Diet selection by immature green turtles (*Chelonia mydas*) at Bahía Magdalena foraging ground in the Pacific Coast of the Baja California Peninsula, México

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In order to determine if eastern Pacific green turtles (Chelonia mydas) exhibit feeding preferences samples of recently ingested food items were compared to the food resources available in the marine environment where C. mydas congregates. Stomach samples were collected by conducting gastric lavage and, at the same time, vegetation transects were conducted during spring and winter. Green turtles in our study selectively consumed seaweeds, with Codium amplivesiculatum and Gracialaria textorii as preferred species. Differences in the consumption of species were found across the two mentioned seasons and were consistent with changes in the availability of different algae species in the environment. Based on these results, it is recommended that sea turtle conservation plans along the Baja California Peninsula include Pacific coastal mangrove channels with a high diversity of algae species as priority areas for protection.

Keywords: diet, selectivity, Chelonia midas, seaweed, sea grass, México

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INTRODUCTION

Diet preferences and food selection of sea turtles have been poorly studied in spite of the relevance of these kind of studies for understanding ecological requirements and management strategies, especially for endangered species because the better understanding of their habitat might be part of the successful conservation strategies. The green turtle (also known as black or east Pacific green turtle), *Chelonia mydas*, has been listed as endangered throughout its range (Hilton-Taylor, 2000) due to human-related causes such as habitat modification, egg poaching, and incidental and direct capture of juveniles and adults on fisheries (Caldwell, 1962; Cliffton *et al.*, 1982; Gardner & Nichols, 2001). Green turtles occur along the western coast of North and South America (Cliffton *et al.*, 1982).

In early stages of the green turtle development, invertebrate items are more consumed (while they are still in open ocean), but when they become juvenile and start living in coastal areas they are known to feed primarily on sea grasses and/or marine algae, but also occasionally consume animal material (Bjorndal, 1997; Seminoff *et al.*, 2002; López-Mendilaharsu *et al.*, 2003, 2005) and mangrove vegetation and fruits (Limpus & Limpus, 2002). Geographical differences in food

Corresponding author: R. Riosmena-Rodriguez Email: riosmena@uabcs.mx preferences of green turtles are possible to observe when comparing what has been reported for Australia (mostly sea grasses and a few seaweeds: see Garnett *et al.*, 1985; Forbes & Limpus, 1993; Brand-Gardner *et al.*, 1999; Read & Limpus, 2002), Hawaii (sea grasses and red seaweeds: see Balazs, 1980), Colombia (sea grasses and seaweeds: see Amorocho & Reina, 2007), the Caribbean (sea grasses: see Bjorndal, 1980), and an Arabian feeding ground (sea grasses: see Ross, 1985). The above differences in diet composition are likely related to availability of the species in the environment (Echavarria *et al.*, 2006) but also to the nutritional composition of each species (Villegas-Nava, 2006; McDermid *et al.*, 2007). However, no studies on diet preferences by green turtles exist along the Pacific coast of North and South America using the approach of direct comparison between availability vs ingestion.

The extent to which the diet of green turtles is determined by selective feeding (Garnett *et al.*, 1985; Ross, 1985; Brand-Gardner *et al.*, 1999) or by food availability (Garnett *et al.*, 1985; Balazs *et al.*, 1987) of different diet species has been addressed in several studies, but much research is needed to elucidate the relationships between the nutrition and diet preferences of sea turtles (Villegas-Nava, 2006; McDermid *et al.*, 2007). As variation in diets in green turtles in different foraging grounds may affect net nutritional gain (Bjorndal, 1997) and consequently growth rate, understanding diet selection is critical for assessing habitat quality and thus making decisions (Groombridge & Luxmoore, 1989; Hirth, 1997; NMFS & USFWS, 1998) on which habitats must be

protected to enhance green sea turtles' chances of survival. Bahía Magdalena has been identified as a high priority area for conservation, because most of the human practices being developed in this area represent an important threat to the high biodiversity including endangered species such as sea turtles (Arriaga *et al.*, 1998). In this paper we analyse the diet selection of green turtles in the region of Bahía Magdalena, during two seasons, winter and spring, relating relative abundance of potential food items in the environment to the food items that are ingested. This is the first study on diet selection in the eastern Pacific Ocean.

MATERIALS AND METHODS

Study site

Bahía Magdalena (24°15′N-25°20′N and 111°20′W-112°15′ W; Figure 1) is a coastal bay approximately 1390 km², located on the west coast of the Baja California Peninsula, México. As a result of seasonal marine upwelling it is a highly productive lagoon that is sheltered from Pacific waters by two barrier islands, Magdalena Island and Margarita Island (Sanchez-Rodriguez *et al.*, 1989). Sea surface temperatures (SSTs) in Bahía Magdalena experience substantial seasonal variation, reaching a maximum of 28°C in late summer (September) and a minimum of 19°C in March (Lluch-Belda *et al.*, 2000). Estero Banderitas is a mangrove channel located on the northwestern side of Bahía Magdalena; it is characterized by a series

of large and small islands lined with mangroves and sandy beaches. Due to the limited rainfall from May through to October of only 0–50 mm, it has a dry climate with many deserts lying behind the mangroves. Estero Banderitas is fairly shallow with the depth ranging from 0.5 m to 8 m, depending on the tides, which are classified as mixed semi-diurnal. In this region, the seasonal marine upwelling provides a continuous availability of plant nutrients, which allows for the productivity of a diverse range of fauna and flora, yet the primary source for nutrients derives from the mangroves in the area.

Vegetation sampling

The study was conducted between January and May, 2002 at the region known as Estero Banderitas, where green turtles were commonly present. Two representative sampling sites were chosen: Punta Entrada $(24^{\circ}51'39''-43''N)$ and $112^{\circ}7'51''-55''W)$ and Isla Conchalito $(24^{\circ}54'37''-41'')$ y $112^{\circ}7''-49''$ (Figure 1). During the present study we visited the area twice, once in winter (January) and once in spring (May) to look at the seaweed community. The per cent cover of the marine vegetation was estimated along three 50 m transects (perpendicular to the coast) at the two different locations (2 seasons \times 3 transects \times 2 locations = 12 transects). Above ground biomass was also estimated along each transect; the vegetation was collected from five randomly selected 0.25 m² quadrats (total of 60 quadrats). Physical data such as SST, and water depth were recorded at each sampling location.

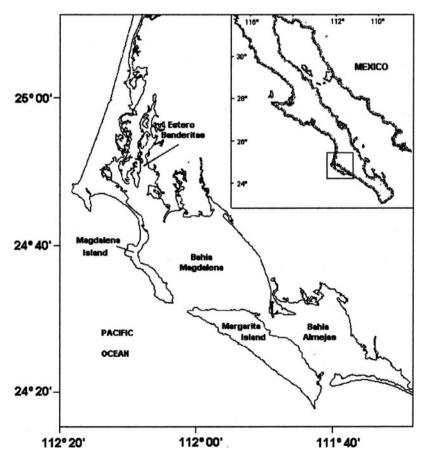


Fig. 1. Bahía Magdalena - Almejas lagoon complex, Baja California Sur, México.

Samples were preserved in 4% buffered formalin/seawater solution. In the laboratory the vegetation samples were separated and identified to the lowest possible taxonomic level based on a combination of taxonomic keys cited in Riosmena-Rodriguez & Paul-Chávez (1997). The per cent cover was calculated by dividing the distance occupied by each species along the transect by the total length of the transect (50 m). During each season (winter and spring) per cent cover values of each plant species along transects were averaged across sites. To estimate the above ground biomass we measured the relative sample volume of each species or taxonomic group collected from quadrats following the procedure of water displacement in a graduated cylinder.

Turtle capture and measurements

Two entanglement nets ($100-130~\text{m}\times7~\text{m}$; mesh size = 60~cm) were set during a 24-h period once per month from January 2002 to May 2002 at the same location; June was not possible to sample because of logistical problems. Each net was monitored at 1-2~h intervals depending on the intensity of the current. Entangled turtles were removed from the net and transported to the beach. The straight carapace length (SCL; $\pm~0.1~\text{cm}$) was measured using calipers, taken from the anterior notch to the posterior tip of the supracaudal scute. Turtles with SCL $\geq~77.3~\text{cm}$ were considered mature based on the mean size of nesting females at the closest major rookery (Colola, Michoacán; Figueroa *et al.*, 1993). Turtles were also weighted ($\pm~0.5~\text{kg}$) prior to release.

Diet analyses

Diet samples of recently ingested food items were collected by conducting gastric lavage according to the methods of Forbes & Limpus (1993). All turtles captured from the study area were in good condition after the sampling and were released at the site of capture. All food material obtained was preserved in a 4% formalin solution in seawater. Eaten groups were identified to the lowest possible taxonomic level based on a combination of taxonomic keys cited in Riosmena-Rodriguez & Paul-Chávez (1997).

Each diet item was quantified by volume (V), measured by water displacement, and frequency of occurrence (F) (Hyslop, 1980). Relative volume and frequency of ocurrence (Hyslop, 1980) were also calculated.

These two measures (volume and frequency) were combined to calculate two indices: simple resultant index (R_s) and weighted resultant index (R_w) (Mohan & Sankaran, 1988) according to the following:

$$\begin{split} R_s &= (V^2 \times F^2)^{1/2} \times 100 \\ & \Sigma (V^2 \times F^2)^{1/2} \\ R_w &= Q(V^2 \times F^2)^{1/2} \times 100 \\ & \Sigma Q(V^2 \times F^2)^{1/2} \\ Q &= 45 - I\theta - 45I; \, \theta = tan^{-1}(V/F) \end{split}$$

where V = per cent volume; F = per cent frequency of occurrence.

Dietary selection was determined with an assessment procedure formulated by Johnson (1980). This method includes the Waller–Duncan (W statistic) test for differences among ranks in relation to selection, which indicates preference for foods. This procedure provides a measure of the relationship between availability of a food resource in the environment and the utilization of that resource, which is expressed as T-bar values (averaged rank differences). The smallest T-bar value indicates the most preferred resource.

Statistical analysis

Regression analyses were used to detect the relationship between the above-ground biomass and per cent cover of plant species. Volume percentages of food items consumed were arcsine root transformed to improve normality and variance homogeneity and then a two-way analysis of variance (ANOVA) was conducted between seasons (winter and spring) and principal diet components. A Tukey honestly significant difference multiple comparison test for unequal sample size was used when significant differences were detected from the ANOVA (Sokal & Rolhf, 1995).

RESULTS

Vegetation composition and abundance

Sea surface temperature during this period ranged from 20°C to 22°C, mean water depth ranged from 2.8 to 5.8 m. Sixteen plant species were identified along 12 transects belonging to 3 different taxonomic groups (Chlorophyta, Rhodophyta and Phaeophyta). The number of species was higher in winter (13 species) than in spring (9 species).

Based on biomass data the predominant species in winter was *Amphiroa beauvoisii* (%V = 27.5 \pm 25.6%) followed by *Gracilaria vermiculophylla* (%V = 15.7 \pm 14.2%) and *Aspagaropsis taxiformis* (%V = 14.6 \pm 8.5%). Spring was dominated by *Caulerpa sertularioides* (%V = 28.2 \pm 5.1%), *Amphiroa* sp. (%V = 26.5 \pm 26.5%) and *Gracilaria vermicullophylla* (%V = 25.7 \pm 25.7%).

Vegetation cover data showed that in winter *Amphiroa* sp. was the predominant species along transects (10.8 \pm 10.8%) followed by *Aspagaropsis taxiformis* (5.0 \pm 1.8%). During spring *Caulerpa sertularioides* and *Amphiroa beauvoisii* were present in mean per cent cover of 6.7 \pm 3.7% and 4.8 \pm 4.8%, respectively. All other species were less than 0.9% (Figure 2).

Per cent cover and above-ground biomass showed a positive correlation ($r^2=0.62$) so per cent cover was considered to be a good estimator of the species availability within the study area.

Turtle capture and diet analyses

A total of 15 turtles of *Chelonia mydas*, were live-captured in the Estero Banderitas. Mean SCL was 59.9 cm (standard error (SE) = 1.9; range = 48.0-75.6 cm). All the turtles analysed through this technique were immature individuals (SCL < 77.3 cm). Mean oesophageal lavage sample volume was 8.8 ml (SE = 5.2; range = 1-80.5 ml).

The diet of green turtles in Banderitas was composed of 9 prey items (7 species of algae, fragments of mangrove

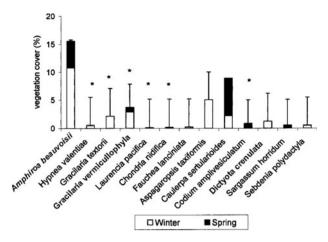


Fig. 2. Seasonal variation (winter and spring) of algae species collected in Banderitas Channel, Bahía Magdalena, México. Values represent the average per cent cover from 6 transects each season. (*) indicate species consumed by black turtles in this region.

roots and an unidentified sponge), but only 7 of these items were considered major diet constituents (volume \geq 5% in at least one sample; Garnett et~al.,~1985): Gracilaria vermiculophylla, Gracilaria textorii, Chondria nidifica, Laurencia vermiculophylla, Ulva lactuca, Codium amplivesiculatum and an unidentified poriferan (Table 1). Two additional diet items (Hypnea valentiae and mangrove fragments) were present in trace amounts. Food items from turtles captured in the Estero Banderitas were arranged by its $R_{\rm w}$ values in order of decreasing importance as: C. amplivesiculatum > G. textorii > G. vermiculophylla > L. vermiculophylla > U. lactuca > C. nidifica and the unidentified sponge (Figure 3).

The volume of various prey species recovered from diet samples differed significantly ($F_{6,91} = 16.7$, P < 0.000). The dominant species overall collected from lavage samples during winter and spring were *Codium amplivesiculatum* (%V = 48.3% F = 73.3) and *Gracilaria textorii* (%V = 36.1% F = 80).

Green turtles have a trend that suggests differences between the compared seasons in the mean relative volumes of food items consumed ($F_{6.01} = 6.5$, P < 0.000). Gastric

Table 1. Seasonal variation (winter and spring) in the diet of green turtles *Chelonia mydas* captured in the Estero Banderitas Bahía Magdalena, México. Values represent per cent relative volume (%V), frequency of occurrence (%F), number of stomach where the seaweed was present (No., out of the 15 collected) and N = number of stomachs.

Diet item	Winter $(N = 9)$			Spring (N = 6)		
	%V Mean (SE)	No.	%F	%V Mean (SE)	No.	%F
Gracilaria vermiculophylla	17.6 (11.7)	3	33.3	1.5 (1.0)	2	33.3
Gracilaria textorii	51.3 (12.5)	8	88.9	13.6 (7.8)	4	66.7
Chondria nidifica	1.0 (1.0)	1	11.1	_	_	-
Laurencia pacifica	_	-	-	4.7 (4.7)	1	16.7
Ulva lactuca	2.2 (2.2)	1	11.1	_	-	-
Codium amplivesiculatum	27.8 (11.1)	6	55.6	78.6 (8.8)	6	100.0
Unidentified poriferan	-	-	-	1.5 (1.5)	1	16.7

SE, standard error.

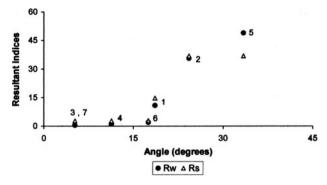


Fig. 3. Resultant indices R_s and R_w plotted against the angle for food items in lavage samples of green turtles (N = 15) captured in the Estero Banderitas, from January to May, 2002. 1, *Gracilaria vermiculophylla*; 2, *Gracilaria textorii*; 3, *Chondria nidifica*; 4, *UUlva lactuca*; 5, *Codium amplivesiculatum*; 6, *Laurencia pacifica*.

lavage samples collected during winter were dominated by G. textorii (V = 51.3% and F = 88.9%) and C. amplivesiculatum (V = 27.8% and F = 55.6%). In spring the opposite occurred: C. amplivesiculatum (V = 78.6% and F = 100%) was the most abundant and frequent component of the diet (P < 0.0002) and G. textorii (V = 13.6% and F = 66.7%) was the second (Table 1). There were no differences in the consumption of G. textorii between the two seasons (P= 0.08) but the consumption of C. textorii between the winter (textorii between

Diet selection

Relative volumes of food items in lavage samples were compared with the food available based on per cent cover of each plant species in winter and spring in the Estero Banderitas.

Only species consumed by turtles in the area were considered in the analysis, and the algae Caulerpa sertularioides, Amphiroa sp. and Aspagaropsis taxiformis because their availability in the study area was relatively high. Species were consumed in different proportions in the Estero Banderitas. The ranked order of food preference by these turtles during winter (January-March 2002) and spring (April-May 2002) are displayed in Table 2. In winter C. amplivesiculatum, G. textorii, U. lactuca and C. nidifica were consumed more than available (selectively eaten) based on negative Tbar values (Table 2). Codium amplivesiculatum was the most preferred species followed by G. textorii, but averaged rank differences showed that the preference for C. amplivesiculatum was significantly greater than G. textorii. Significant differences in ranks were also found between G. textori, U. lactuca and C. nidifica (W = 1.74, P < 0.05) during winter. Differences in preference ranks between G. vermiculophylla and C. sertularioides were not significant, even though G. vermiculophylla was little consumed relative to availability and C. sertularioides was not consumed by any turtle. Finally A. taxiformis and Amphiroa beauvoisii were avoided despite their great abundance in the study area.

During spring only three algae were selectively eaten: C. amplivesiculatum, G. textorii and G. vermiculophylla; significant differences in ranks were only found between C. amplivesiculatum and G. vermiculophylla (W=1.74, P<0.05). Similarly to winter the test showed that

Table 2. Dietary selection of marine algae by green turtles in the Estero Banderitas, during winter and spring.

Diet item	Winter (1	(e = N	Spring $(N = 6)$		
	Tbar ^{1,2}	Rank	Tbar ^{1,2}	Rank	
Codium amplivesiculatum	-4.7 a	1	-2.9 a	1	
Gracilaria textorii	$-3.1_{\rm b}$	2	-2.4_{ab}	2	
Ulva lactuca	-2.0 c	3	_	_	
Laurencia pacifica	_	_	$-1.1_{\rm b}$	3	
Chondria nidifica	$-0.9_{\rm d}$	4	_	_	
Gracilaria vermiculophylla	1.2 e	5	-0.8_{c}	4	
Caulerpa sertularioides	2.5 e	6	3.2 f	6	
Aspagaropsis taxiformis	3.5 f	7	_	_	
Amphiroa sp.	4.5 g	8	2.4 d	5	

¹Negative Tbar indicates use > availability.

G. vermiculophylla (not even mentioned on the Figure as important or present in either season) was not selectively eaten and *Amphiroa* sp. and *C. sertularioides* were avoided despite their great availability in the study area (Figure 2).

DISCUSSION

The diet of immature green turtles in the Estero Banderitas was composed almost exclusively of marine algae (7 of the 9 different food items consumed) and all of the preferred species are known to be associated to rhodolith beds in dense quantities (Iglesias-Prieto et al., 2003). The number of stomachs analysed (15 in total) is a good representative in relation to the calculated density of turtles for the area (300 according to Brooks, 2005). Data obtained from seasonal vegetation surveys throughout the year (López-Mendilaharsu et al., 2003, 2005) showed an increase in the number of the available species during winter and spring compared to the other seasons (Santos Baca & González, 2005). But despite the great availability of potential food species recorded during these months, turtles concentrated their foraging efforts upon a small fraction of species. Moreover only 2 species (the green alga Codium amplivesiculatum and the red alga Gracilaria textorii) turned out to be the predominant diet components accounting for 84.4% of turtles' diet (which were the most abundant). Seasonal differences in the consumption of these two species during winter and spring were due to their variable abundance within the study area. In winter the availability (% cover) of G. textorii was higher than C. amplivesiculatum, which was reflected in lavage samples in which G. textorii prevailed over C. amplivesiculatum. In spring the availability of C. amplivesiculatum was slightly greater than G. textorii and the same pattern was reflected in the lavage. This result might indicate that the turtles were feeding according to the abundance. Nevertheless, both species were consumed in relatively large amounts compared to their availability in the environment, and also the coverage of these resources in the environment were far less abundant compared to other species which were not consumed (i.e. Amphiroa sp. and Caulerpa sertularoides) both in winter and spring. Therefore, this indicates that the turtles were feeding selectively within the channel. The channels are extremely diverse in the area (Figure 1) and will be used for the turtles to rest and feed from items that accumulate there after high tide and also will grow associated with the patchy rhodolith bed present in the area (Riosmena-Rodriguez personal observation).

Codium amplivesiculatum was the preferred species by the green turtles during winter and spring, even though its availability in the study area was very low. However, it is probable that the coverage and biomass values of C. amplivesiculatum have been underestimated due to the fact that this species appears in channels of stronger current located out of the transects (Dawson, 1950; Holguín-Acosta, 2002), or there is the possibility that the turtles are not just feeding in the estuary and this species is more abundant in other parts of Bahía Magdalena. Gracilaria textorii was the second most preferred species after C. amplivesiculatum in winter. In this particular case the availability of G. textorii in the environment was higher than C. amplivesiculatum, resulting in a stronger selection of C. amplivesiculatum. Also, G. vermiculophylla was the third food species in order of importance overall, but according to Johnson's test this algae was less preferred than any of the other species consumed. These results indicate that ecological interpretations of the results from lavage samples may be misleading without the knowledge of the composition and abundance of the available vegetation in the environment.

Species such as *C. nidifca* (winter) and *L. pacifica* (spring) were selectively eaten, mostly as a result of their presence in very small quantities (% cover less than 0.2) or due to their absence (as *U. lactuca*) in the environment. *Gracilaria vermiculophylla* was selectively consumed during spring but it was more frequently found in diet samples during winter. This result indicates that this algae was ingested in slightly higher proportions than available during spring, but not during winter when its availability was greater.

The two most abundant algae species in the environment Amphiroa sp. and C. sertularioides were not consumed by any turtle suggesting that they were avoided because of their size or for their basic proximal components (Villegas-Nava, 2006). Interestingly, C. sertularioides has been reported in other green turtle diet studies in the Atlantic (Ferreira 1968; Sazima & Sazima, 1983) and in the western Pacific Ocean (Garnett et al., 1985) but its contribution in the bulk of their diets was less than 0.1% and can be considered as incidental consumption. Nutritional assays had shown strong differences among species (McDermid et al., 2005, 2007), where the balance between fibre nitrogen related substances and/or freshwater supply is the key to understand the selectivity.

In conclusion, although immature green turtles in the Estero Banderitas feed over a great array of algae species, they have preferences for some specific algae species whose availability and abundance showed marked fluctuations throughout the year (López-Mendilaharsu et al., 2003, 2005). Diet preferences and diet diversity changes across seasons coincided with seasonal changes in vegetation biomass. In this respect, the availability of certain resources influenced the selection of the food. However, in the case of sea grasses the impacts of El Niño-Southern Oscillation events and global warming (Echavarria-Heras et al., 2006) are clearly present in Bahía Magdalena with a reduction of the density of Zostera marina over the years (Riosmena-Rodriguez, unpublished data) and the increment of water temperature (Lluch-Belda et al., 2000). An implication of such apparent selectivity in relation to habitat degradation, such as contamination by residual and thermal waters or

 $^{^{2}}$ Taxa not sharing common letters differed in preference (P < 0.01).

habitat damages by ships, leading to diminished abundance of such species may be detrimental to the turtle's quality of nutrition. Mangrove fringes within the Bahía Magdalena complex have been highlighted as priority areas where conservation efforts should be focused due to the lack of regulation and management of the natural resources (Arriaga *et al.*, 1998).

Based on these results, it is recommended that recovery goals for green turtle populations along the Baja California Peninsula should include Pacific coastal mangrove channels and rhodolith beds that are a common element in the bay and in many mangrove areas over the Mexican Pacific. These areas have a high diversity of algae species and should be made priority areas for protection.

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