Chapter

First Report on the Diversity of Epizoic Algae in Larval of Shellfish Gastropod *Aliger gigas*

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Abstract

Epibiosis occur frequently on the shells of some marine crustaceans, which often serve as substrate for various species of algae, there is few information on the associations between these. The objective of this study was to determine if the gastropod mollusk *Aliger gigas* (formerly *Lobatus gigas*) in larval had some sort of the association with algal. To the above was carried out collecting egg masses in the environment, the larvae were cultivated in seawater filtered 5 μm. The algal material found was observed in electron microscopy, for its identification and quantification. We analyzed 60 larvae aged 2–44 days for analyzing the structure of the shell and its epibionts. Of the larvae analyzed, 50 larvae presented epizoic. The algae community consisted of 28 taxa, and composed of 25 diatoms (Bacillariophyta) and three cyanophytes (Cyanobacteria). The H′ diversity values fluctuated between 0.2 a 1.2. The dominant and frequent species were formed by diatoms: *Nitzschia panduriformis var. minor*, *Halamphora* sp. and *Cyclophora* sp.

Keywords: cyanophyte, diatom, epibiont, *Aliger gigas*

1. Introduction

Epibiotic is a type of association in which an organism lives on the surface layer of another organism called basibiont, these nonparasitic organisms are known as epibionts [1, 2]. The shells of gastropod and bivalve mollusks represent a suitable habitat for the settlement of various species of algae, viruses or fungi [3–6]. Different studies have focused on epiphytic diatoms of grasses and marine macroalgae [7–9]; in copepods of the species *Farranula gibbula*, the epibiotic diatom *Pseudohimantidium pacificum* has been observed [10]. Very little information exists on symbiotic associations between algae and crustaceans or marine planktonic mollusks, being able to cite what was observed in *Peringia ulvae* (formerly *Hydrobia ulvae*) and diatoms *Cocconeis placentula* and *Achnanthes lemmermannii*, also cyanophytes and bacteria in its Shell [5]. Based on the above, the objective of this work was to identify epizoic species present in the shells of the larval stages of the marine gastropod mollusk, *Aliger gigas*.

2. Material and methods

An egg mass of *Aliger gigas* was incubated in filtered seawater with a 5 µm mesh, until hatching. Later, the larvae were cultured with seawater filtered with a 50 µm mesh and fed with *Nannochloropsis oculata* (Ochrophyta, Eustigmatophyceae) at a concentration of 1 000 cells per larva, at a density of 100 larvae/L. The larvae were fixed in glutaraldehyde, cacodylate and dehydrated in alcohols from 70 to 100% and dried at a critical point. The shells of 60 larvae between 2 and 44 days old were processed. The specimens were observed in a JEOL field emission scanning electron microscope (JSM-7600F), of the National Laboratory of Nano and Biomaterials of Cinvestav IPN Mérida, the presence of epizoic algae was analyzed and to its quantification was carried out. For the identification of phytoplankton, the works of [11–16], among others. The AlgaeBase system was consulted to verify accepted taxonomic names [17].

To obtain the relative abundance index, the proportion of abundance of each species (organism number) was quantified in relation to the total abundance of organisms counted in each larva of different ages [18]. The contribution of the abundance of the epizoic algae species of each larva was determined by means of the SIMPER analysis [19]. This analysis determines the species that most contribute to the similarity between sample. A cumulative similarity discrimination value of 90% was applied. Based on the composition and abundance of the epizoic algae species, the community was characterized by the following descriptors: to evaluate the diversity, the species richness of Margalef (S), the Shannon-Wiener index (H′) and Pielou's equity (J') considering to according to in accordance with [20], through the ODI program of the Interdisciplinary Center for Marine Sciences, Department of Plankton.

To obtain dominance of the species, an Olmstead & Tukey test was used [21]. The dominant, constant, occasional and rare species were determined from the relationship between the densities of the organisms and their frequencies of appearance. The statistical programs used were Primer-E and R.

3. Results

Of the 60 specimens of *A. gigas* larvae analyzed, 83% presented epizoic algae. The epizoic algae community consisted of 28 taxa, made up of 25 diatoms and three cyanophytes. It should be noted that one of the recorded diatom species *Cylindrotheca closterium* is considered a species that can be harmful and forms algal blooms (**Table 1**).

3.1 Specific diversity

The diversity values H′ fluctuated between 0.9 and 1.2. The 28-day-old premetamorphic larval shells presented the highest value of H′ 1.2 with an equity of J′ 0.4, and a species richness of S 14. These values of H′ 1.2 with a J 0.5 and an S 9, were slightly higher in the 30-day-old larvae, which already had foot formation. For the 20-day-old larvae, H 'was 1.1, J' was 0.4 and S was 11 and in the 18-day-old larvae, H 'was 0.9, J' was 0.4 and S was 9 species (**Figure 1**, **Table 1**). Following the same behavior, the youngest veliger larvae, 8 days old, presented the lowest diversity with values of H 'of 0.2, J' of 0.42 and S of 4 species (**Figure 1**, **Table 1**).

3.2 Dominant species

Based on the Olmstead and Tukey test, the epizoic algae community consisted of 17 rare species, followed by five common, three abundant and three dominant

Larva age in days	**8**	**15**	**16**	**18**	**20**	**26**	**28**	**30**	**36**	**42**	**%FR**	**At.**
Number of larvae analyzed	**4**	**2**	**9**	**1**	**2**	**2**	**10**	**2**	**6**	**6**		
Bacillariophyta												
Amphora sp.	0	0	0	0	0	0	0	2.5	0	0	10	R
Cocconeis lineata	0	0	0	0	0.1	1.5	0	3	0	0	30	R
Cocconeis scutellum	0	0	0	0	0.1	2	0.1	0	0	0	30	O
Craspedostauros sp.	0	5	0	0	0	0	12	0	0.1	0	30	R
Cyclophora sp.	0	66	10	3	5	1.9	0.3	15	76	70	90	D
Cylindrotheca closterium	1	7.5	1.5	5	4.4	1.3	0.3	0	0	0	70	C
Entomoneis paludosa	0	0	19	3.6	7	17	16	11	8.5	4	80	C
Halamphora coffeaeformis	0	0	0	0	0	0	0	0	0.1	0.1	20	R
Halamphora sp.	0	1.7	47	30	10	7	0.2	0	0	0	70	D
Haslea tsukamotoi	0	0	0	0	0	1	0.1	7.5	0	1	40	R
Hippodonta pseudacceptata	0	0	0	0	54.3	0	0	0	0	0	10	R
Hyalosynedra sp.	0	0	0	0	0	0.4	0.1	0	0	1.2	30	R
Licmophora sp.	0	0	0	0.3	0	0	0	0	0	0	10	R
Navicula radiosa	0	0	0	2.6	0	19	1	9	0	0	40	O
Nitzschia dissipata	0	0	0	0	0.1	0	0.1	0	0	0	20	R
Nitzschia inconspicua	0	0	0	0	0	8	0	0	0	2.8	20	R
Nitzschia linearis	0	0	0	0	0	0	0	0	0	0.3	10	R
Nitzschia microcephala	0	0	0.4	0	0	0	0.1	0	0	0	20	R
Nitzschia panduriformis var. minor	64	18	8	51	9	4.5	1.1	20	7.5	0.2	100	D
Nitzschia sp.	0	0	3.6	4	5	0	0	0	0	0	30	O
Pleurosigma sp.	5	0	0	0.5	0	0	0	0	0	0	20	R

Psammodictyon panduriforme	0	0	2.2	0	0	0	0	0	0	0	10	R
Pseudachnanthidium sp.	0	0	0	0	0	0.3	0	0	0	0	10	R
Scalariella sp.	0	0	8	0	0	0	0	0	0.2	0.1	30	R
Stephanodiscus minutulus	0	0	0.3	0	0	0	0	0	0	0	10	R
Cyanophyta												
Haloleptolyngbya sp.	0	0	0	0	5	32	68	30	7.6	20	60	C
Richelia intracellularis	0	0	0	0	0	0	0	2	0	0	10	R
Arthrospira sp.	0	1.8	0	0	0	4.1	0.6	0	0	0.3	40	R
S	**4**	**6**	**10**	**9**	**11**	**14**	**14**	**9**	**7**	**11**		
H′	**0.2**	**0.7**	**0.4**	**0.9**	**1.1**	**0.7**	**1.2**	**1.2**	**0.5**	**0.7**		
J′	**0.2**	**0.4**	**0.2**	**0.4**	**0.4**	**0.3**	**0.4**	**0.5**	**0.3**	**0.3**		

Table 1.
Percentage of the relative abundance of the epizoic algae community in the shells of Aliger gigas larvae. % FR: Percentage of the relative frequency. At: Attribute, D: D: Dominant, C: Constant, O: Occasional and R: Rare. S: Species richness, H′: Diversity and J′: Equity.

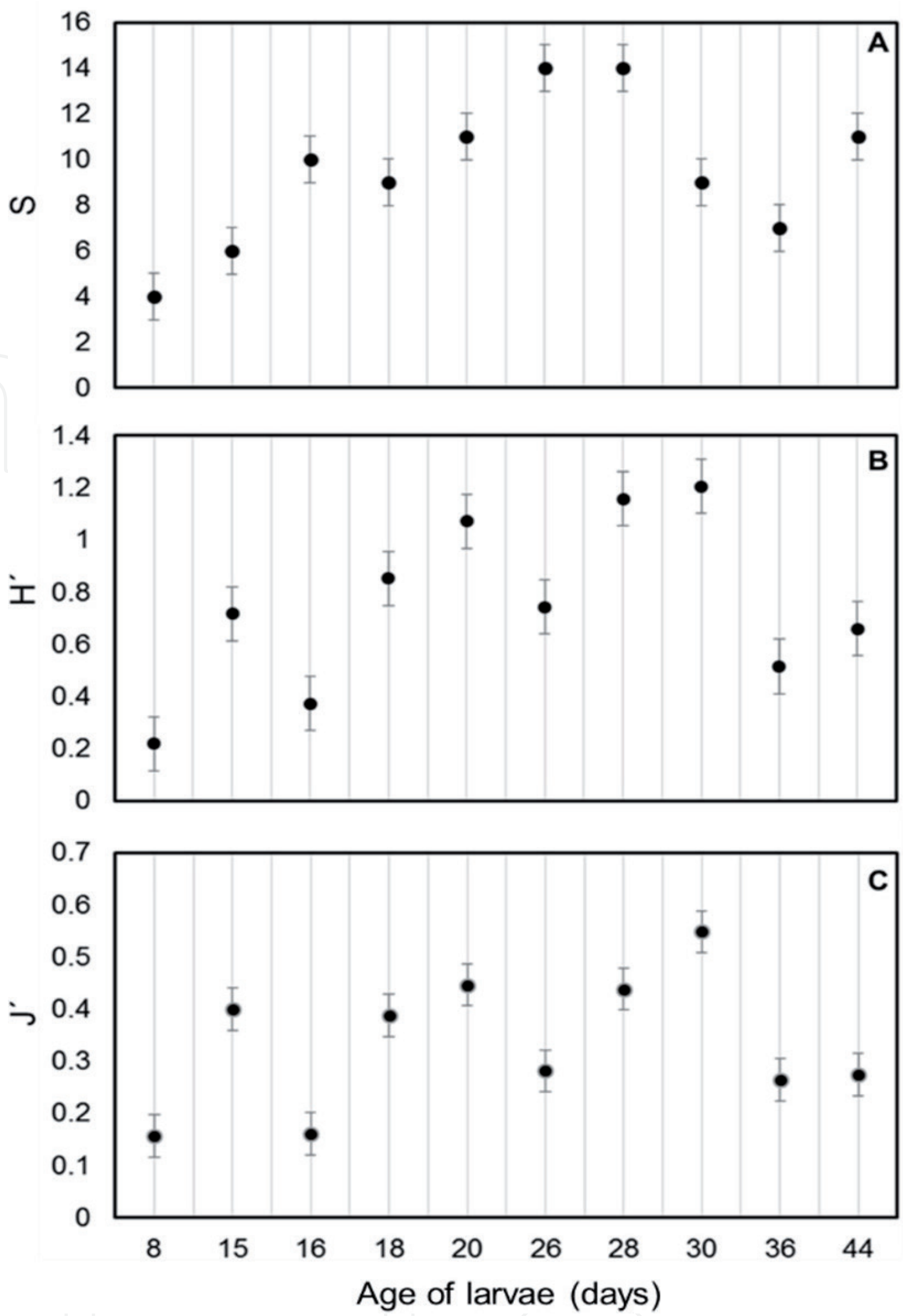

Figure 1.
(A) Species richness (S), (B) diversity (H′) and (C) equity (J') of the epizoic algae community in the shells of Aliger gigas larvae.

species (**Table 1**). The dominant species were made up by the diatom *Nitzschia panduriformis var. minor*, whose highest relative abundance was 68% in 8-day-old larvae; *Hippodonta pseudacceptata* with a relative abundance of 54% in 20-day-old larvae; *Halamphora* sp. with a relative abundance of 47% in 16-day-old larvae and *Cyclophora* sp. with 70 and 76% in larvae of 36 and 44 days respectively (**Figure 2**, **Table 1**). In addition, of the cyanophyte *Haloleptolyngbya* sp. with a relative abundance of 68% in 28-day-old larvae (**Figure 2**, **Table 1**).

3.3 Characteristics of *Aliger gigas* larvae, observed in the development of this work

The two to five-day old larvae have a shell formed by two turns in a spiral presenting small granule at the apex and a velum characterized by having two lobes and the right tentacle, corresponding to a young veliger larva. The shell of eight-day-old

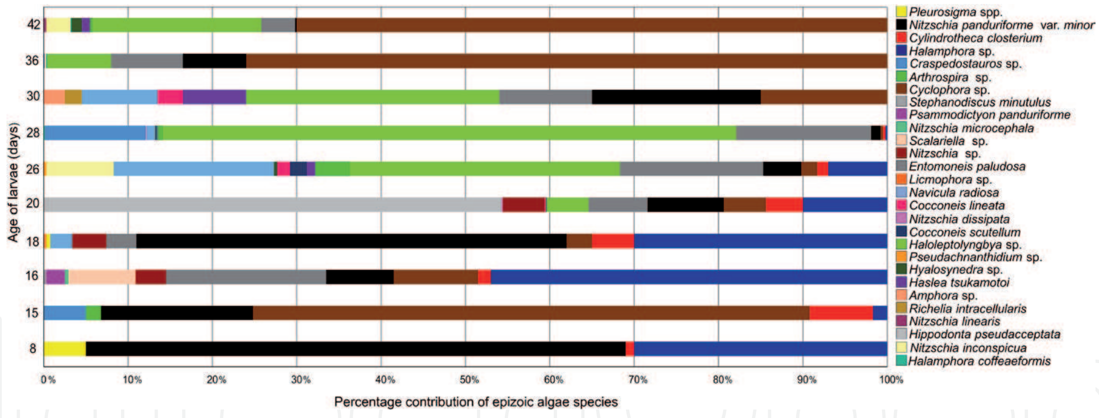

Figure 2.
SIMPER analysis, percentage contribution of epizoic algae species from the shells of the larvae of the marine gastropod mollusk Aliger gigas.

larvae is characterized by having three coils, showing well-defined lines of ornamentation on the body of the shell. As regards its development; the velum has four lobes; with the right tentacle well differentiated and the formation of the left tentacle.

The 15–18-day old larvae have a carapace with three and a half turns in the spiral, the body has four parallel lines ending at the end of the siphon channel and is highly ornamented. The velum has six lobes, tentacles, and proboscis. In larvae from 20 to 28 day of development, their shell has three and a half turns, with a band of uniform striations on the body of the shell, the radula is already observed and the velum begins the process of reabsorption therefore the larva begins to have a creeping shape beginning its benthic phase. It is a stage known as a precompetent larva. Regarding larvae between 30 and 44 days old, at this stage the larvae are ready to metamorphosis (30 days). Post-metamorphic larvae or post larvae of 44 days, present a foot with an active crawling behavior. The shell is characterized by presenting four turns, with a well-developed band of striae, the proboscide and the radula are present and active, as is the foot with its operculum.

4. Discussion

Diatoms have been reported in the literature as the main group of epizoic microalgae species attached to different types of animals that can be copepods [10, 22–24]; cladocerans [25], hydrozoans [26–27], krill [28] even in whales [29–30]. Diatoms are also present in diving birds [31–32] and reptiles such as crocodiles [33].

As mentioned by [34], the first phase of the colonization of a substrate occurs mainly by bacteria with diatoms, fungi and protozoa; which generate a film on the surface of the basibiont. The results of this study showed that the shells of larvae of the marine gastropod mollusk, *Aliger gigas*, provide an adequate and frequent substrate for the settlement of epizoic microalgae, in the case of diatoms and cyanophytes. A dominance of diatom species was also observed. *Nitzschia panduriformis var. minor* was reported in 8-day-old larvae, *Halamphora* sp. was present in 16-day-old larvae, *Hippodonta pseudacceptata*, in larvae of 20 days and *Cyclophora* sp., in larvae of 36 and 44 days. Obtaining low values of diversity H′(0.2 to 0.9) and J' (0.2 to 0.5), in these phases of the larvae. Margalef [35] mentions that diversity is low when there is a dominance of some species.

Likewise, it was found that the diatom and cyanophyte populations were not stable. It is interesting to note that, although the larvae were under the same culture conditions, in the larvae of different ages, the structure of their epizoic microalgae community changed. In larvae less than 20 days old, the cells of the dominant microalgae were shed from the larvae shells allowing the colonization of other microalgae; on the other hand, in larvae of 20 to 30 days a higher H′ diversity of 1.0 to 1.2 was reported. In another study with gastropods, carried out by [6] reviewed the shells of seven gastropods (*Alvania lineata*, *Bittium reticulatum*, *Clanculus cruciatus*, *Columbella rustica*, *Gibbula adansoni*, *Nassarius incrassatus* and *Jujubinus striatus*), reporting a richness of 19 to 25 species and a high J' equity of 0.70 to 0.80 and [36] in *Lepidochelys olivacea* (olive ridley) shells, recorded a diversity of 1.1 to 2.1 and a high J' equity of 0.56 to 0.86.

From what was observed in this study, the size and structure of the shell of *A. gigas* larvae on the different days of development provide a substrate for the epizoic microalgae.

The two to five-day old larvae did not have microalgae, the size of the shell is small, thin, smooth, the shell is formed through a transient amorphous calcium carbonate that acts as a precursor in the aragonite crystallization sequence [37, 38]. In addition, the time for colonization is still short.

In the case of larvae from 26 to 44 days, the highest species richness was reported, the shell is larger about 1 200 μm with greater ornamentation and with greater adhesion surface. Especially the 44-day-old larvae present a periostracum, outermost layer of the shell composed of an organic matrix [38, 39], which offers a better substrate, rich in proteins, which permit the growth of epizoic microalgae. In 28-day-old larvae, the number of spirals and shell ornamentation may be the factors that support the presence of *Haloleptolyngbya* sp., in addition to the mucus secreted by the microalgae themselves to adhere to the substrate. The 30 to 36-day old larvae showed a lower species richness and a dominance of *Cyclophora* sp. This diatom forms colonies in a zigzag shape, occupying the entire larva shell and preventing other microalgae from adhering. In several studies they have agreed to point out that gastropod shells are good microenvironments, due to their different structures and sizes [6]. Size does influence the colonization of epizoic microalgae, observing that the largest shells (*Bittium reticulatum*, *Gibbula adansoni*, *Columbella rustica* and *Clanculus cruciatus*) presented greater abundance and the small shells (*Alvania lineata*, *Nassarius incrassatus* and *Jujubinus striatus*) higher species richness, unlike what was found in this work.

Some microalgae produce allelopathic substances that inhibit the growth of others [40]. This could explain why some larvae had fewer epizoic microalgae than others, or for the fact that some had successfully colonized earlier and no longer left space for the colonization of more species. In addition to the changes in the abundances of the epizoic microalgae community, it is important to study the physical and chemical factors that influence their succession and to analyze whether *A. gigas* larvae fed on the epizoic microalgae reported. Epizoic microalgae associated with the velum of the larvae analyzed in this study were reported, *Cylindrotheca closterium*, *Hippodonta pseudacceptata* and *Cyclophora* sp.

The ecology studies of epizoic microalgae on the larvae of *A. gigas*, allows to know which species of phytoplankton or phytobenthos are present in the system where these larvae inhabit of the Mexican Caribbean. There are few studies focused on the study of diatoms and even less if they are found as epizoic microalgae. As the knowledge of the factors that regulate the competitive ability of the different epizoic microalgae species increases, the degree of interaction between them and their basibiont will also be understood.

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