

Non-selective *in situ* grazing of diatoms by juvenile Green Abalone (*Haliotis fulgens* Philippi, 1945) in the Baja California Peninsula

Consumo *in situ* no selectivo de diatomeas por juveniles de abulón azul (*Haliotis fulgens* Philippi, 1945) en la Península de Baja California

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ABSTRACT

For experimental or rearing purposes, young abalones are fed benthic diatoms successfully during their early life stages without considering if the diatoms provided are found in the abalone habitat, nor if grazing selectivity exists. Our aim in this study was to gather further evidence that would indicate whether young *Haliotis fulgens* graze diatoms *in situ* selectively. Thirty-four specimens of young *H. fulgens* were collected in a fishing site in BCS, México on two dates. We identified the diatoms in their gut contents (144 species) and determined their relative abundances. Fourteen diatoms (80% of the total abundance) were considered most important and twelve were among the most important in the diet of young abalone in BCS. The diversity values (H') of the diatoms in their guts ranged from 1.05 to 4.47, depending on dominance, which reflected the structure of diatom assemblages *in situ*. The above observations and the low similarity measured between samples show that species richness and equitability are similar even if the species composition of diatoms ingested by young abalone differs between specimens. The results lead us to conclude that grazing of diatoms by young *H. fulgens* is non-selective, and that the structure of diatom assemblages is more important than the particular taxa in their diet.

Key words: Diatoms, abalone, *in situ* grazing, diversity, selectivity.

RESUMEN

Durante sus etapas tempranas de vida los abalones jóvenes son alimentados exitosamente con diatomeas bentónicas sin considerar si las especies suministradas se hallan en su medio natural, o si existe algún tipo de preferencia o selectividad por ciertas diatomeas. El objetivo de este estudio fue recabar evidencias acerca de si los juveniles de *Haliotis fulgens* Philippi presentan un consumo selectivo de diatomeas cuando se alimentan *in situ*. Se recolectaron treinta y cuatro juveniles de *H. fulgens* en un sitio de pesca de BCS, México en dos fechas. Se disecaron y se examinaron contenidos intestinales. Se identificaron 144 taxa de diatomeas y se determinaron sus abundancias relativas; 42% fueron epifitas. Catorce taxa representaron el 80% de la abundancia total; de estos 12 han sido propuestos como los

más importantes en la dieta de juveniles de abulón en la costa W de BCS. Los valores de diversidad (H') variaron entre 1.05 y 4.47 y dependieron más de la dominancia que de la riqueza de especies, reflejando la diversidad de diatomeas en el hábitat. Estas observaciones y la baja similitud medida entre contenidos intestinales, muestran que la riqueza y diversidad de especies de diatomeas ingeridas por juveniles de abulón serán similares aun cuando la composición de especies de diatomeas sea distinta entre especímenes. Esto nos permite concluir que la ingestión de diatomeas por juveniles de abulón es no selectiva y que la estructura de las asociaciones de diatomeas es más importante que los taxa específicos en su dieta.

Palabras clave: Diatomeas, abulón, consumo *in situ*, diversidad, selectividad.

INTRODUCTION

In México, as well as in other countries, natural populations of abalone (*Haliotis* spp.) have been much depleted. Thus, research on abalone biology and ecology has been focused on the recovery of abalone fisheries by proposing an aquaculture approach to the problem (Searcy-Bernal & Salas-Garza, 1990). However, it has been noted that much of the aquaculture practice concerning feeding of abalone, particularly juveniles, is highly empirical, and that its *in situ* feeding habits have not been well documented (Siqueiros Beltrones *et al.*, 2004).

During early life stages, cultured abalones are successfully fed benthic diatoms, whether for experimental or rearing purposes. However, this practice does not require that the diatoms provided occur naturally in their natural habitat, nor it matters if young abalones show preference for any particular diatom species.

Updated inventories indicate that many species of benthic diatoms, which represent a food source for abalones during their early stages, occur in their rocky environment (Siqueiros-Beltrones & Valenzuela-Romero, 2004; Siqueiros-Beltrones *et al.*, 2004; Siqueiros-Beltrones *et al.*, 2005). In Thailand, *Haliotis*

asinina thrives in a habitat (coral reef) where few small macroalgae exist (*Cladophora* sp., *Centroceras* sp. and *Ectocarpus* sp.); thus *H. asinina* has to feed mostly on benthic diatoms throughout its life (Sawatpeera *et al.*, 1998). However, most studies on abalone diet in their natural environment examine the gut contents of adult specimens (Serviere-Zaragoza *et al.*, 1998), which feed mostly on macroalgae (Tutschulte & Connell, 1988). Consequently, few studies have focused on the *in situ* diet of young abalone whether it is macroalga based (Guzmán del Prío *et al.*, 2003) or diatom based (Siqueiros-Beltrones *et al.*, 2004). Recently gut content analyses (size 10 to 100mm) of *Haliotis fulgens* Philippi and *H. corrugata* Wood in BCS, México showed up to 9 species of macroalgae and very few diatoms in their diet (Guzmán del Prío *et al.*, 2003). However, the reexamined samples revealed that abundant diatoms were present. This observation prompted the examination of young specimens of *H. fulgens* and *H. corrugata* from the same locality. As a result 98 diatom taxa were recovered from the gut contents, mostly epiphytes, out of which 37 showed to be numerically more important in their diet (Siqueiros-Beltrones *et al.*, 2005). The low intra- and inter-specific similarity of diatom taxa measured in the gut contents from the last study posed no evidence of *in situ* selective grazing on diatoms by young abalone. The aim of this study was to gather further evidence for determining if young *Haliotis fulgens* feeding on diatoms is non-discriminative in their natural environment.

Because our null hypothesis (H_0) stated that diatom feeding by young *H. fulgens* in their natural habitat is non-selective it required supportive evidences; thus, the following subordinate hypotheses were contrasted: 1) Abalone gut contents will show high species richness and diversity of ingested diatoms as do the assemblages in the wild, thus reflecting non-discrimination in grazing; 2) Species composition of diatoms in the gut contents will differ between abalone specimens and likewise from the substrate samples because of the patchy distribution of benthic diatom assemblages; this should be reflected in low similarity index values.

MATERIAL AND METHODS

Juvenile specimens of *H. fulgens* were collected at Clam Bay, an abalone fishing site inside a small cove located 6 km SE of Bahía Tortugas. Maximum depth is measured at 6 m and is protected

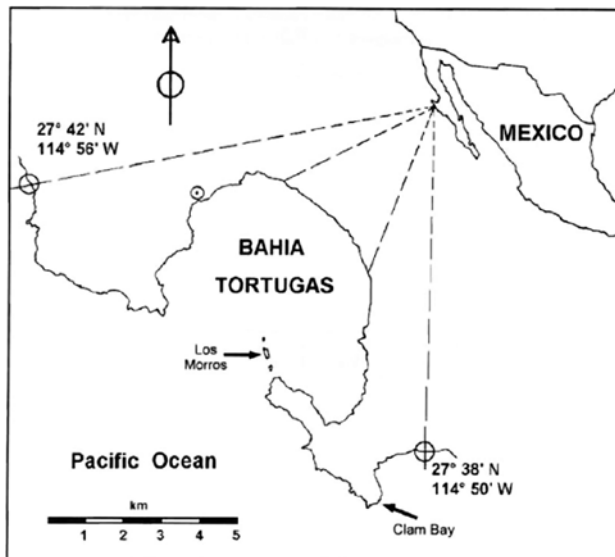


Figure 1. Location of sampling site, Clam Bay in B.C.S., Mexico.

Table 1. Relative (R.A.) and accumulated (A.A.) abundances of the most numerous diatom taxa found in the gut contents of young *Haliotis fulgens*.

SPECIES	R. A. (%)	A.A. ($\Sigma\%$)
1.- <i>Rhoicosphenia genuflexa</i> (Kützing) Medlin	11.0	11.0
2.- <i>Cocconeis californica</i> var. <i>californica</i> Witkowski, Lange-Bertalot <i>et</i> Metziltin	10.5	21.5
3.- <i>Eunotogramma</i> sp.1	9.0	30.5
4.- <i>Cocconeis speciosa</i> Gregory	8.8	39.3
5.- <i>Navicula</i> sp. 1	6.3	45.6
6.- <i>Gomphoseptatum aestuari</i> (Cleve) Medlin	6.2	51.8
7.- <i>Hyalinella</i> sp. 1	5.8	57.6
8.- <i>Gomphonemopsis pseudoexigua</i> (Simonsen) Medlin	4.0	61.6
9.- <i>Cocconeis costata</i> var. <i>hexagona</i> Grunow in Van Heurck	3.9	65.5
10.- <i>Grammatophora hamulifera</i> Kützing	3.4	68.9
11.- <i>Cocconeis dirupta</i> var. <i>flexella</i> (Janish <i>et</i> Rabenhorst) Grunow	3.3	72.2
12.- <i>Cocconeis diminuta</i> Pantocsek	3.1	75.3
13.- <i>Tabularia investiens</i> (W. Smith) Williams <i>et</i> Round	2.7	78.0
14.- <i>Cocconeis costata</i> var. <i>pacifica</i> (Grunow) Grunow	2.4	80.4

from wave action and NW winds. The specimens were collected at wadding depth during low tide by surveying the rocky beach. Due to abalone scarcity, the search for specimens demanded a thorough examination of rocks exposing the underside and retrieving them with a spatula. In this manner 34 specimens (16-74 mm in length) collected in November 2003 (27) and in June 2004 (7) were preserved in 70% ethanol.

We dissected the specimens under a stereoscope and removed their guts. The gut contents of each specimen (sample) were extracted by holding one extreme in place while pressing with a dissecting needle length-wise. Gut contents were then transferred with a pipette into a test tube. To oxidize, the organic matter of each sample was treated with a mixture of commercial alcohol and nitric acid at 1:3:5 (Siqueiros-Beltrones & Voltolina, 2000); these proportions varied according to the organic matter in each sample. The oxidized material was rinsed with distilled water to a pH \geq 6. The clean diatom frustules were mounted on two permanent slides per sample using Pleurax.

Diatoms were identified using scientific reports from studies made in similar localities in the region (Siqueiros-Beltrones, 2002; Siqueiros-Beltrones *et al.*, 2004, 2005; Witkowski *et al.*, 2000). The relative abundances for all diatom taxa in each sample were estimated (n = 500, Siqueiros-Beltrones, 2002).

To contrast our Ho, evidence was gathered based on the analyses of species composition and structure of diatom assemblages found in the gut contents of the collected specimens. Species diversity and equitability were estimated using Shannon's H' and Pielou's J', along with Simpson's 1- λ (Brower & Zar, 1984). Similarity between gut contents was measured

on the basis of presence/absence of taxa with Jaccard index (Magurran, 1988), and also considering their relative abundances

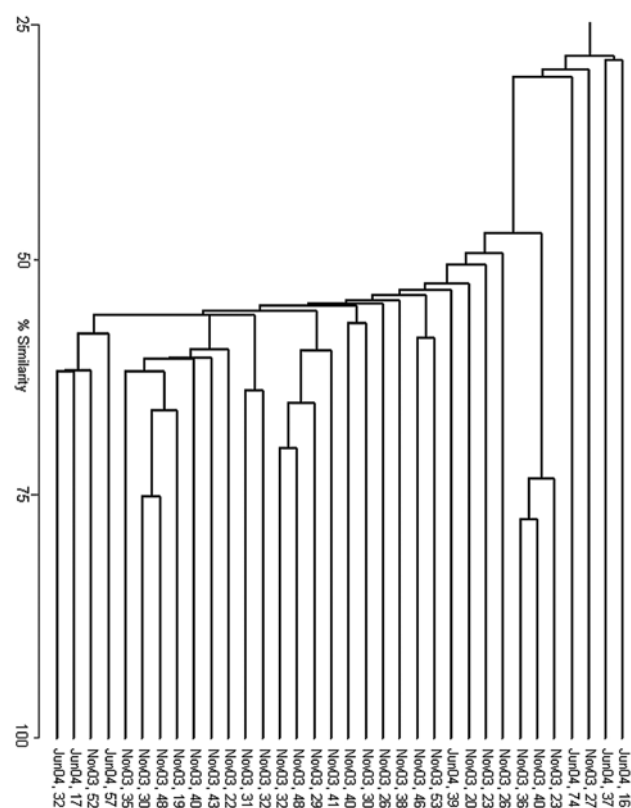


Figure 2. Sample similarity using Bray-Curtis including all the diatom taxa found in the gut contents of young *H. fulgens* collected in November 2003 and June 2004. Specimen size in mm appears on floor axis after the date.

Table 2. Sizes (mm) of the 34 juvenile specimens of *Haliotis fulgens* collected, and species richness (S), diversity (H' y H' max), equitability (J') and dominance (λ) values for diatom assemblages retrieved from their gut contents.

Date	mm	S	H'	H' max	J'	λ
June 2004						
	16	39	2.8	4.9	0.58	0.27
	17	30	4.2	5.3	0.79	0.09
	32	48	4.4	5.6	0.78	0.09
	37	33	3.1	5.0	0.62	0.19
	39	39	3.8	5.3	0.72	0.15
	57	28	3.5	4.8	0.74	0.13
	74	12	1.0	3.6	0.29	0.68
November 2003						
	19	30	3.1	4.9	0.63	0.19
	20	47	3.7	5.6	0.66	0.16
	22	28	3.1	4.8	0.64	0.25
	23	32	3.0	5.0	0.59	0.27
	23	43	4.2	5.4	0.77	0.10
	26	34	4.1	5.1	0.82	0.08
	26	28	2.5	4.8	0.52	0.39
	27	15	1.5	3.9	0.39	0.44
	29	22	3.5	4.5	0.79	0.11
	30	29	3.6	4.9	0.73	0.16
	30	20	2.6	4.3	0.61	0.27
	31	40	3.5	5.3	0.66	0.18
	32	39	3.8	5.3	0.72	0.12
	32	40	4.5	5.3	0.84	0.06
	35	22	2.3	4.5	0.51	0.32
	36	28	2.6	4.8	0.54	0.37
	38	25	3.4	4.6	0.73	0.15
	40	42	4.2	5.4	0.78	0.08
	40	39	3.9	5.3	0.74	0.10
	40	20	2.1	4.3	0.49	0.43
	41	38	4.0	5.2	0.76	0.10
	43	24	2.8	4.6	0.62	0.20
	46	29	2.7	4.9	0.56	0.31
	48	36	4.1	5.2	0.80	0.09
	48	30	3.3	4.9	0.67	0.17
	52	48	4.3	5.6	0.77	0.08
	53	35	3.5	5.1	0.68	0.16

using Bray-Curtis index (Brower & Zar, 1984). In a first approach, we included all taxa in the analyses and constructed the dendrograms by simple union. In a second approach, only the most abundant taxa were considered, *i.e.*, those representing 80% of the total abundance (N). Also, following Siqueiros-Beltrones *et al.* (2005), similarity was measured between diatom assemblages in the gut contents of the June specimens and from a rock.

RESULTS

Overall, 144 diatom taxa were identified as mostly epiphytes, 89 taxa in June 2004 and 130 taxa in November 2003. Lower species richness (S) in June samples could relate to smaller sample size. Over 80% of total abundance (N) was represented by only 14 taxa of epiphytic diatoms (Table 1). Table 2 shows how the distribution of the relative abundances among the taxa determined the structure of the assemblages. High dominance values in certain samples were due to the high numbers of *Cocconeis californica*

var. californica Witkowski *et al.*, *Eunotogramma* sp. 1 and *Rhoicosphenia genuflexa* (Kützing) Medlin; these are epiphytic forms that reached up to 50% of the total abundances. Diversity values were high in general (H' mean = 3.3) although ranging from 1.05 to 4.47, while H' max values reflected the highly variable species richness among the samples from 3.6 (S=12) to 5.6 (S=48). In agreement with our H1, high diversity values are supportive of (Ho) non-selective grazing of diatoms by juvenile *H. fulgens*.

Similarity between the gut contents of all the specimens measured both with the Jaccard and Bray-Curtis indices was very low, and dendrograms showed no group formation even though the shared presence of dominant taxa should have promoted it (Fig. 2). This may also indicate that grazing of diatom taxa was indiscriminate in accordance with H1. The similarity analysis based on the 14 most abundant taxa, yielded (high) values of 75 to 93% (Fig. 3) with the Jaccard index, while the Bray-Curtis yielded a wider range of variation (32 to 83%) due to the difference in proportional

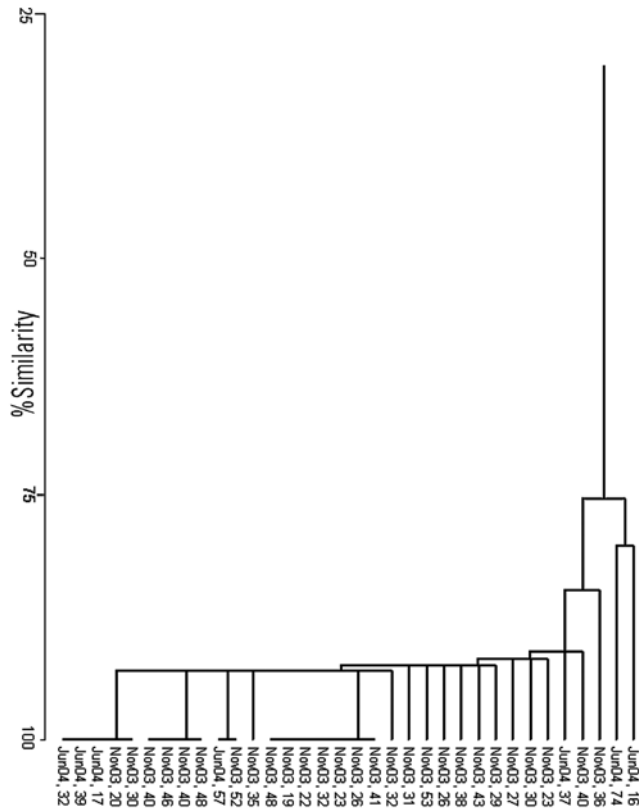


Figure 3. Sample similarity using Jaccard's index based solely on the 14 most important taxa found in the gut contents of the collected specimens.

representation of the same (common and dominant) diatom taxa in different samples (Fig. 4). This relation usually reads the other way around in the derived dendrograms when comparing Jaccard and Bray-Curtis indices. Moreover, similarity between the gut contents of the June specimens and the available diatom flora from a rock substratum (89 taxa) based on Jaccard's similarity was very low (>50%). Although this could indicate selective feeding by the abalone, the difference in S is an important factor.

The Bray-Curtis index indicated certain similarity between less than half the specimens. This is attributed to the presence of different important taxa in the gut contents of each abalone specimen (Figure 5). Thus, high similarity in the presence/absence of taxa and high S lead us to infer no diatom taxa are preferred by juvenile *H. fulgens*, and that the importance of certain diatom taxa in their gut contents detected by the Bray-Curtis index depends on the patchy distribution of diatoms, that is, on whatever taxon is abundant in a given diatom patch. This agrees with our hypothesis that the young of green abalone feed non-selectively on diatoms in the wild.

DISCUSSION

High species richness of the diatoms found in the gut contents of *H. fulgens* juveniles further supports the proposal that they repre-

sent an important component of their diet (Siqueiros-Beltrones *et al.*, 2005), which has seldom been considered as such for young and adult haliotids as opposed to what is accepted for post-larva abalone (Siqueiros-Beltrones & Voltolina, 2000). Moreover, epiphytic diatom represented 42% of the total species richness. Considering that juvenile abalone feed largely on macroalgae, the abundance of diatoms (epiphytes) shows their particular importance in their diet. Moreover, *Haliotis asinina* thrives in a habitat (coral reef) in Thailand where few small macroalgae exist (*Cladophora* sp., *Centroceras* sp. and *Ectocarpus* sp.); thus, it has to feed mostly on benthic diatoms throughout its life (Sawatpeera *et al.*, 1998), whether these are epiphytes or not. Several diatom taxa that may be considered important in the diet of young and adult abalone occur commonly on rocky substrata. But the few *in situ* studies available have noted the importance of only certain (although imprecisely identified) diatoms (*Cocconeis* spp.) in the diet of postlarva abalone, specifically on coralline algae covering the rock (Daume *et al.*, 1997, 1999; Kawamura *et al.*, 1998).

However, in the central region of the Baja California Peninsula 42 species of macroalgae associated to rocks, which serve as refuge and food for *Haliotis* spp., have been identified (Carreón-Palau, 2000). Thus, epiphytic diatom forms are highly available, and young abalone feed commonly on macroalgae surfaces (pers. obs.).

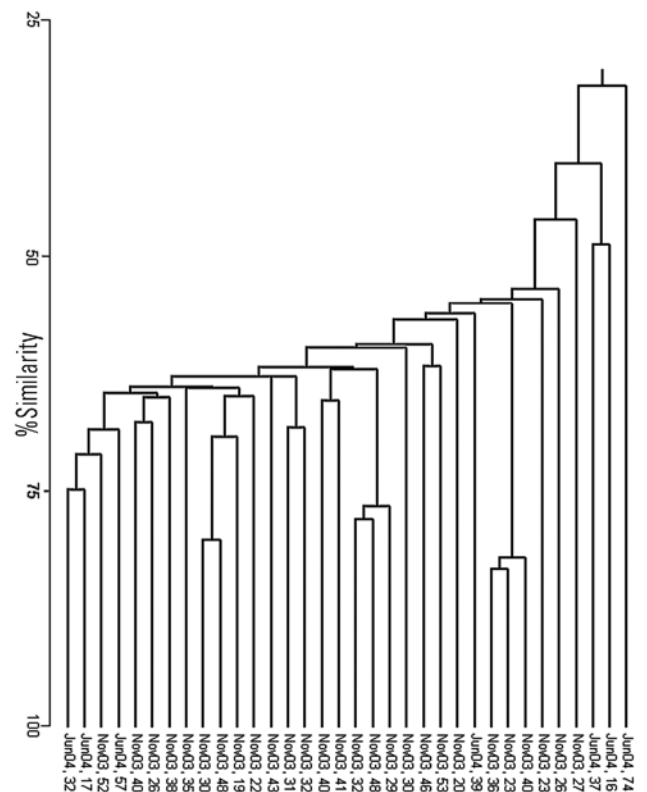


Figure 4. Sample similarity using Bray-Curtis index based solely on the 14 most important taxa found in the gut contents of the collected specimens.

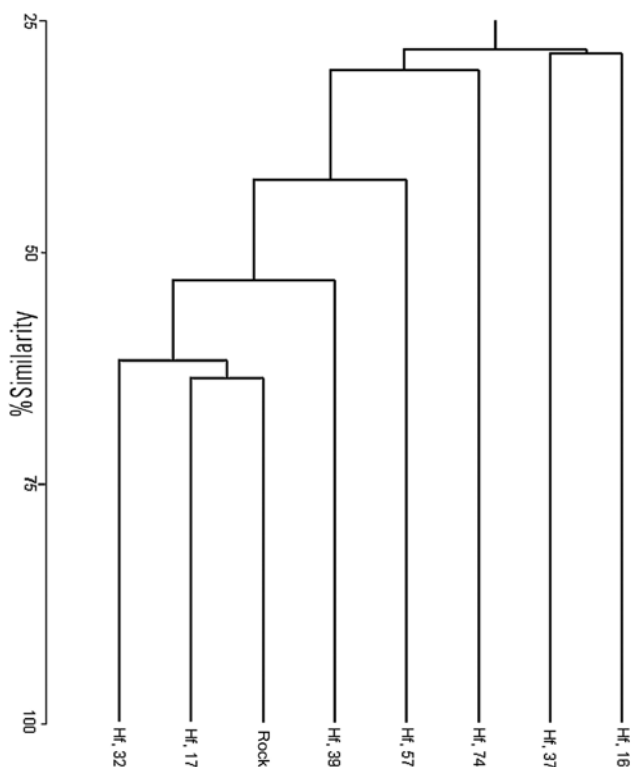


Figure 5. Similarity groups based on Bray-Curtis index. There are values of over 50% between the rock sample and less than half of the gut content samples from June.

Most diatom taxa in the present study are epiphytes, where 95% of the diatom total abundance is represented by 38 taxa, very similar to what Siqueiros-Beltrones *et al.* (2005) reported. In both studies *Cocconeis speciosa*, *Gomphoseptatum aestuari*, *Gomphonemopsis pseudoexigua*, *Cocconeis costata* var. *hexagona*, *Grammatophora hamulifera*, *Cocconeis diminuta*, *Tabularia investiens*, *Cocconeis costata* var. *pacifica*, *Rhoicosphenia adolphi*, *Campylopyxis garkeana*, *Grammatophora marina*, and *Cocconeis scutellum* (Table 1), whose nutritional contribution should be assessed, stood out in the gut contents of young abalone (*H. fulgens* and *H. corrugata*), and are thus considered a natural component of their diet in the Pacific coast of Baja California Sur, Mexico.

The observed species richness (S) was higher (46 more taxa) than the one reported from *H. corrugata* and *H. fulgens* gut contents by Siqueiros-Beltrones *et al.* (2005). Although these authors examined 12 specimens (98 taxa), our June sample size was only 7, yielding 89 taxa; nonetheless, it shows that a correspondence does exist between S and the number of abalone specimens examined. However, collection of young abalone in the wild poses great difficulty because of their scarceness and legal restrictions.

The structure analysis of the diatom samples showed that few abundant taxa and many rare or uncommon taxa charac-

terized the diatom assemblages in the gut contents, and 5% of the total abundance included over 100 diatom taxa. Can these be considered accompanying flora of lesser importance in the diet of *H. fulgens* juveniles during the studied seasons? Or, is this diversity required for their optimal growth? This could explain why young abalone grow faster in the wild (Guzmán del Prío *et al.*, 2004).

Our observations agree with Siqueiros Beltrones *et al.* (2005), who indicated that only a tenth of the diatom taxa in the gut contents of young abalone are abundant (important), suggesting that these might be selected during grazing. However, our interpretation is that the importance of certain diatom taxa in the gut contents of juvenile *H. fulgens* depends not on selectivity but is more dependent on non-discriminative grazing biased by the (patchy) distribution of diatoms in the wild. This is what the similarity dendrograms from this and previous studies (Siqueiros Beltrones *et al.*, 2005) show, *i.e.*, very low similarity between the gut contents of abalone specimens from the same or different species or with the surrounding flora. Thus, whatever diatom species is abundant in a certain diatom patch will be better represented in the ingested material and, according to recent diatom species inventories (Siqueiros Beltrones & Valenzuela-Romero, 2004; Siqueiros-Beltrones *et al.*, 2005), it is likely that many other diatom taxa turn out to be important constituents of young abalone diet depending on the time of the year, considering that the total inventory of diatom taxa in the abalone habitat reaches over 320 taxa (Siqueiros-Beltrones *et al.*, 2004). However, certain selectivity may occur due to differential ability of young abalone to digest the ingested diatoms, where smaller forms may pass their digestive system intact (Kawamura *et al.*, 1998); or break the diatom walls with their radula (Roberts *et al.*, 1999), which is related to the different degree of silicification of the available diatoms.

The above warns on the complexity of the relationships between young abalone and diatoms, which may surpass the implications of our hypothesis (even though it could not be refuted) and require higher resolution analyses. However, the range of variation of the diversity measurements similar to that reported by Siqueiros Beltrones *et al.* (2005), permits us to infer that the structure of diatom assemblages that serve as food for young *Haliotis* spp. is more important than the particular taxa involved. High diversity of diatoms in the gut contents reflects high diversity of diatom assemblages *in situ*. The low values of H' observed might reflect grazing on monospecific patches of certain taxa, which result also in high dominance values (λ). In general, diversity values are high, supported by corresponding values of J' (and low dominance) indicating that diatom taxa are represented homogeneously. The H' values for benthic diatom assemblages with a stable structure generally range between $1 < H' < 5$ (Siqueiros-Beltrones, 2005). These variations may represent succession stages and reflect a heterogeneous distribution whose permanence shows its stability far from an improbable H_{max} under normal conditions (Siqueiros-

Beltrones, 2005). Such stability appears to favor many grazing species found in rocky habitat, including abalone.

The results of this study may be used as a reference when choosing a better diet for abalone juveniles under culture conditions for rearing or research purposes, whether looking for a particular species or an assemblage of those present in the abalone habitat or in their diet.

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