

ARTICLE

Diatom species diversity in the diet of healthy and sick specimens of adult *Haliotis fulgens* and *Haliotis corrugata*

Diversidad de especies de diatomeas en la dieta de especímenes sanos y enfermos de adultos de *Haliotis fulgens* y *Haliotis corrugata*

David Siqueiros-Beltrones¹, Uri Argumedo-Hernández², Nurenkaya Vélez-Arellano³ and Federico Andrés García-Domínguez³

¹Departamento de Plancton y Ecología Marina, Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, Av. Instituto Politécnico Nacional S/N, Col. Playa Palo de Santa Rita, La Paz, BCS, México. dsiquei@ipn.mx

²Departamento de Economía, Universidad Autónoma de Baja California Sur, km 5.5 Carretera al sur, La Paz, BCS, México

³Departamento de Pesquerías y Biología Marina, Instituto Politécnico Nacional, Centro Interdisciplinario de Ciencias Marinas, Av. Instituto Politécnico Nacional S/N, Col. Playa Palo de Santa Rita, La Paz, BCS, México

Resumen. Se analizó la diversidad de especies de diatomeas en la dieta de abulones azul (*Haliotis fulgens*) y amarillo (*H. corrugata*) del NW de México. Esta se referenció con valores de índices de condición y hepatogonádico (considerados como indicadores de la salud y cambios reproductivos, respectivamente) en ambas especies de abulón, incluyendo especímenes enfermos con síndrome de deshidratación (WS). Las hipótesis enunciaban que: a) las asociaciones representadas en contenidos intestinales de *H. fulgens* vs. *H. corrugata* mostrarían una estructura típica con muchos taxa raros y pocas especies abundantes; b) que no habría diferencia en la estructura de dichas asociaciones de diatomeas; y c) que las asociaciones de diatomeas en abulones con síndrome de deshidratación serían similares a las de especímenes sanos. Para contrastar las hipótesis se examinaron abulones sanos, recolectados entre febrero 2012 y febrero 2013, y especímenes enfermos recolectados entre marzo y mayo 2012. Los especímenes se pesaron y se midieron para calcular los índices de condición y hepatogonádico. Para analizar la diversidad de especies de diatomeas en contenidos intestinales de abulones se disecaron especímenes de 7 fechas de muestreo entre marzo y septiembre 2012. Las muestras de diatomeas se describieron con base en la abundancia relativa de las especies, índices de diversidad y de similitud. Para este período, 19 taxa de diatomeas comprendieron el 80% de las abundancias relativas; los más abundantes fueron *Cocconeis scutellum*, *Gomphonemopsis pseudexigua*, *Grammatophora gibberula*, *Bacillaria socialis* y *Striatella unipunctata*. La diversidad de especies de diatomeas fue alta en todas las muestras. Estos valores y la similitud medida entre muestras indican que ambas especies de abulón se alimentaron de las mismas asociaciones de diatomeas disponibles *in situ* a lo largo de este período. Las muestras de diatomeas de especímenes enfermos representaron asociaciones similares a las de especímenes sanos. Por lo tanto, no hubo evidencias para rechazar ninguna de las hipótesis planteadas.

Palabras clave: Diatomeas epifitas, diversidad de especies, abulones, Península de Baja California

Abstract. Diatom species diversity in the natural diet of healthy adult green abalone (*Haliotis fulgens*) and pink abalone (*H. corrugata*) from NW Mexico was analyzed and, referenced with values of condition index and hepato-gonadic index, used respectively as indicators of the health and reproductive season for the abalone. Likewise observations were done in abalone specimens affected with withering syndrome (WS). Our hypotheses stated that: a) the diatom assemblages represented in the gut contents of *H. fulgens* vs. *H. corrugata* would show a typical structure with few abundant taxa and many rare or uncommon species; b) no difference would be found between the structure of diatom assemblages in the gut contents of *H. fulgens* and *H. corrugata*; and c) diatom assemblages in abalone specimens with WS would be similar to those of healthy specimens. To test our hypotheses we examined healthy abalones collected from February 2102 to February 2013, and specimens with WS collected from March to May 2012. All specimens were measured and weighted and their condition and hepato-gonadic indices calculated. To analyze the diatom species diversity in gut contents of the abalone, specimens from 7 sampling dates from March to September 2012, were dissected. The diatom samples were described using species relative abundances, diversity indices and similarity between samples. For this period 19 diatom taxa comprised 80% of the relative abundances; the most abundant were *Cocconeis scutellum*, *Gomphonemopsis pseudexigua*, *Grammatophora gibberula*, *Bacillaria socialis* and *Striatella unipunctata*. High diversity values were estimated for diatom samples in all abalone specimens. Similarity and diversity values indicate that throughout this period both abalone species fed on the same diatom assemblages available *in situ*. Likewise, diatom samples in the guts of specimens affected by WS represented diatom assemblages similar to those of healthy specimens. No evidence was thus at hand for rejecting our hypotheses.

Key words: Epiphytic diatoms, species diversity, abalone, Baja California Peninsula

INTRODUCTION

The lucrative abalone (*Haliotis* spp.) fishing industry from the middle of the XXth century in the Mexican north western region collapsed more than 40 years ago (Salas-Garza & Searcy-Bernal 1992) due to overexploitation, including pouching, but also to unforeseen changes in climate regime at that time. This most likely overran an unaware management that experienced sadly the lack of information on the complex ecology of said species. The above prompted concerned scientists to undertake autoecological studies mainly on restoration of abalone populations mainly through projects in aquaculture.

Adding to the management problem of this resource, the disease known as withering syndrome (WS) that affected the *Haliotis* spp. populations in the 1980's enhanced the difficulty in recovering the abalone industry. According to a review by García-Esquivel *et al.* (2011) to support a study on green abalone, specimens with withering disease show the following clinical signs: weakness, lethargy, shrinking of foot muscle, mantle retraction, poor gonad development, inability to tightly adhere to the substrate, and death. All this suggested a strong pathogen disease association. Said pathogen infects the epithelial cells lining the gut and enzyme-secreting cells of the digestive diverticula causing the inability to eat in severely ill organisms.

Scientific research on the feeding habits of *Haliotis* spp. in the western coast of the Baja California peninsula (Mexico) began formally at the end of the 1990's with efforts directed toward determining their *in situ* diet. Some studies described the diet of young abalone (Serviere-Zaragoza *et al.* 1998, Guzmán del Prío *et al.* 2003), while others focused on adults (Mazariegos-Villarreal *et al.* 2012). All of which basically provided inventories of the macroalgae species found in their digestive tracts.

Concerning the diatom component in the diet of abalone, even after 2000, most studies consisted mainly of *in vitro* observations on early abalone juveniles or their post-larvae. Soon after, the first comprehensive inventories of diatoms available for abalone feeding in the wild became published (Siqueiros-Beltrones *et al.* 2004). Actual observations of the *in situ* feeding of young abalone on diatoms provided detailed species identifications (Siqueiros-Beltrones *et al.* 2004, 2005), while the lookout for selectivity in their feeding on certain diatom taxa increased the list of taxa included in their diet whilst discriminating the most important (Argumedo-Hernández & Siqueiros-Beltrones 2010).

Concerning adult abalone, it has been strongly accepted for decades that their diet is based solely on macroalgae, particularly on *Macrocystis pyrifera* for this region. However, the potential importance of diatoms in the diet of adult abalone has been recently proposed (Siqueiros-Beltrones 2002, Siqueiros-Beltrones *et al.* 2004), supported by the fact that the macroalgae that they feed on, including *M. pyrifera*, represent a substratum that is densely covered by epiphytic diatoms. Allegedly, these would provide important nutritional elements to the adult abalone, either complementary or essential.

Notwithstanding the need for carrying out chemical analyses of said nutritional elements, it is first necessary to determine the association structure of those epiphytic diatoms that are actually forming part of the *in situ* diet of adult abalone (*Haliotis* spp.). This requires first to make a precise taxonomic inventory as the one supporting this study (Siqueiros-Beltrones & Argumedo-Hernández 2015). The objective was to determine the species relative abundances and the species diversity of the diatoms identified in the gut contents of adult specimens of *Haliotis fulgens* (Philippi, 1845) and *Haliotis corrugata* (Wood, 1828) in their natural habitat. Previous results support our assumption that the diatom species found in the gut contents of both abalone species accurately represent their diverse availability *in situ* (Siqueiros-Beltrones & Argumedo-Hernández 2015).

The observations on the diatom based diet of healthy specimens of these abalone species and their time variations were referenced with measurements depicting their physical condition throughout a year, as well as with the characteristics related to their reproductive changes as seen in the hepato-gonadic index. Our hypothesis (H_0) stated that no difference would be found in the structure of diatom assemblages represented in the gut contents of *H. fulgens* as compared to *H. corrugata*, inasmuch they have similar feeding habits in a shared habitat. However, because a change in feeding habits may occur during the onset of the WS, similar observations were carried out in sick specimens, both for their gut contents as well as for their physical and reproductive condition, in order to record any anomaly in their diet that may be related to this disease. In this case our null hypothesis read that the diatom assemblages in the gut contents of specimens affected with WS would be similar to those described for healthy specimens.

MATERIALS AND METHODS

HEPATO-GONADIC AND CONDITION INDICES

Monthly samplings from February 2012 to February 2013 were carried out in the vicinity of La Bocana (26.0625 N and 112.287 W), located on the NW coast of Baja California Sur (BCS), Mexico (Fig. 1). Around 30 specimens of *Haliotis fulgens* and 30 of *H. corrugata* were collected on each date by scuba diving from a depth of 8 to 12 m. Size and weight of the specimens varied between 90 mm and 190 mm and from 90 to 900 g, respectively. Additionally, 20 specimens (18 *H. corrugata* and 2 *H. fulgens*) showing symptoms of the WS were collected, 9 in March, 9 in April, 1 in May and 1 in July 2012 (Table 1). All specimens were kept for 2 or 3 days in abalone culture tanks at the Cooperativa Progreso de La Bocana, BCS and afterwards transported to the laboratories at CICIMAR-IPN in La Paz, BCS where they were measured and dissected.

With these, indicators of the abalone health condition and reproductive changes were used. The condition index provides a general view of the specimen's physical condition; taking the mean value as reference, higher

values indicate that the organisms are in good health whilst low values indicate loss of weight, inasmuch it is calculated by dividing the fresh weight less the shell of the organism between its total weight, and expressed as a percentage. The hepato-gonadic index, used as an indicator of reproductive status (reproductive season) was estimated by dividing the weight of the hepato-gonad complex by the weight of the specimen without shell and expressed as a percentage (Webber & Giese 1968, Villalejo-Fuerte & Ceballos-Vázquez 1996, Zetina *et al.* 2000).

DIATOM SAMPLES

For determining the diatom based diet of adult *Haliotis fulgens* and *H. corrugata*, out of the overall sampling period, 7 sampling dates: March, April, May, June, July, August and September 2012 were selected arbitrarily. From these, samples of gut contents were taken from only 118 specimens; 11 of these were affected by WS (Table 1). Guts were extracted, longitudinally opened with a scalpel and their contents retrieved using a 5 ml plastic pipette. Gut contents were placed in a 250 ml plastic bottle with 80% ethyl alcohol.

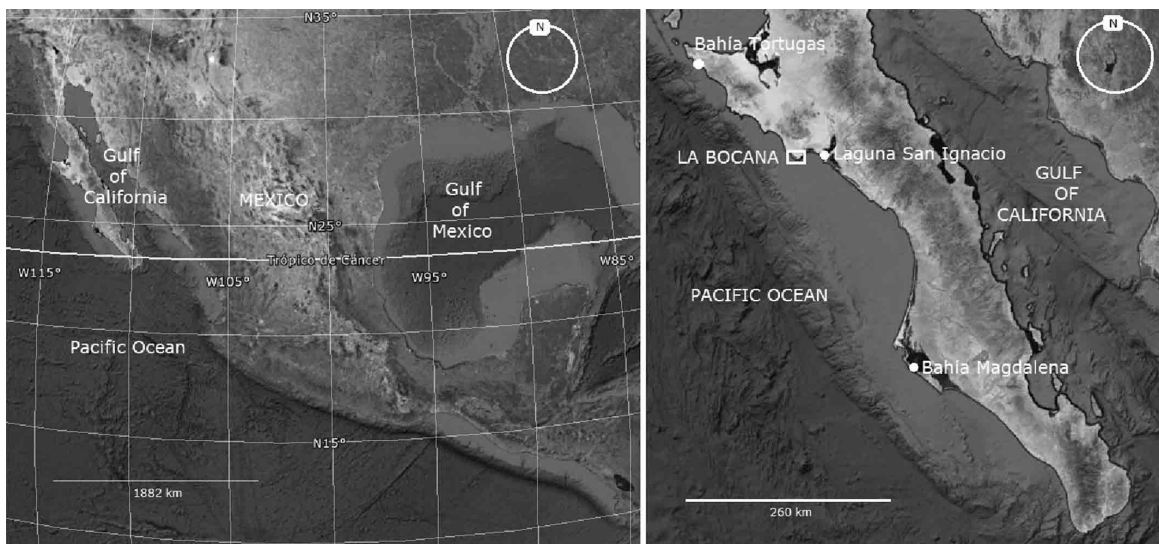


Figure 1. Location of the abalone site La Bocana in the western coast of the Baja California Peninsula, where specimens of *Haliotis fulgens* and *H. corrugata* were collected / Localización del sitio abulonero La Bocana en la costa oeste de la Península de Baja California, donde se recolectaron especímenes de *Haliotis fulgens* and *H. corrugata*

Table 1. Sampling dates, number of specimens, and average size of examined adult *Haliotis fulgens* (Hf) and *H. corrugata* (Hc) from La Bocana, BCS, Mexico. SL= number of slides for identifying diatoms in their gut contents; ND= no data; WS= sick specimens; H= healthy specimens; N= specimens included in the compound sample / Fecha de muestreo, número de especímenes, talla promedio de los adultos examinados de *Haliotis fulgens* (Hf) y *H. corrugata* (Hc) de La Bocana, BCS, México. SL= número de laminillas utilizadas para la identificación de las diatomeas en los contenidos intestinales; ND= sin datos; WS= especímenes enfermos; H= especímenes sanos; N= especímenes incluidos en la muestra compuesta

Date (2012)	Species	N	Health	SL	Size mm
March	Hc	9	WS	ND	144
	Hf	10	H	5	125
	Hc	10	H	5	127
April	Hf	1	WS	2	154
	Hc	8	WS	5	143
	Hf	10	H	5	114
	Hc	10	H	5	114
May	Hc	1	WS	2	148
	Hf	5	H	5	128
	Hc	5	H	5	137
June	Hf	10	H	5	138
	Hc	10	H	5	141
July	Hc	8	H	5	131
	Hf	8	H	5	139
	Hf	1	WS	2	136
August	Hc	3	H	5	148
	Hf	10	H	5	141
September	Hf	4	H	5	139
	Hc	4	H	5	121

Specimens from the same species collected in a same date represented a single compound sample, *i.e.*, one of *H. fulgens* and one of *H. corrugata* for each sampling date. To identify diatoms 5 sub-samples or repetitions were separated from the base sample and each one was deposited in a test tube. Then the organic matter from the samples was oxidized using a mixture of nitric acid and commercial alcohol at an initial proportion of 1:3:5 (Siqueiros-Beltrones & Voltolina 2000) which varied according to the amount of organic matter in each sample. After oxidation the samples were rinsed repeatedly with purified water until reaching a pH \geq 6. From each subsample 2 double permanent slides were mounted using the synthetic resin Pleurax (IR= 1.7).

STRUCTURE OF DIATOM ASSEMBLAGES

Diatoms were identified at 1000 \times under a microscope with

phase contrast and planapochromatic optics, using the references in Siqueiros-Beltrones & Argumedo-Hernández (2015). To estimate the relative abundances (RA) of the diatom taxa 300 valves were counted (n) in a slide representing a sample. Based on said RA species were classified as: abundant (> 25 valves), very common (6-25), common (3-5) and rare (1-2), and community parameters were estimated for the diatom assemblages, such as diversity using Shannon's H', Pielou's J' and Simpson's 1- λ for each sample. Also, similarity between the samples was measured with Jaccard's index which considers only presence-absence of species, and with the Bray-Curtis index which considers both presence-absence of taxa and their RA (Brower *et al.* 1984).

RESULTS

The values of the hepato-gonadic index in both abalone species showed the same pattern, a pronounced peak in August-September which depicts both populations reproductive season, followed by a steep decrease in October which reflects spawning events (Fig. 2A). Values are lower for *H. fulgens* than for *H. corrugata*, which indicates that the weight of the hepato-gonadic gland is proportionally lower in comparison with the organisms overall weight. Much lower values were measured for specimens with WS indicating a diminished development of the hepato-gonadic gland as a symptom of the disease. Albeit a similar pattern as in healthy specimens was apparent.

In relation to the condition index, Figure 2B shows that for *H. fulgens* specimens condition remains stable throughout the year with slight variations in April, August and November (2012), and February 2013. The *H. corrugata* values are lower, which indicate that the weight of the individuals without the shell is much lower than its overall weight; this is because *H. corrugata* has a thicker shell that exhibits more fouling than the *H. fulgens* specimens. A pronounced peak in is also observed in May-September 2012 for *H. corrugata*, which indicates that the specimens were gaining weight, whilst a small decrease in the index value occurred in November. Sick specimens however, showed much lower values compared to those of healthy *H. fulgens* and *H. corrugata* specimens. This makes evident the WS symptoms inasmuch there is a loss of weight and a decrease in weight and size of the callus. However, the specimens collected in April showed higher values than those from previous dates, coinciding with that observed in healthy specimens.

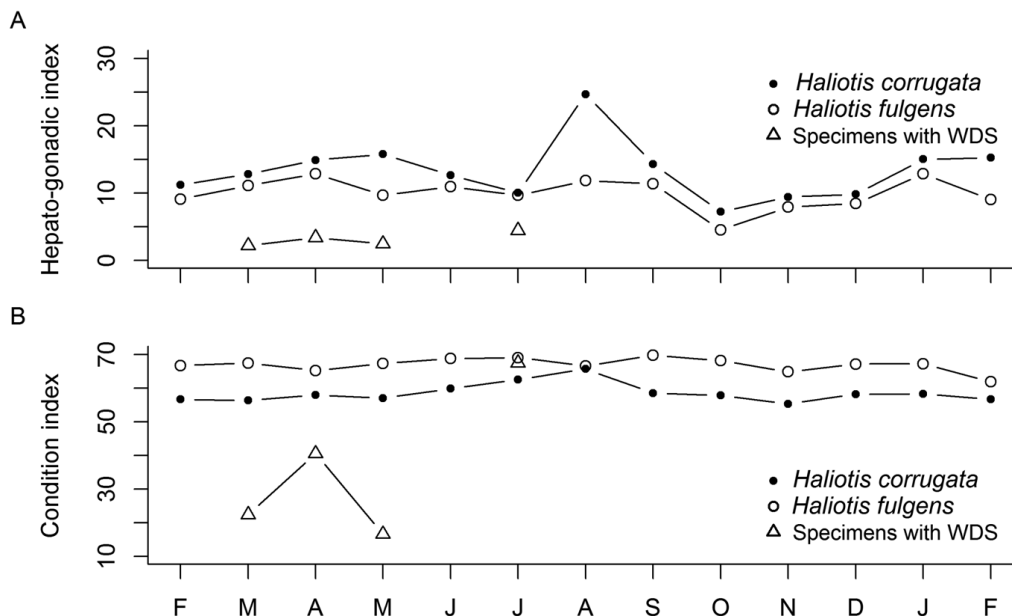


Figure 2. A) The hepato-gonadic index values in both abalone species show the same pattern: a pronounced peak in August-September, and a steep decrease in October. Values are lower for *H. fulgens*. Condition index values (B) show that in *H. fulgens* the condition of the organisms remains stable throughout the year with slight lows in April, August and November 2012 and February 2013. Apparently, values were lower for *H. corrugata* / A) Índice hepatogonadal para ambas especies presentan la misma tendencia: pico pronunciado en agosto-septiembre y marcada disminución en octubre; los valores son más bajos en *H. fulgens*. El índice de condición (B) muestra que en *H. fulgens* la condición de los organismos permanece estable a lo largo del año con ligeras disminuciones en abril, agosto y noviembre 2012, y febrero 2013. Aparentemente, en *H. corrugata* los valores son menores

DIATOM DIET ANALYSIS

Gut content analysis of both species of abalone yielded 149 diatom taxa (species and varieties), mainly epiphytic forms (Siqueiros-Beltrones & Argumedo-Hernández 2015). The best represented genera were *Cocconeis* with 17 species, *Navicula* (15), *Amphora* (15) and *Nitzschia* (11) that sum up around 45% of the floristic inventory. Gut content samples of healthy specimens of *H. corrugata* from August and September did not contain sufficient diatom valves for the quantitative analysis, nor did the April *H. fulgens* specimens with WS. Other samples that yielded quantifiable preparations had low number of diatoms albeit this was observed both for healthy and sick specimens. Absence of diatoms in the guts was observed both in healthy and sick abalone. In the latter, this occurred in specimens from April, which showed peaks in the condition and hepato-gonadic indices (Fig. 2). Diatom species richness (S) in the gut contents appears higher for the *Haliotis fulgens* suggesting a wider trophic spectrum, whilst in *Haliotis corrugata* S is similar to that of the sick specimens, most of which are of the same species.

STRUCTURE OF THE DIATOM ASSEMBLAGES

In accordance with our hypothesis, the diatom taxocenosis gathered from the gut contents of *H. fulgens* and *H. corrugata* adult specimens was composed by few abundant taxa and many (55) rare and common taxa depicting a typical benthic diatom assemblage. Overall, the main represented taxa were *Grammatophora gibberula*, *Cocconeis scutellum*, and *Gomphonemopsis pseudexigua*, among the 23 species comprising 80% (N=1800) of the total RA (Table 2).

While high abundances of taxa such as *Bacillaria socialis* and *Striatella unipunctata* coincide for the *H. fulgens* and *H. corrugata* samples from April, that of *G. pseudexigua* coincides in the July samples for both abalone species. The abundance of this diatom species and of *C. scutellum* and *Tabularia investiens* remained constant throughout the observed dates. In contrast, *Rhoicosphenia genuflexa* showed an abundance peak in the *H. corrugata* sample from May (Table 3). But in general, the RA of the diatom taxa in every sample (date) were characterized by few abundant taxa and many rare or uncommon species.

Table 2. Diatom taxa (24) representing 80.5% of the overall relative abundances (RA) in the gut contents of *Haliotis fulgens* and *H. corrugata* from La Bocana, BCS, Mexico / Taxones de diatomeas (24) que representaron el 80,5% del total de la abundancia relativa (RA) en los contenidos intestinales de *Haliotis fulgens* y *H. corrugata* de La Bocana, BCS, México

Diatom taxa	RA
<i>Gomphonemopsis pseudexigua</i>	13.50
<i>Grammatophora gibberula</i>	13.33
<i>Cocconeis scutellum</i>	8.51
<i>Tabularia investiens</i>	4.30
<i>Rhoicosphenia genuflexa</i>	4.09
<i>Bacillaria socialis</i>	3.92
<i>Grammatophora oceanica</i>	3.86
<i>Navicula parva</i>	3.30
<i>Striatella unipunctata</i>	3.11
<i>Ehrenbergia granulosa</i>	2.67
<i>Nitzschia constricta</i>	2.32
<i>Navicula cincta</i>	2.05
<i>Nitzschia frustulum</i> v. <i>perminuta</i>	1.88
<i>Halamphora coffeaeformis</i>	1.76
<i>Amphora pusio</i> v. <i>parvulus</i>	1.67
<i>Gomphoseptatum aestuari</i>	1.65
<i>Navicula directa</i>	1.62
<i>Cocconeis diminuta</i>	1.53
<i>Cocconeis speciosa</i>	1.16
<i>Amphora pediculus</i>	1.14
<i>Cocconeis dirupta</i>	1.11
<i>Podosira stelliger</i>	1.09
<i>Grammatophora marina</i>	0.97
Cumulative relative abundance	80.50

Table 3. Diatom species (31) representing > 80% of the relative abundances in gut contents of *Haliotis fulgens* and *H. corrugata*, for healthy and sick (WS) from La Bocana, BCS, Mexico / Especies de diatomeas (31) que representaron > 80% de la abundancia relativa en contenidos intestinales de *Haliotis fulgens* y *H. corrugata* sanos y enfermos (WS) de La Bocana, BCS, México

Diatom taxa	Hf	Hc	WS
<i>Grammatophora gibberula</i>	18.17	13.06	
<i>Cocconeis scutellum</i>	12.29	6.27	2.42
<i>Gomphonemopsis pseudexigua</i>	12.18	11.74	19.59
<i>Bacillaria socialis</i>	5.49	3.62	
<i>Tabularia investiens</i>	3.84	4.08	5.81
<i>Grammatophora oceanica</i>	3.80	5.58	
<i>Navicula cincta</i>	3.38		
<i>Striatella unipunctata</i>	3.03	5.01	
<i>Nitzschia frustulum</i> v. <i>perminuta</i>	2.54		2.06
<i>Nitzschia constricta</i>	2.32	2.53	1.97
<i>Navicula directa</i>	2.25	1.04	
<i>Amphora pusio</i> v. <i>parvulus</i>	2.22	1.55	
<i>Cocconeis diminuta</i>	1.83	1.04	1.52
<i>Podosira stelliger</i>	1.37	1.32	
<i>Grammatophora hamulifera</i>	1.34		
<i>Cocconeis dirupta</i>	1.30		
<i>Catacombas gallionii</i>	1.19		
<i>Grammatophora marina</i>	0.95	1.38	
<i>Cocconeis placentula</i>	0.88		
<i>Cocconeis speciosa</i>	0.88		3.22
<i>Rhoicosphenia genuflexa</i>		10.88	3.58
<i>Gomphoseptatum aestuari</i>		4.32	
<i>Melosira nummuloides</i>		2.13	
<i>Amphora bigibba</i>		1.44	
<i>Navicula</i> cf. <i>agnita</i>		1.44	
<i>Halamphora coffeaeformis</i>		1.32	5.19
<i>Nitzschia distans</i>		1.27	
<i>Nitzschia dissipata</i> v. <i>genuina</i>		1.04	
<i>Navicula parva</i>			16.82
<i>Ehrenbergia granulosa</i>			12.70
<i>Amphora pediculus</i>			5.81
Relative abundances	2840	1738	1118
Cumulative relative abundances	81.27	82.05	80.68

The estimated values used to describe the assemblage structure (Table 4) show high species diversity of diatoms where the major components are high species richness and low dominance. In a single case (Hc June) the equitability contributed the most to the high diversity, having the overall high value of J' . And, as with the RA the measured values throughout the sampling period (Fig. 3) agree with our hypothesis, inasmuch the diversity values and its components fall within the ranges ($1 > H' < 5$) that characterize typical benthic diatom assemblages (Siqueiros-Beltrones 2005). Thus, these are considered to be accurately represented in the analyzed gut contents, while indicating that the available diatom flora in the

Table 4. Estimated values of the indices that describe the structure of diatom assemblages represented in the gut contents of *Haliotis fulgens* (Hf) and *H. corrugata* (Hc) collected at La Bocana, BCS. S= Species richness; N= Sample size; J' = Pielou's equitability; H' = Shannon's diversity; λ = Simpson's dominance; $1-\lambda$ = Simpson's diversity; W= specimens with WS; ND= no diatoms present; NP= not processed for diatoms / Valores calculados de los índices que describen la estructura de asociaciones de diatomeas representadas en contenidos intestinales de *Haliotis fulgens* (Hf) y *H. corrugata* (Hc) de La Bocana, BCS. S= Riqueza de especies; N= Tamaño de muestra; J' = Equidad de Pielou; H' = Diversidad de Shannon; λ = Dominancia de Simpson; $1-\lambda$ = Diversidad de Simpson; W= especímenes con síndrome de deshidratación; ND= Sin diatomeas; NP= No procesados para diatomeas

Sample	S	N	J'	H'	λ	$1-\lambda$
Hc March NP						
Hf March	28	302	0.647	3.11	0.193	0.807
Hc March	34	322	0.769	3.92	0.113	0.887
Hf April	45	404	0.642	3.53	0.169	0.830
Hf April W ND						
Hc April	35	331	0.781	4.00	0.091	0.909
Hc April W	39	333	0.576	3.05	0.282	0.718
Hc May W	30	429	0.716	3.52	0.153	0.847
Hf May	61	397	0.773	4.58	0.084	0.916
Hc May	24	370	0.550	2.52	0.304	0.696
Hf June	34	374	0.752	3.83	0.134	0.866
Hc June	39	390	0.806	4.26	0.076	0.924
Hf July	41	304	0.703	3.77	0.150	0.850
Hc July	37	325	0.691	3.61	0.169	0.831
Hf July W	34	356	0.556	2.83	0.330	0.671
Hf August	23	347	0.549	2.48	0.299	0.701
Hc August ND						
Hf September	40	315	0.759	4.04	0.092	0.908
Hc September ND						

abalone environment is highly diverse. Also, in agreement with our hypothesis no difference is apparent in the structure of diatom assemblages represented in the gut contents of *H. fulgens* as compared to *H. corrugata*, nor in the gut contents of specimens with WS.

SIMILARITY BETWEEN SAMPLES

Similarity measurements between samples based on presence/absence of taxa suggests that both abalone species feed on the same diatom species available in their shared habitat, inasmuch the highest similarity observed was between samples of either species from the same date (Fig. 4). This comprises also the gut content samples from specimens with WS, and although the April sample of sick *H. corrugata* showed the minimum similarity, the other 2 samples fall in indiscriminately. Likewise, this agrees with the outcome of the Bray-Curtis index (Fig. 5), thus showing that the more abundant diatom taxa are distributed in similar proportions among the gut content samples from the same dates. The high relative abundances of certain diatom taxa from the specimens with WS (Table 4) do not suggest any other type of relation.

DISCUSSION

No apparent coincidences could be appreciated between parameter measurements of the diatom samples and the condition or hepato-gonadic indices of either abalone species that could suggest changes in feeding habits throughout the sampling period. However, such constancy strongly suggests that adult abalone feed non-selectively on the epiphytic diatoms available *in situ* as juveniles do (Siqueiros-Beltrones *et al.* 2004, 2005), and supports the representativeness of the diatom based diet for adults of both abalone species, which applies to both healthy and sick specimens.

There are few studies that relate reproductive aspects of haliotids with their diet; most refer to the macroalgae-based diet (Muñoz & Ambler 2012, Mardones *et al.* 2013). Until now, most observations on the role of diatoms in the diet of abalone have referred to juveniles or post-larvae. This precludes any comparative analysis inasmuch no studies on the diatom based diet of adult abalone were available until now. Also, much limitation has existed hitherto with the gathering of enough abalone specimens from their natural environment for studying. Thus, although our results are strongly exploratory, they are well supported by the numerous specimens used in our observations.

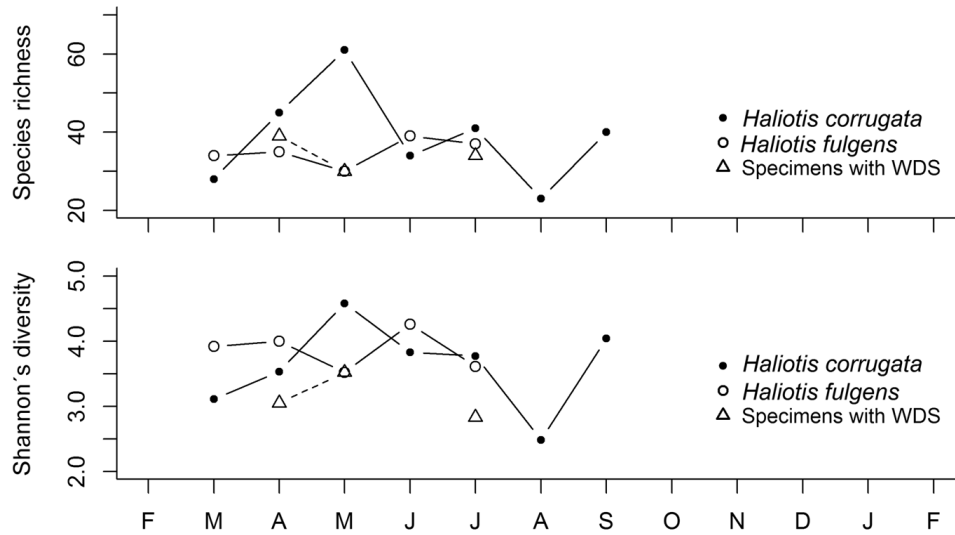


Figure 3. Species richness and species diversity (H') values for the diatom assemblages represented in the gut contents of adult abalone collected at La Bocana, BCS, Mexico at different dates / Valores de riqueza y diversidad (H') de especies para las asociaciones de diatomeas representadas en contenidos intestinales de abulones adultos recolectados en La Bocana, BCS, México en fechas distintas

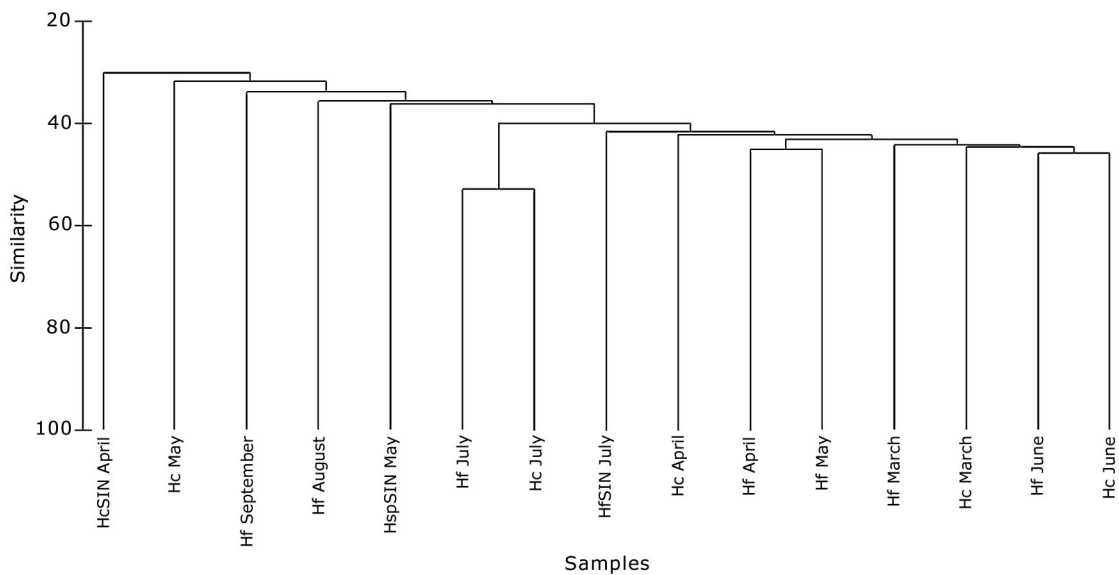


Figure 4. Similarity between diatom samples in the gut contents of *Haliotis fulgens* and *H. corrugata* collected at La Bocana, BCS, based on presence/absence of taxa (Jaccard's index). SIN= specimens with WS / Similitud entre muestras de contenidos intestinales de *Haliotis fulgens* y *H. corrugata* recolectados en La Bocana, BCS, de acuerdo a presencia y ausencia de taxones (índice de Jaccard). SIN= especímenes enfermos

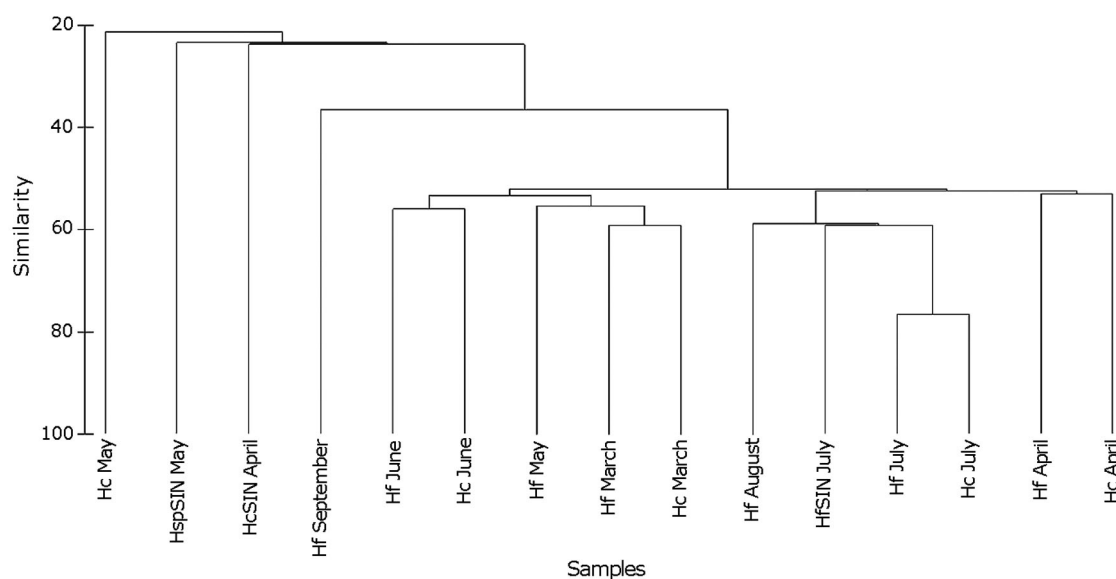


Figure 5. Similarity between diatom samples in the gut contents of *Haliotis fulgens* and *H. corrugata* collected at La Bocana, BCS, based on the relative abundances of shared taxa (Bray-Curtis index). SIN= specimens with WS / Similitud entre muestras de diatomeas de contenidos intestinales de *Haliotis fulgens* y *H. corrugata* recolectados en La Bocana, BCS, de acuerdo con abundancias relativas de los taxones compartidos (índice de Bray-Curtis). SIN= especímenes enfermos

The diatom species richness measured in gut contents of the 2 abalone species from this study (149) compared to other studies dealing with juveniles *Haliotis* spp. (150) is very similar (Siqueiros-Beltrones *et al.* 2004, 2005). Representative taxa from this list are provided in an iconographic catalog supporting this study (Siqueiros-Beltrones & Argumedo-Hernández 2015). However, the assumption that the natural diatom assemblages are represented accurately in the samples from gut contents of abalone may be questionable, inasmuch the total number of diatom taxa found in gut contents of the adult *H. fulgens* and *H. corrugata* examined represent only half of the diatom taxa recorded *in situ* in the same region (Siqueiros-Beltrones *et al.* 2004). Differences in feeding habits could explain this, as well as the heterogeneity of the habitat as with other grazing molluscs such as chitons (Siqueiros Beltrones & Argumedo-Hernández 2012). On the other hand, 24 out of the 37 diatom taxa previously recorded as the most abundant in gut contents of young abalone from Bahía Tortugas and Bahía Asunción, north of La Bocana (Siqueiros-Beltrones *et al.* 2005) were listed in our study, which suggests that both adults and juveniles feed mostly on the most common macroalgae harboring these epiphytic diatom species.

The main diatom taxa in this study such as *Cocconeis scutellum* and *Gomphonemopsis pseudexigua* have been reported as such in previous studies, but *Grammatophora gibberula* for example, stands out as a new important taxon in the diet of abalone. Whilst, taxa such as *Cocconeis costata* var. *pacifica* and *C. speciosa* recorded as abundant in gut contents of abalone juveniles (Siqueiros-Beltrones *et al.* 2005) were absent or very scarce. Higher frequency sampling as well as longer term (year to year) representations are required to determine if diatom assemblage variations are responsible for the observed discrepancies.

The high values of diatom species diversity and the similarity between samples of gut contents from both abalone species and from healthy or sick specimens represent typical benthic diatom assemblages (Siqueiros-Beltrones 2005) and support our hypothesis that no difference would be found in the structure of the diatom assemblages represented in the gut contents of *H. fulgens* as compared to *H. corrugata*. This suggests that the diatom assemblages in the shared habitat are the same and that their feeding habits are very similar. Also, it is most likely that adult abalone feed non-selectively on the available diatom assemblages, as it has been observed

with juveniles, although most diatoms are epiphytic forms suggesting that abalone go for the macroalgae. It is also probable that the higher frequency of certain diatom taxa in the samples varies seasonally and in shorter periods depending on the growth of the host macroalgae. Unlike our present study which is mostly exploratory and, thus, not designed to apply rigorous statistical techniques Higher frequency observations based on simultaneous sampling are needed to test the hypothesis that no differences exist in the diatom based diet of abalone adults and juveniles of either species, supported on *a priori* selected statistical tests.

In relation to the health of the abalone specimens, even though our second hypothesis on the diet of abalone with WS is not refuted, *i.e.*, that the diatom assemblages in sick specimens would be similar to those of healthy specimens, our reasoning dealt with the feeding anomalies leading to their malnutrition and eventual death. The 'normal' contents in their guts, as shown by the presence of representative diatom samples, along with the lower values in the condition index suggest more of a digestive inability than a feeding inability. Observation of fresh samples from fecal material may serve to clear this out in a later study.

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