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EPIPHYTIC DIATOMS OF *Macrocystis pyrifera* (L.) C. AG. FROM THE BAJA CALIFORNIA PENÍNSULA, MÉXICO

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ABSTRACT. Blades of *Macrocystis pyrifera* and other kelp species used to feed young abalone (*Haliotis* spp.) in culture were observed in order to determine the species of epiphytic diatoms. For comparison rocks and sediments were also surveyed. The dominant taxon on *M. pyrifera* blades was *Cocconeis* cf. *britannica*. Other important species were *Cocconeis speciosa, Gomphonemopsis pseudoexigua,* and *Climacosphenia moniligera*, which was associated to a colonial *Navicula* sp. None of these species were observed on the other kelp species or on rocky substratum. Even though *Cocconeis* cf. *britannica* was very abundant it represents a new record for the Baja California Peninsula coasts. Other first records are *C. speciosa, Pseudogomphonema kamtschaticum, Rhoicosphenia adolphi* and *Okedenia inflexa*, albeit this one from sediments and rocky substratum. The absence of *Cocconeis* cf. *britannica* from other substrata and other kelp from the area suggests that it may have a preference for *M. pyrifera* as substrate. These observations should be considered when assessing the kelp´s nutritional value for abalone feeding.

Key words: Epiphytic diatoms, *Cocconeis* cf. *britannica*, *Macrocystis*, Abalone, New records, Baja California Península.

Diatomeas epífitas de *Macrocystis pyrifera* (L.) C. Ag. de la Península de Baja California, México

RESUMEN. Se observaron láminas de *Macrocystis pyrifera* y otras especies de algas pardas utilizadas para alimentar abulones en cultivo, con el ojetivo de determinar las especies epífitas de diatomeas. También se analizaron rocas y sedimentos con fines comparativos. *Cocconeis* cf. *britannica* fue la especie dominante y se observó en abundancia cubriendo las láminas de *M. pyrifera*. Otras especies importantes fueron *Cocconeis speciosa*, *Gomphonemopsis pseudoexigua*, así como *Climacosphenia moniligera* asociada a colonias de *Navicula* sp. Ninguna de estas especies se observó sobre las otras feofitas o en sustrato rocoso. A pesar de su abundancia, *Cocconeis* cf. *britannica* constituye un nuevo registro para las costas de la Península de Baja California, al igual que *C. speciosa, Pseudogomphonema kamtschaticum* y *Rhoicosphenia adolphi*, así como *Okedenia inflexa*, pero éste último para sustrato rocoso y sedimentos. La ausencia de *Cocconeis* cf. *britannica* en otras feofitas y otros sustratos de la zona sugiere que tiene una preferencia por *M. pyrifera* como sustrato. Esto debe ser considerado al estimar el valor nutricional de esta macroalga como alimento de abulones.

Palabras clave: Diatomeas epífitas, *Cocconeis* cf. *britannica, Macrocystis*, Abulón, Nuevos registros, Península de Baja California.

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INTRODUCTION

The Baja California Peninsula extends over a wide latitudinal range comprising warm-temperate, subtropical and tropical conditions, including a transitional zone. Several extensive surveys of benthic diatoms along its coasts yielded more than 800 taxa of epiphytic, epilithic, epipsammic and epipelic forms (Siqueiros Beltrones, 1988, 1990, 1994, 1999, 2000; Siqueiros Beltrones & Ibarra Obando, 1985; Siqueiros Beltrones *et al*., 1991; Siqueiros Beltrones & Morzaria Luna, 1999; Siqueiros Beltrones & Sánchez Castrejón, 1999; Siqueiros Beltrones, 2002). However, many locations and different substrata still need to be surveyed.

Combining the interest on diatom taxonomy with abalone aquacultural projects has offered the possibility for exploring a particular type of habitat and ecological relationship inasmuch diatoms play an importan role in abalone feeding. A recent study which related

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in vitro diatomological analyses with the grazing activity of pink abalone (*Haliotis rufescens* Swainson) post-larvae (Siqueiros Beltrones & Voltolina, 2000) showed that diatom films grown for feeding the post-larvae were of epipelic nature. This led to the study of diatom growths on rocks were abalone thrive (Siqueiros Beltrones, 2000), in order to find better candidates as a food source for abalone.

Given the normal habitat of abalone, epilithic diatoms would be the primary choice, although most of the substrate is occupied by diverse species of macroalgae with a great degree of epiphytism by diatoms, which have been observed to be ingested by pink abalone and chitons of the genus *Stenoplax* sp. (Siqueiros Beltrones & Valenzuela Romero, 2001).

Abalone (*Haliotis* spp.) has a strong preference for drift *Macrocystis pyrifera* (L.) C. Ag. blades, and in abalone farming *Macrocystis* is succesfully fed to juveniles and adults (Ebert & Houk 1984, Hahn 1989, Mazón Suástegui *et al*., 1992, McBride, 1998). This offered the possibility for surveying the epiphytic diatom associations which live on the blades of *Macrocystis pyrifera* and other kelps that are used to feed *Haliotis* spp., and to determine which species of epiphytic diatoms may be suggested as a potential diet for the monoculture feeding of abalone.

MATERIAL AND METHODS

Fronds of *Macrocystis pyrifera* (L.) C. Ag., *Egregia menziesii* (Turner) Areschoug, and *Eisenia arborea* Areschoug were collected at different dates from sites located in the Punta Eugenia-Bahía Tortugas area and Eréndira (Fig. 1; Table 1). All were stored dry to feed juvenile abalone under laboratory conditions in La Paz and Bahía Tortugas. Additionally, blades of *Macrocystis* and *Eisenia*, and three submerged rocks (≈ 1 kg) were collected from the same site at B. Tortugas during low tide (Z = 0.5 m) for comparing the epiphytic and epilithic diatoms (Table 1). Sediments samples from a seawater runoff in the B.Tortugas laboratory were also collected.

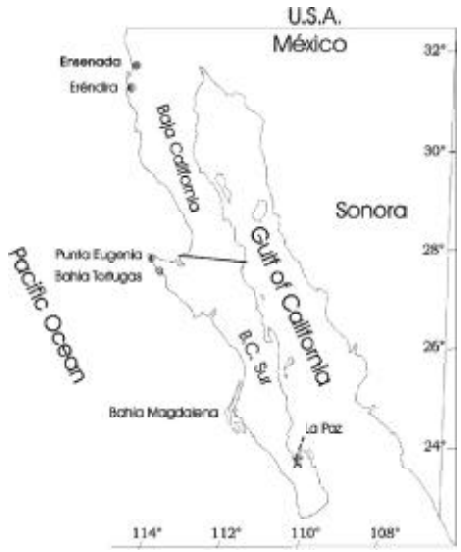

Figure 1. Location of the sampling areas for *Macrocystis pyrifera*, *Egregia menziesii, Eisenia arborea*, and rocky substratum in the abalone (*Haliotis* spp.) fishing zones of the Baja California Peninsula.

Figura 1. Localización de las zonas de *Macrocystis pyrifera, Egregia menziesii, Eisenia arborea* y sustrato rocoso en las áreas de pesca de abulón (*Haliotis* spp.) de la Peninsula de Baja California.

At least 20 dried and rehydrated blades of the three kelp species (10 *M. pyrifera*, 5 *E. menziesii*, 5 *E. arborea*) were analyzed. Several blades chosen at random were used for the floristic analysis of epiphytic diatoms. The humidified blades of three kelp species were scraped using a glass slide concentrating the resulting microalgae and mucus. Additionally, sections of the outermost layer (epidermis) of *M. pyrifera* blades were peeled off and observed under 100X and 450X with a phase contrast microscope, to determine the number of cells of the dominant species per mm^2 (n=5) in 5 blades (N=25) of each sampling date. The *M. pyrifera* blades were also observed under a dissecting microscope. For floristic comparison, the epilithic diatoms were detached from the collected rocks by applying ultrasound for 30 seconds.

All the samples were oxydized with a 1:3:1 mixture of sample, nitric acid and commercial alcohol (Siqueiros Beltrones & Voltolina, 2000), which produces a mild exothermic reaction yielding well cleaned diatom frustules.

Table 1. Localities and sampling dates for the kelp species used to feed young abalone under culture conditions in La Paz and Bahía Tortugas, B.C.S. *Synoptic samplings.

Tabla 1. Localidades y fechas de muestreo de las especies de feofitas utilizadas para alimentar juveniles de abulón en cultivo, en La Paz y Bahía Tortugas, B.C.S. *Muestreos sinópticos.

	Locality	Date
Macrocystis pyrifera (L.) C. Ag	Punta Eugenia	May, August, November 2000
	Bahía Tortugas, B.C.S.	January, February*, 2001
	Eréndira, B.C.	September 2000
Eisenia arborea Areschoug	Punta Eugenia-Bahía Tortugas, B.C.S.	August, November 2000
		February*, 2001
Egregia menziesii (Turner) Areschoug	Bahía Tortugas, B.C.S.	September, December 2000
Submerged rocks	Bahía Tortugas, B.C.S.	February*, 2001

These were rinsed several times with distilled water until pH >6, and used to prepare permanent slides using Meltmount as mounting media (R.I.=1.7), now housed in the diatom collection (Diatomario) of the Museo de Historia Natural de la Universidad Autónoma de Baja California Sur (MHNUABCS).

Phase contrast microscopy at 1000X was used to identify the diatom taxa based on classic and recent literature, particularly Schmidt *et al*., (1874-1959), Peragallo & Peragallo (1897-1908), Hendey (1964), Laws (1988), Romero & Rivera (1996), Siqueiros Beltrones (2002), and Witkowsky *et al*. (2000).

RESULTS

All the kelp samples showed good appearance, except for the September sample of *M. pyrifera* from Eréndira, which looked deteriorated and showed heavy fouling with bryozoans (old blades). Also, many colonies of hydrozoans harbouring abundant frustules of a *Cocconeis species* were observed and studied separately. The January and February *M. pyrifera* blades had a more greenish and thinner appearance.

The most abundant epiphytic diatom on the *M. pyrifera* blades by far was *Cocconeis* cf. *britannica*, which formed an extensive mosaic covering the whole blade surface and apparently served as substratum for the above and other diatom species. This remained constant from May until November.

The estimates of cell abundance of *Cocconeis* cf. *britannica* on the *M. pyrifera* blades varied between 3000 and 4000 frustules/mm^2. The Eréndira (Sep.) blades showed a much more scarce population, in the January and February blades this species was scarce while other taxa were absent. This corresponded with a far smaller amount of mucus scraped from the surface.

Observations of the *M. pyrifera* blades under the stereoscope revealed discrete colonies or tufts of *Climacosphenia moniligera* Ehrenberg spaced throughout most of the *M. pyrifera* blades. Further observations using a 45X objective revealed branched tubular colonies (Fig. 2) of *Navicula* sp. (Fig. 4 p, q, r) which served as a fixing point for *C. moniligera* (Fig. 4 m, n). These became highly abundant in the November samples, reaching between 60 to 200 ind./mm^2, always associated to the *Navicula* sp. colonies.

Also abundant on the *M. pyrifera blades* was *Gomphonemopsis pseudoexigua* (Simonsen) Medlin (Fig. 3 c - g) , while *Cocconeis speciosa* Gregory (Fig. 4 i - l), a first record for the peninsula, occurred frequently but it was not abundant. Other common taxa included *Pseudogomphonema kamtschaticum* (Grun.) Medlin, *Rhoicosphenia adolphi* A. S., which represents new records for the peninsula, and *Tabularia investiens* W. Smith.

Although *G. pseudoexigua* is considered a rare taxon by Round *et al*. (1990), in our

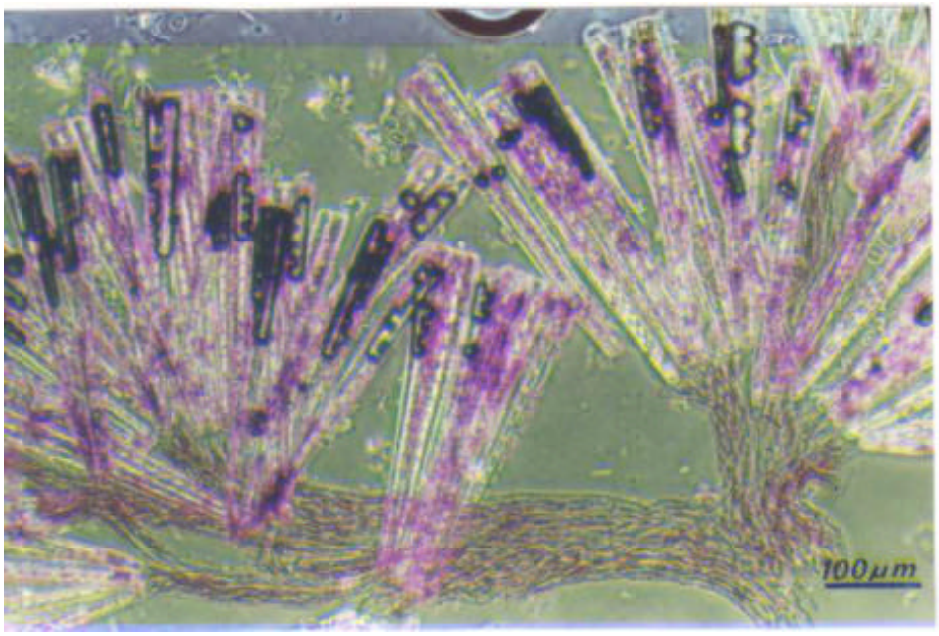

Figure 2. Colony of *Climacosphenia moniligera* Ehrenberg atatched to a *Navicula* sp. colony growing on blades of *Macrocystis pyrifera* from Punta Eugenia-Bahía Tortugas, B.C.S.

Figura 2.Colonia de *Climacosphenia moniligera* Ehrenberg adherida a una colonia de *Navicula* sp. Sobre las láminas de *Macrocystis pyrifera* de Punta Eugenia-Bahía Tortugas, B.C.S.

samples it was abundant, and in addition many specimens were larger than the upper limit given in the literature. This taxon was absent from the August blades, while *Cocconeis speciosa* seemed to increase its frequency. Both taxa were very scarce in September, and the *G. pseudoexigua* specimens were smaller (<20 µm), but became frequent again in the November samples.

In constrast, the samples from the surfaces of *Egregia* and *Eisenia* blades did not yield an epiphytic diatom flora and the few diatoms observed were different from those of the *M. pyrifera* taxocoenosis. Likewise, diatoms from the rocky substratum showed a very different composition with only a few valves or fragments of the *M. pyrifera* diatom flora. Many forms were epipelic, such as *Okedenia inflexa* (Brébisson) Eulenstein ex De Toni (Fig. 4 v) which is recorded for the first time for the Baja California Peninsula, and was abundant in the damp sediments outside the B. Tortugas laboratory. Common epilithic forms such as *Cocconeis dirupta* Gregory (Fig. 4 u) and *Cocconeis dirupta* var. *flexella* (Jan.) Rabenhorst were frequent on the rocks but were not present on *Macrocystis*.

Description of *Cocconeis* cf. *britannica* Naegeli ex Kützing

Because this species could prove useful for feeding abalone in the laboratory, precise identification is required, based on its description and on iconographic references. The dissimilar valves of *Cocconeis* cf. *britannica* are elliptic, with biseriate striae that become tetraseriate and broader toward the margin of the pseudoraphe or sternum valve (Fig. 4 a-h). This appears as a marginal ring that is better defined and narrower in larger specimens. It has a narrow axial area or sternum that becomes slightly wider at the center. In most cases the raphe valve appears more delicate except for the marginal striae. The raphe is strait, unlike the raphe of *Cocconeis britannica* which is described as slightly bent (Hendey, 1964; Witkowsky *et al.*, 2000). The observed valves in our samples vary in size from 15 to 70 µm in lenght and from 11 to 65 µm in width. The abo-

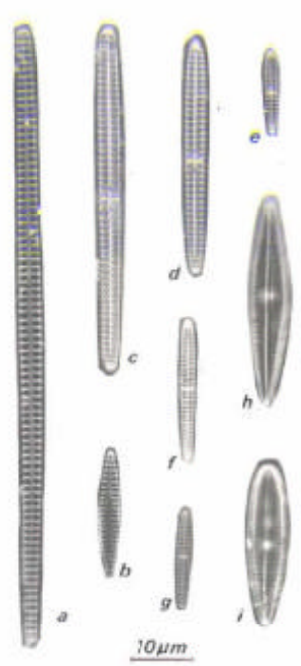

Figure 3. Other abundant and common diatom species found on *Macrocystis pyrifera* blades from Punta Eugenia-Bahía Tortugas, B.C.S. a-b) *Tabularia investiens* W. Smith; c-g) *Gomphonemopsis pseudoexigua* (Simonsen) Medlin; h-i) *Pseudogomphonema kamtschaticum* (Grunow) Medlin.

Figura 3. Otras especies comunes y abundantes encontradas sobre láminas de *Macrocystis pyrifera* de Punta Eugenia-Bahía Tortugas, B.C.S. a-b) *Tabularia investiens* W. Smith; c-g) *Gomphonemopsis pseudoexigua* (Simonsen) Medlin; h-i) *Pseudogomphonema kamtschaticum* (Grunow) Medlin.

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nimum size of 25-30 µm for Lar-
≥35 µm) are more circular (lenght/width <1.2). The smaller specimens of our samples (25 µm or smaller), exhibit a L/W proportion around 1.4. Small specimens have 7 marginal and 9 axial striae/10µm on the sternum valve, while large specimens have 6 marginal and 8 axial striae/10µm.

DISCUSSION

Although further studies are needed to precisely determine the taxon for this species (i.e. scanning electron microscopy observations), the identification here provided helps to discriminate it from similar taxa and to characterize the floristics of an hitherto unsurveyed substratum. To date *Cocconeis* cf. *britannica* had not been recorded in any of the studies carried out along the Baja California Peninsula which comprise various habitats, i. e. epiphytic, epipelic, epilithic, in productive and in harsh environments, in spite of its high abundance on the *M. pyrifera* blades.

Hendey (1964) and Witkowsky *et al.* (2000) indicate a widespread distribution for *C. britannica* Naegeli ex Kützing along the Atlantic coasts, Northern Atlantic, Mediterranean Sea and Gulf of Mexico. For the northern hemisphere in the Eastern Pacific we have no knowledge of a record for this taxon.

For the Baja California Peninsula, recently analyzed assemblages from rocky and algal substrata (Siqueiros Beltrones, 2000; Siqueiros Beltrones & Valenzuela Romero, 2001) and later observations are yielding still new records but not of this taxon or of *Cocconeis britannica*. Such was also the case for *C. speciosa, Pseudogomphonema kamtschaticum* and *Rhoicosphenia adolphi*, which are recorded for the first time in the region. Likewise *Okedenia inflexa*, was observed abundantly in sediments and rocks, constitutes another new record for the region. Along with the new records for the dominant species, these data are indicators of the need for much more taxonomic surveys of benthic diatoms along the Baja California Peninsula.

The high abundances of *C. moniligera* and *G. pseudoexigua* surpass any previous observation in the region. Their frequency in different dates coupled to the ageing of *M. pyrifera* blades evidentiates an interesting successional process of the diatom taxocoenosis. Also, the colonies of the hydrozoan *Eucopella caliculata* (Hincks) containing abundant frustules of *Cocconeis notata* Petit, suggests an interesting partnership (Siqueiros Beltrones *et al*., 2001) occurring in the epiphytic community of the blades.

This is the first study on epiphytic diatoms of *M. pyrifera* in the region, which may explain why *Cocconeis* cf. *britannica* has been overlooked although, because of its high abundance on the *M. pyrifera* blades, the probability of

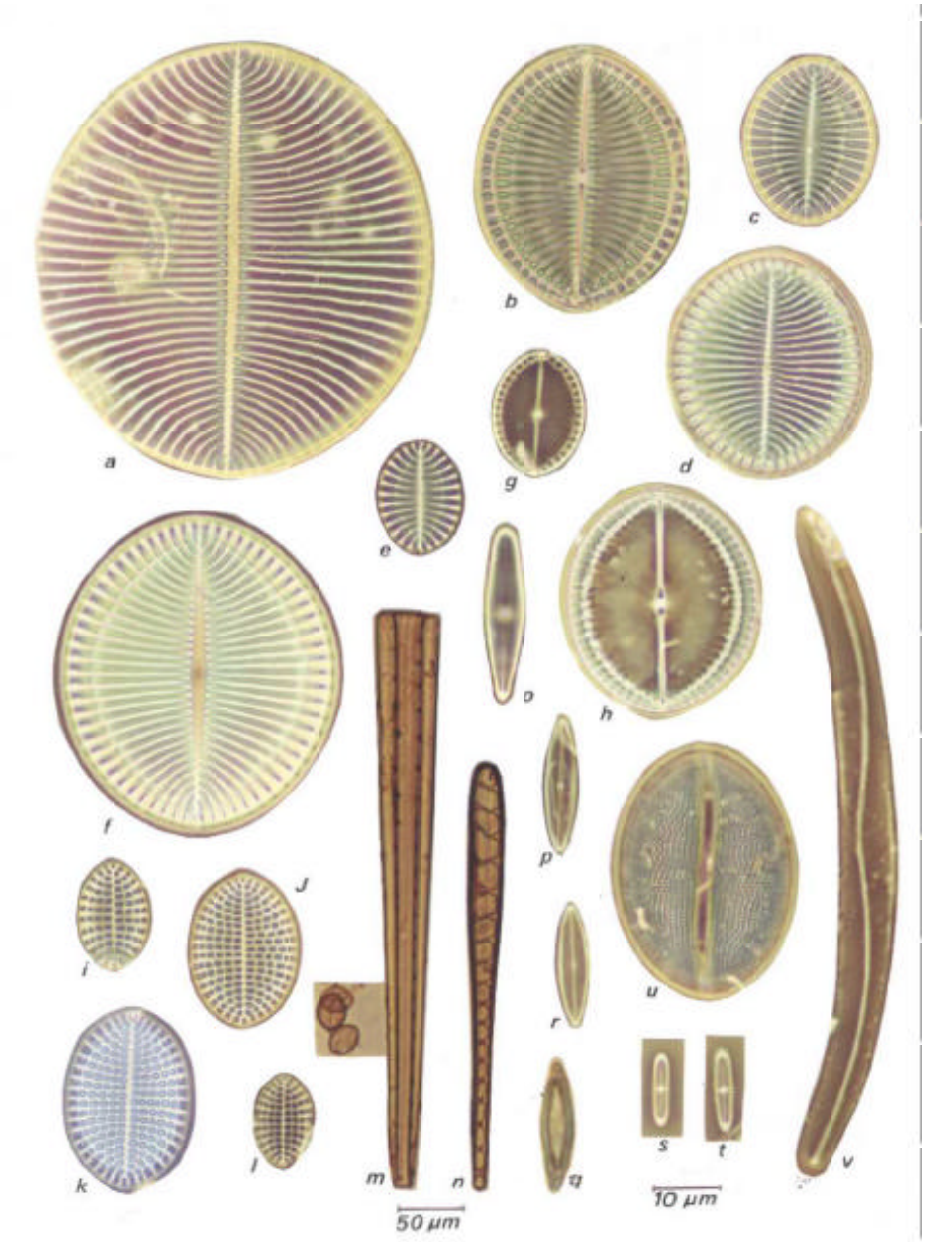

Figure 4. Array showing the different sizes of cf. *britannica* *Ma-* from Punta Eugenia-Bahía Tortugas, B.C.S. a) Largest size observed, sternum-valve (uncommon); b) men observed; g, h) Sternum ra- *Cocconeis speciosa* Gregory, ; m) *Climacosphenia moniligera* and *C* *britannica* for size reference; n) , valve view; o) *Pseudogomphonema kamtschaticum* Medlin; p, q, r) Colony forming *Navicula* *Rhoicosphenia adolphi* A. Schmidt; u) Gregory and v) *Okedenia inflexa* *M. Pyrifera*.

. Arreglo que muestra la variación en tamaño de Naegeli ex Kützing sobre las láminas de de Punta Eugenia-Bahía Tortugas, B.C.S. a) Tamaño máximo observado, valva del pseudorafé (esternón) (rara); b) Valva del rafé observada a través de la valva del esternón; c, d, f) Tamaños más pequeño observado; g, h) Valvas del rafé. i-l) *Cocconeis speciosa* *Climacosphenia moniligera* Ehrenberg, vista conectiva y . cf. *britannica* *C. moniligera,* vista valvar; o) (Grunow) Medlin; p, q, r) *Navicula* célula viva); s, t) *Rhoicosphenia adolphi* *Cocconeis dirupta* Gregory y v) (Brébisson) Eulenstein ex De Toni, de sustrato rocoso, no encontrada sobre

finding detached frustules on other substrata, at least as occasional contaminants, should be high. This taxon appears to be a preferential epiphyte, but the specificity of the host-epiphyte relation with *M. pyrifera* has to be confirmed observing other species of macroalgae. It is also necessary to follow the successional process of diatoms on *M. pyrifera* comparing the different localities and using association structure analysis, and to confirm that the other kelp species do not exhibit a similar fouling by diatoms.

The abundance of *Cocconeis* cf. *britannica* on *M. pyrifera* blades (and that of *C. monilifera, G. pseudoexigua*, and the other species present) should be considered when assessing the nutritional value of this kelp species for feeding abalone in culture. It has been shown that certain epiphytes, although not diatoms, may significantly enrich the diet of small abalone (Trevelyan *et al.*, 1998). Other studies show that abalone post-larvae scrape diatoms (*Cocconeis scutellum* Ehr.) off coralline algae (Daume *et al.*, 1997) and in sites were macroalgae are scarce adult abalone may feed mostly on benthic diatoms (Sawatpeera *et al.*, 1998).

Preliminary observations show that abundant valves of *Cocconeis* cf. *britannica* are found in the gut contents of young green abalone (*Haliotis fulgens* Philippi) fed *M. pyrifera* blades in the laboratory, as much as other *Cocconeis* species are in the guts of *H. rufescens* from natural rocky substratum (pers. obs.).

Finally, strong interest exist on isolating and mass growing benthic diatoms with the purpose of feeding the best possible diet to young abalone under culture conditions. *Cocconeis* cf. *britannica* might be a good choice for the monospecific feeding of abalone. Researchers interested in isolation and mass production of potentially useful species for this purpose should consider it as a good candidate. Likewise, the fact that it blooms on the *M. pyrifera* blades should also be considered when developing the best isolating and production media for this species.

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