Colonization of Fiber-glass Plates by Benthic Diatoms from Subtidal Sediment off the Coast of Yucatán, México

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INTRODUCTION

Micro- and macrofouling on large and small boats and other surfaces in the sea represent an important economic and environmental problem (López-Fuerte et al., 2017). Biofouling has received considerable attention in many countries since the middle of the former century (e.g. Woods Hole Oceanographic Institution, 1952); however, in México, its interest is still incipient. Although most of the attention on this process has focused on macrofouling biota, it is known that the initial microfouling film plays a definite successional role. At this stage, dia-

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toms constitute a key element, in part because of their high biomass (Cooksey & Wigglesworth-Cooksey, 1995; López-Fuerte et al., 2017) and by the secreted exopolymers films that promote the ensuing macrofouling, conditioning the original surface for settling larvae of invertebrates. Hence, it is necessary to know precisely which are the primary microfouling diatom species in order to understand the ecological processes that may give an insight into treating or preventing the fouling problem.

The source of benthic diatoms as major microfoulers on alternative substrate, placed in the marine environment, maybe the water column itself and the nearby substrate such as sediments (Fernandes et al., 1999). However, many diatom species which turn out to be abundant on said surfaces are not considered typical from rocky substrata or other hard surfaces but are epipelic forms thriving in sediment (Siqueiros-Beltrones, 2002). Among alternative substrate, diatoms may have specific preferences (Mitbavkar & Anil, 2000), e.g., a higher diatom recruitment has been observed on (hydrophobic) fiberglass than on (hydrophilic) glass surfaces (Patil & Anil, 2005a), although heavy fouling has been observed on silicon treads used as adhesive (Siqueiros-Beltrones, 2002).

For the Gulf of Mexico and Caribbean Sea (Mexico), few floristic studies on benthic diatoms exist. Navarro & Hernández-Becerril (1997) compiled a check-list of diatom species that gathered both phytoplanktonic and benthic forms. Later studies have been carried out in the Yucatan Peninsula that address several issues, from species composition of epiphytic diatoms on *Thalassia testudinum* in which López-Fuerte et al. (2013) identified 87 taxa, to the recording of new taxa (Hernández-Almeida et al., 2013), including the epipelic form *Navicula lusoria* in the coast of Quintana Roo (Hernández-Almeida et al., 2014). And, in particular, the study by López Fuerte et al. (2017) where colonization processes of diatom assemblages for monthly periods were observed. However, their results were directed toward recording the properties of antifouling paint applied to their alternative substrata. Thus, further ex professo research on benthic diatom floristics is still required to identify the species composition of the microfouling diatom assemblages and their source, while observing the colonization process itself. According to the above, our objective was to elaborate a floristic list of benthic diatoms that colonize alternative substrata such as fiber-glass, and those that thrive in the bottom sediment to test our hypothesis that epipelic (sediment) diatom taxocenoses constitute the source for the colonization of alternative substrata.

**MATERIAL AND METHODS**

The Port of Telchac is located north of the Yucatan Peninsula. The area is influenced by a Caribbean current that travels E-W along the north coast of the peninsula known as the Yucatan Current (Logan et al., 1969) (Fig. 1). The littoral consists of sandy beaches with a mild slope (Contreras et al., 1998; Alonzo et al., 2006), where turtle-grass *Thalassia testudinum* is common and abundant, along with filamentous chlorophytes (Herrera-Silveira & Morales-Ojeda, 2009) and rhodophytes (Zubía et al., 2007).

There, from November 2013 to February 2014, six fiber-glass plates (9.8×5×0.4 cm) suspended from a stainless-steel supporting structure were laid over the bottom sediment at a depth of 10 m over four
months to be colonized by diatoms. One plate was retrieved at intervals of 1, 3, 4, 8, 12 and 16 weeks. To compare assemblages from the distinct substrata, natural sediment was simultaneously collected around the supporting structure for the sampling of epipelagic diatom taxa with the aid of a Petri dish (López-Fuerte & Siqueiros Beltrones, 2006). Diatoms were brushed off from both sides of the plates and placed into a test tube. Both the brushed off material and the sediment were processed to remove the organic matter by oxidizing the sample with a mixture of ethanol and nitric acid at a ratio of 1:3:3 (Siqueiros Beltrones, 2002). After the oxidizing reaction, the samples were rinsed with distilled water until reaching a pH>6. Cleaned diatoms were mounted on permanent slides using Pleurax (RI=1.7); two slides were mounted for each side of each plate and sediment.

Diatom species identification was carried out at 650× and 1000× under a Zeiss Primo Star optical microscope with phase contrast illumination and plan achromat optics, with a mounted Axio Cam MRc5 camera. Diatom taxa were identified following Foged (1975, 1978, 1984), Peragallo & Peragallo (1908), Siqueiros Beltrones (2002), Siqueiros Beltrones & Hernández-Almeida (2006), López-Fuerte et al. (2010), and Stidolph et al. (2012). Then, relative abundances of the taxa were estimated (N=250) for each plate and sampling weekly. Overall abundance for each species was computed by adding the number of counted valves for each week. A total of 2815 valves were counted in the samples from the fiberglass plates (1,3,4,8,12 and 16), and 1748 valves in the sediment samples. Presence/absence similarity analysis was conducted using the Bray-Curtis index between the samples from all dates. Also, by accessing a floristic database for fouling diatoms published by López-Fuerte et al. (2017) the similarity between both species lists was measured (Primer 7 software).

RESULTS

Floristics. Overall, 88 benthic diatom taxa including species and varieties comprised within 42 genera were identified growing on the fiberglass plates and sediment (Table 1). Two species are new records for the Mexican littorals, Cocconeis latestriata (Fig. 20) and Navicula uniseriata (Fig. 37), and six could not be identified to species category. The plates alone harbored 82 taxa, while in the sediment 77 taxa were recorded. On both substrata, around 70% were pennate forms. A total of 47 taxa are new additions, increasing the floristic list for the area to 210 taxa. An iconographic catalog is presented as reference taxa (Figs. 2-31).

Colonization. In the first week of colonization, the pioneer (and abundant) taxa were Cocconeis scutellum var. parva (119), Paralia sulcata (31), Dimeregramma australis (25) and Cocconeis thalassiana (23). While in the sediment, Cymatosira lorentziana (259), Paralia sulcata (252), Cocconeis scutellum var. parva (247), Dimeregramma australis (141), were the most abundant taxa. Overall, the most abundant diatom taxa found on the fiberglass plates were: Cocconeis scutellum var. parva (538), Cymatosira lorentziana (448), Paralia sulcata (422), Dimeregramma australis (185), Actinopychus senarius (143), Grammatophora marina (125) and Shionodiscus oestrupii (123). During the colonizing process, the abundant primary taxa from the initial phase became scarcer within a month, while less common taxa from the initial phase became abundant (Fig. 52). In general, species of Amphora, Biddulphia, Cocconeis, Fragilaria, Grammatophora, Gyrosigma, Mastogloia, Trachyneis, Triceratium that dominated the initial phase were outnumbered by species of Achnanthes, Auliscus, Campylodiscus, Diploneis, Ehrenbergia, Lyrella, Navicula, Nitzschia, Terpsinoë in the following stages. Thus, two groups were defined by the Bray-Curtis similarity test, one clustering the taxocenoses from weeks 1, 3, 4 (initial phase), and a second group that included distinct taxa from weeks 8, 12, 16 (Fig. 53). In the initial phase, 22 taxa were observed exclusively, while 18 taxa were added in the next month.

Qualitatively, the similarity measured between species composition growing on the fiberglass plates and that in the sediment reached 83.7%, while quantitatively they exhibited a similarity of 60%, showing that these taxa grow much alike on both substrata.

DISCUSSION

This study focused on determining the species composition of diatom assemblages that form biofilms on fiber-glass surfaces during the initial phases of colonization. Our purpose was to generate basic information to understand better and potentially overcome the problem of biofouling on surfaces of boats and industrial equipment submerged in the marine environment. Such basics imply setting objectives on knowing the species composition or floristics of the primary micro-foulers, i.e., benthic diatoms and their provenance, and observing its changes over time. Floristically, our number of taxa surpasses those of similar studies elsewhere, e.g., Redekar & Wagh (2000) recorded 49 species and 19 genera, while Patil & Anil (2005b) identified 71 species and 38 genera, and Mitbavkar & Anil (2007) recorded 35 diatom taxa growing on fiber-glass plates.

In contrast, our species richness is half of that recorded (170) by López-Fuerte et al. (2017) both for sediment and fiber-glass plates (and one third fewer genera). In sediment, they identified 115 taxa against our 77, while, on fiber-glass plates, they identified 133 taxa against our 82. These differences in the number of taxa are due likely to four factors: (1) the mooring period, that in their study it extended for 18 months and in ours for 4 months; (2) the material of the plates, that in their study was fiber-glass plates coated on one side with antifouling acrylic paint, while in ours it was untreated fiber-glass; and (3) the degree of contact of plates with sediment, because in their study the supporting structure was PVC stands whose sta-
Table 1. Floristic list of fouling diatoms growing on fiber-glass plates and surrounding sediment in Telchac beach, Yucatan, Mexico. *Taxa observed in sediment samples; Δ, taxa not recorded in López-Fuerte et al. (2017); NR, new records.

| Order                           | Genera                                                                 |
|---------------------------------|------------------------------------------------------------------------|
| Bacillariophyta                 | Lyrella henneydi (W. Smith) Stickle & D.G. Mann                        |
| Class Coscinodiscophyceae       | Lyrella irrigata (Greville) D.G. Mann                                  |
| Subclass Thalassiosiphoeidae    | Lyrella lyra (Ehrenberg) Karajeva                                      |
| Order Thalassiosireas           | Petronæs granulata (Bailey) D.G. Mann                                 |
| Family Thalassiosireas          | Petronæs plagiostoma (Grunow) D.G. Mann                               |
| Ehrenbergiavula granulosa (Grunow) Witkowski | Order Mastogloiales        |
| Shionodiscus oestrupii A.J. Alverson, S.H. Kang & E.C. Theriot | Family Mastogloiacese       |
| Thalassiosira decipiens (Grunow) Jørgensen | Mastogloia binotata (Grunow) Cleve                                  |
| Thalassiosira eccentrico (Ehrenberg) Cleve | Mastogloia erythraea (Grunow) D.G. Mann                               |
| Family Stephanodiscaceae        | Mastogloia fallax Cleve                                              |
| Cyclotella atomus Hustedt * Fig. 9 | Mastogloia fimbriata (T. Brightwell) Grunow *                         |
| Subclass Coscinodiscophyceae    | Mastogloia gibboa Brun * Fig. 48                                      |
| Order Melosireas                | Mastogloia punctatissima (Greville) Ricard                               |
| Family Hylodiscaceae            | Mastogloia varians Hustedt *                                            |
| Order Paráles                   | Order Achannthales                                                  |
| Family Parálaceae               | Family Achannthaceae                                                 |
| Paralia sulcata (Ehrenberg) Cleve |                                                      |
| Order Coscinodiscase            | Family Cocconeidoaceae                                               |
| Class Fragilarioideae           | Cocconeis britannica Naegeli                                         |
| Subclass Fragilarioideae        | Cocconeis disculloides Hustedt * Fig. 26                               |
| Order Rhamphoneidales           | Cocconeis fluminensis var. subimpleta H.Peragallo & M.Peragallo *    |
| Family Rhamphoneidaceae         | Cocconeis latestriata Hustedt * NR Fig. 20                             |
| Biddulphia biddulphiana (Ehrenberg) Ehrenberg | Cocconeis peloides Hustedt *                                       |
| Biddulphia biddulphia (Ehrenberg) Ehrenberg * | Cocconeis scutellum var. parva (Grunow) Cleve *                     |
| Order Rhamphoneidales           | Cocconeis thalassiana Romero & López-Fuerte *                         |
| Family Rhamphoneidaceae         | Order Naviculace                                                      |
| Cymatosira Lorenziana Grunow    | Family Sciototidae                                                    |
| Class Fragilariophyceae         | Diploneis chersonensis (Grunow) Cleve *                               |
| Subclass Fragilariophyceae      | Diploneis didyma (Ehrenberg) Ehrenberg *                             |
| Order Fragilariaceae            | Diploneis splendida (Ehrenberg) Ehrenberg *                           |
| Family Fragilariaceae           | Diploneis papula var. constriucta (Ehrenberg) Ehrenberg *             |
| Fragilaria sp.                  | Diploneis littoralis (Donkin) Cleve *                                 |
| Opephora pacifica (Grunow) Petit | Diploneis obliqua (J.-J. Brun) Hustedt *                              |
| Opephora schwartz (Grunow) Petie ex Pelletan | Diploneis schmidtii Cleve * Fig. 35                                    |
| Order Rhamphoneidales           | Diploneis smithii Cleve *                                            |
| Family Rhamphoneidaceae         | Diploneis sp. 1 *                                                    |
| Cymatosira campanulata Grunow   | Diploneis sp. 2 *                                                   |
| Cymatosira limnetes Grunow      | Family Naviculace                                                     |
| Psammodiscus nitidus (W. Gregory) Round & D.G. Mann | Navicula cuspidata var. ambigua (Ehrenberg) Cleve *                 |
| Order Rhodophymatales           | Navicula (Lyrella) clavata var. distenta (Kuntze) Hustedt             |
| Family Rhodophymatales          | Navicula longis (Gregory) Ralfs ex Pritchard                         |
| Diploneis chersonensis (Grunow) Cleve | Navicula sp. Bory de Saint-Vincent *                                 |
| Diploneis didyma (Ehrenberg) Ehrenberg * | Navicula uniseriata Östrup * NR Fig. 37                              |
| Diploneis littoralis (Donkin) Cleve * | Trachyneis aspera (Ehrenberg) Cleve *                               |
| Diploneis sp. 1 *               | Trachyneis velata A. Schmidt *                                        |
| Diploneis sp. 2 *               | Family Pleurosigmaetaceae                                            |
| Diploneis sp. 2 *               | Pleurosigma formosum W. Smith *                                       |
| Diploneis sp. 2 *               | Pleurosigma normani Ralfs *                                           |
| Diploneis sp. 2 *               | Gyrosigma balticum (Ehrenberg) Rabenhorst *                          |
| Diploneis sp. 2 *               | Family Plagiotripidae                                                 |
| Diploneis sp. 2 *               | Tropidonæs pusilla (W. Gregory) Cleve*                               |
| Diploneis sp. 2 *               | Family Sellaphoraceae                                                 |
| Diploneis sp. 2 *               | Fallacium nummularia (Greville) D.G. Mann *                          |
| Diploneis sp. 2 *               | Family Amphipleuraceae                                                |
| Diploneis sp. 2 *               | Halamphora coffeiformis (C. Agardh) Levkov *                         |
| Diploneis sp. 2 *               | Family Pinnulaceae                                                    |
| Diploneis sp. 2 *               | Oestrupia powellii (Lewis) Heiden ex Hustedt *                        |
| Diploneis sp. 2 *               | Order Thalassiosiphyscales                                            |
| Diploneis sp. 2 *               | Family Catenulaceae                                                   |
| Diploneis sp. 2 *               | Amphora amoena F. Hustedt *                                           |
| Diploneis sp. 2 *               | Amphora arenaria Donkin *                                             |
| Diploneis sp. 2 *               | Amphora cingulata Cleve *                                            |
| Diploneis sp. 2 *               | Amphora immarginata Nagumo *                                         |
| Diploneis sp. 2 *               | Amphora obtusa W. Gregory *                                           |
| Diploneis sp. 2 *               | Amphora ostrea var. lineata Cleve *                                  |
| Diploneis sp. 2 *               | Amphora proteus Gregory *                                            |
Table 1. Continued.

| Order Bacillariales | Family Bacillariaceae |
|---------------------|-----------------------|
| Bacillaria socialis (Gregory) Ralfs * | Δ Fig. 16 |
| Nitzschia acicularis (Kützing) W. Smith * | Δ |
| Nitzschia fluminensis Grunow * | Δ Fig. 38 |

| Order Rhopalodiales |
|---------------------|
| Family Rhopalodiaceae |
| Rhopalodia gibberula (Ehrenberg) Otto Müller * | Δ |
| Order Surirellales |
| Family Surirellaceae |
| Campylodiscus cf. angularis Ehrenberg ex Kützing * | Δ |
| Psammodictyon constrictum (Gregory) D.G. Mann * | Δ |
| Psammodictyon panduriforme (W. Gregory) D.G. Mann * | Δ |
| Surirella armoricana H. Peragallo & M. Peragallo * | Δ |
| Surirella fastuosa Ehrenberg * | Δ |
| Surirella fastuosa var. reedens (A. Schmidt) Cleve * | Δ |

bility did not guarantee the permanent separation of plates and the bottom sediment, while in our study the plates were hanged on a stable stainless steel supporting structure, not in direct contact with bottom sediment; (4) season, inasmuch their sampling comprised Spring and Summer, while ours was in the Autumn and Winter period. In accordance with this last factor, our floristics has to be considered complementary.

In any case, the 47 taxa added in our study that had not been listed previously, indicate that much floristic work is still required for this area. Notwithstanding, the joint lists reach 210 taxa of fouling diatoms: 158 epipelic forms, and 173 on the alternative substratum. This study and the one by López-Fuerte et al. (2017) have recorded more taxa than earlier studies in the Gulf of Mexico, the Caribbean Sea, and the Yucatan Peninsula (Licea et al., 2011; Navarro & Hernández-Becerril, 1997; Stidolph et al., 2012; Siqueiros Beltrones & Martinez, 2017).

Only seven taxa from the sediment were not observed on the plates backing our assumption that sediment diatom assemblages are the main source of colonizing taxa for this alternative substratum. This is evidenced by the fact that the taxa recorded as abundant on the fiber-glass plates were also abundant on the sediment (Paralia sulcata, Cocconeis scutellum var. parva and Dimeregramma australe).

Although our two new records for the study area were previously reported by Hustedt (1955) for North Carolina (Cocconeis latestritata) and by Foged (1975) for the Caribbean (Navicula uniseriata), they are new for the overall Mexican littorals. Also, the former is listed for the Mexican NW (López-Fuerte & Siqueiros Beltrones, 2016). However, the record is unconfirmed. These newly recorded taxa underline the need for continuing studies on benthic diatom floristics in this and other areas of the Mexican coasts. Previously Hernández-Almeida et al. (2013) reported 9 new records for the Yucatan shores, of which Petroneis plagiostoma, Oestrupia powelli and several species of Cocconeis were commonly observed in the present study, both in sediment and on fiberglass plates.

The primary phase of colonization was characte-
Figures 2-12. At 1000×. 2) Paralia sulcata; 3) Podosira stelligera; 4) Actinoptychus senarius; 5) Psammodiscus nitidus; 6) Shionodiscus oestrupii; 7) Dimeregramma australis; 8) Dimeregramma marinum; 9) Cyclotella atomus; 10) Opephora schwartzii; 11) Odontella aurita; 12) Opephora pacifica. Bar = 10 μm.
Figures 13-27. At 630×. 16) Bacillaria socialis. At 1000×. 13) Amphipentas pentacrinus; 14) Triceratium reticulum; 15) Zygoeceros rhombus; 17) Grammatophora serpentina; 18) Grammatophora marina; 19) Biddulphia biddulphiana; 20) Cocconeis latestriata; 21, 25) Cocconeis britanica; 22) Cocconeis peltoides; 23) Cymatosira lorenziana; 24) Cocconeis thalassiana; 26) Cocconeis disculoide; 27) Fallacia nummularia. Bar = 10 μm.
Figures 28-38. At 1000×. 28) Amphora cingulata; 29) Amphora obtusa; 30) Amphora proteus; 31) Amphora immarginata; 32) Halamphora coffeeaeformis; 33) Diploneis splendida; 34) Diploneis papula var. constricta; 35) Diploneis schmidtii; 36) Mastogloia binotata; 37) Navicula uniseriata; 38) Nitzschia fluminensis. Bar = 10 μm.
Colonization by benthic diatoms

Figures 39-51. At 1000×. 39) Lyrella diffuens; 40) Lyrella clavata var. caribaea; 41) Navicula (Lyrella) clavata var. distenta; 42) Lyrella irroration; 43) Lyrella lyra; 44) Mastogloia fimbriata; 45) Mastogloia splendida; 46) Surtella fastuosa; 47) Mastogloia erythrea; 48) Mastogloia gibbosa; 49) Mastogloia fallax; 50) Petroneis plagiostoma; 51) Petroneis granulata. Bar = 10 μm.
**Figure 52.** Variation of the most abundant taxa during the immersion period.

**Figure 53.** Similarity of diatom assemblages settled on the fiberglass (FG) plates, based on the Bray-Curtis qualitative similarity index. Number, weeks.
opportunity for the settlement of more diatom taxa which prefer a natural alternative, albeit to prove this an ex professo analysis is required.

This study complements and brings up to date the diatom floristics for the study area. However, it is evident that much more floristics research is required in this and other Mexican littorals to eventually provide the taxonomic certainty for conservation and management decision concerning the fouling nature of diatoms.

The above results back our hypothesis that epipelagic diatoms are an important source for the colonization of an alternative substrate. On the other hand, the similarity between the diatom taxocenoses recorded by López-Fuerte et al. (2017) and those from this study was relatively low. Only 25% similarity for sediment samples, and 39% for the fiber-glass plates.

The examination of the sediment adjacent to the structure holding the fiberglass plates rendered an accurate reference on the origin of the diatoms found on the plates, in agreement with our hypothesis, inasmuch as many of the diatom taxa were recorded from sediment. Moreover, the identification of pioneer species in colonization processes, mainly adnate forms, and those that secrete exopolymers that favor the settlement of other life forms provide a basis for other studies related to the control of biofouling.

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Martínez et al.

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