delta^{13}C$ with relation to the diet which could affect the ecological interpretation of the isotopes´ signatures. The lipid extraction makes for a more reliable comparison of tissue samples independently from the quantity of contained lipids. The lipids were extracted in a MARS-5 CEM (Corporation Matthews, NC) microwave oven, as well as with the use of a chloroform-methanol 1:1 solution.

The samples were transformed into gas by combustion, utilizing a Thermoquest NC 2500 elemental analyzer. The gases were sent by a continuous flow of helium to an isotope ratio mass spectrometer, Europa Integra PDZ Europa (Ltd. Cheshire, UK) located in the Laboratory of stable isotopes of the Department of Vegetable Sciences, at the University of California, Davis, California, USA.

The precision and exactness of the method were proven by replicas of commercial standards (International Agency for Atomic Energy). The results were expressed as values of isotopic ratios (δ) in ‰ of the deviation from the samples with respect to the established standards, by means of the following equation (Park and Epstein, 1961):

$$\delta^{15}N \text{ or } \delta^{13}C = \frac{R_{sample}}{R_{standard}} - 1 \times 1000$$

where, R_{sample} is the isotopic ratio of the heaviest stable isotope with relation to the lightest ($\delta^{13}C/\delta^{12}C$ or $\delta^{15}N/\delta^{14}N$), respectively in the sample and $R_{standard}$ is the value of the isotopic ratio for a known standard; in this case the composition of the carbon isotope is referred to as the standard Pee Dee Belemite formation and the nitrogen is reported with relation to the standard atmospheric air. The elemental concentrations of the standards were replicable within a 10%. Considering that the isotopic ratios represent the diet of the integrated organisms over a time of chosen tissue interchange and that the variability amongst the organisms represents the difference between their diets; the trophic position is defined as the intersection of $\delta^{13}C$ and $\delta^{15}N$.

According to Post (2002), $\delta^{15}N$ is related to the Trophic Level (TL); in order to compare our results, the FishBase database (www.fishbase.org, Froese and Pauly, 2006) was consulted for the estimated trophic levels for the species under study. The trophic levels of these species is calculated based upon information derived from the analysis of the stomach contents. The TL extracted from the database was compared to the estimates of $\delta^{15}N$.

RESULTS

Quantification was conducted of the isotopic signatures of carbon and nitrogen for 26 species of demersal fish present in the southern continental shelf of the Gulf of Mexico. A total of 24 species of bony fish and two species of cartilaginous fish were analyzed.

In general a wide variation in the signatures found in two isotopes was observed (Fig. 2, 3). The carbon isotope values indicate a significant difference between two areas (Mann-Whitney test, $p<0.05$). It has presented a larger variation in the samples found in the fish from the Campeche continental shelf, registering values varying from -18.6‰ for the *Pristipomoides aquilonaris* to-12.3‰ for *Scorpaena russula* (Fig. 2). The carbon isotopic values for the species from the Veracruz shelf presented a range of minor variation which oscillated from -17.1‰ en *Gymnothorax nigromarginatus* to -13.8‰ in the *Haemulon plumierii* (Fig. 3). With respect to the signatures

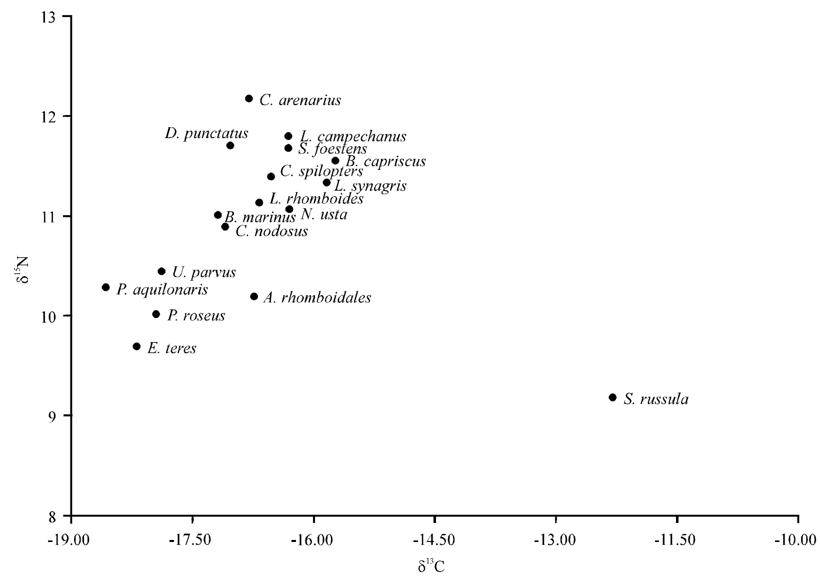

Fig. 2: Trophic position of the dominant species of demersal fish based on the average of ^{15}N and ^{13}C values in the Campeche sound

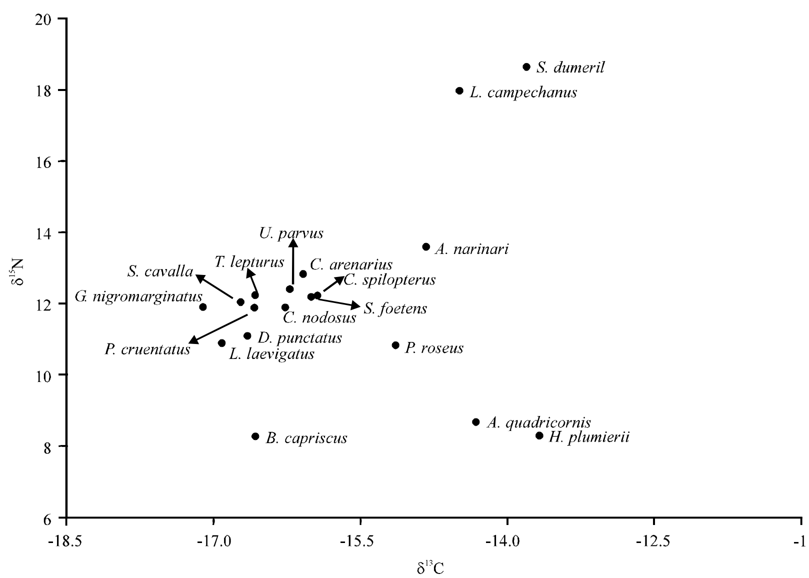

Fig. 3: Trophic position of the dominant species of demersal fish based on the average of ^{15}N and ^{13}C values in the continental shelf of Veracruz

derived from the nitrogen isotope, a minor variation was registered in the samples taken from the Campeche shelf, with values that ranged from a 9.2‰ for *S. russula* to a 12.2‰ for *Cynoscion arenarius* (Fig. 2), while the samples of Veracruz registered a wider range of values, from 8.3‰ in *Balistes capriscus* to 18.6‰ in *Squatina dumeril* (Fig. 3). The Mann-Whitney test also showed a significant difference between areas ($p<0.05$).

Figure 4 shows that the species from the Veracruz continental shelf have higher $\delta^{15}N$ values in relation to the species of the Campeche continental shelf. However, the range of trophic

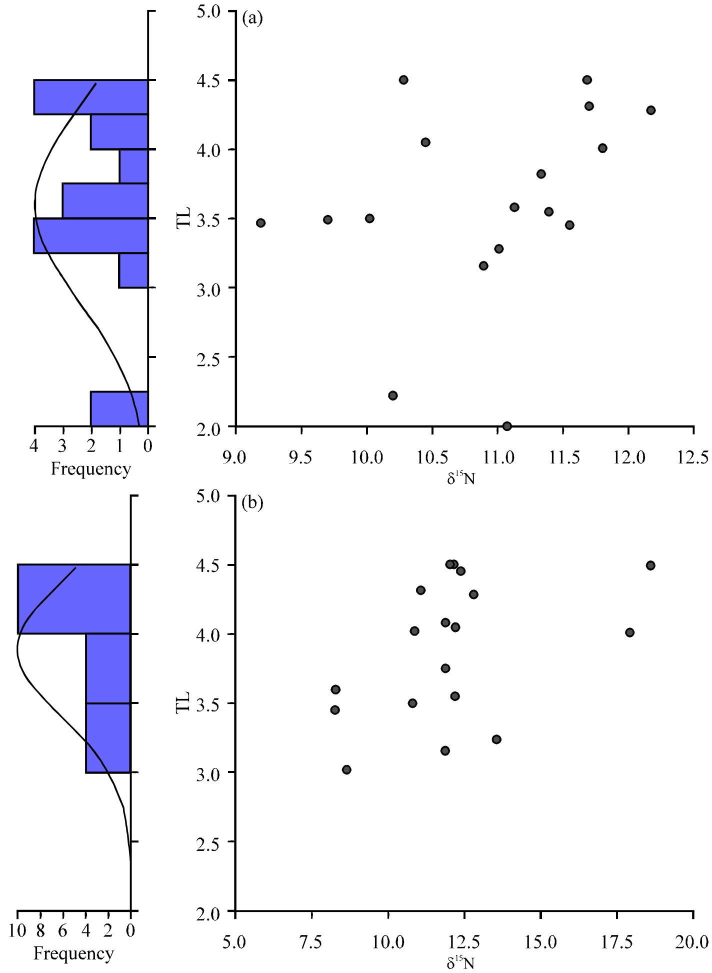

Fig. 4(a-b): Confrontation of the isotopic ratio of ^{15}N vs trophic level of dominant demersal fishes from (a) Campeche Sound (upper panel) and (b) continental shelf of Veracruz (lower panel)

levels was similar in both areas with most species between 3-4.5. Nevertheless, there was not a clear pattern between the isotopic values of nitrogen versus the trophic level. An outstanding aspect in the structure of the species in the Campeche shelf is the presence of consumers of first order with trophic levels of 2.2 and 2.0 (*A. rhomboidalis* and *N. usta*), respectively.

DISCUSSION

The stable isotopes analysis have been utilized to reconstruct diets and to elucidate routes of nutrient sources (Rau *et al*., 1983; Minagawa and Wada, 1984; Owens, 1987; Sholtodouglas *et al*., 1991; Persson and Hansson, 1999; Fredriksen, 2003; Bode *et al*., 2006; Bodin *et al*., 2007; Salomon *et al*., 2008; Tripp-Valdez and Arreguin-Sanchez, 2009).

It was notice that mostly fish dominant species in the Campeche shelf have less enriched signatures in the carbon isotope compared to the Veracruz species (Fig. 2, 3). This fact suggests differences in the use of sources of carbon in each ecosystem. It has been suggested that in Southern Gulf of Mexico lower values of δ^{13}C are correlated with terrestrial origin, in contrast with higher values associated to seagrasses and algae like the primary source of energy (Raz-Guzman and De La Lanza-Espino, 1991, 1993). However, different authors have demonstrated that the isotopic signatures of carbon of the producers of both terrestrial origin and of marine origin present discreet values (Nakano and Murakami, 2001), while primary producers of estuarine influenced environments are more variable (Fry and Sherr, 1984; Owens, 1987; Abu Hena *et al.*, 2004; Kathiresan and Alikunhi, 2011). This is the case of the Campeche Sound which receives a strong influence of Terminos Lagoon, an estuarine complex with a high environmental heterogeneity (Miranda *et al.*, 2005).

On the other hand, Soto *et al.* (2004) pointed out that the southeastern Gulf of Mexico is characterized by an hydrodynamic regimen dominated by an anticyclone spin that when combined with the yearly meteorological conditions, cause processes of stratification or of homogenization of masses of neritic waters, as well as the convergent phenomena against environments subject to the riverside influence of the main rivers, the Coatzacoalcos and the Grijalva-Usumacinta. These oceanographic phenomena promote processes of coupling between the primary and secondary producers (plankton-nekton-benthos)(Yusuf and Webster, 2008). Likewise, Buskey *et al.* (1999) pointed out that the higher enrichment of ^{13}C in the Bank of Campeche is probably due to the contribution of the primary benthonic producers which are often more enriched in carbon than the primary planktonic producers and by the detritus.

Moreover, the highest enrichment of the nitrogen isotope in the Veracruz shelf maybe indicative of a trophic structure dominated by species with high trophic levels, notwithstanding that the species which were analyzed are similar (9 of 26 species are common among areas), the trophic organization is distinct in one and another ecosystem. An additional explanation of these results is in relation with the trophic plasticity documented in many fish species (Day and Mc Phail, 1996; Cutwa and Turingan, 2000; Robinson and Parsons, 2002; Seah *et al.*, 2011; Murugesan *et al.*, 2012). This characteristic allows that many species are adapted to exploit the food resources in several environments, for example an species can live in the bentho-pelagic coupling system (Vander Zanden and Rasmussen, 2001; Oribhabor and Ogbeibu, 2012). Additionally these differences in magnitude of ^{15}N can be due to the atmospheric nitrogen having a differential rate dependent on the ecosystem under study (Saino and Hattori, 1980; Deniro and Epstein, 1981; Mariotti *et al.*, 1984; Owens, 1985, 1987; Altabet, 1988; Croft *et al.*, 1988; Rau *et al.*, 1998) and at least the $\delta^{15}N$ of the organic material of marine origin also depends on the phytoplankton species (composition), physiology, growth rate and migration patterns (Montoya and Mc Carthy, 1995; Escobar-Sanchez *et al.*, 2011).

Although $\delta^{15}N$ is commonly used to estimate the trophic level of organisms (Post, 2002), the fixation of N in the ocean presents a spatial and seasonal variation, specially in coastal areas (Lihan *et al.*, 2011). In this sense, it is probable that the equation proposed by this author does not present a simple linear relation. This effect can be observed in Fig. 4a and b, where the comparisons of the estimates of the trophic level of the species being studied take place with one and another approximation.

In neither of the ecosystems there is a clear pattern between the isotopic ratios of ^{15}N versus the trophic level. However, it is possible to observe a slight increase in the isotopic proportion of nitrogen in regard to the increase of the trophic level. Likewise, it was possible to observe a greater number of species with the highest trophic levels occupying the Veracruz shelf in comparison with the trophic level of the species in the Campeche shelf.

In synthesis it could be said the carbon isotopic signatures of the studied consumers come from different carbon sources, the one from the Campeche shelf probably is influenced more from continental origin while the one from the Veracruz shelf presents a greater influence of marine origin. Finally, even when observed in a general manner, an increase in the isotopic proportion of nitrogen with respect to the increase of the trophic level, the joint analysis does not show a clear linear pattern between these two variables such as it has been suggested in other studies (Cabana and Rasmussen, 1996; Vander-Zanden and Vadeboncoeur, 2002).

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