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Colonization patterns of the date mussel *Lithophaga lithophaga* (L., 1758) on limestone breakwater boulders of a marina

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Abstract

Background and Purpose: *The European date mussel* (Lithophaga lithophaga) *is widespread along the whole Mediterranean rocky coastline where it is frequently but illegally harvested. It is well known that the growth of the date mussel is very slow; however, patterns of recolonization of exploited surfaces have been scarcely investigated. The objective of this study is to assess colonization patterns of the date mussel on limestone boulders which have been in the sea for 19 years. These results could be useful in predicting the reconstitution of natural populations of the date mussel after harvesting.*

Materials and Methods: *Limestone breakwater boulders were placed along the dike of the Marina of Rovinj (northern Adriatic, Istrian peninsula, Croatia) in 1984. Sampling was carried out in summer 2003 by SCUBA diving from six habitats of different inclination and topography: horizontal, inclined, vertical, sheltered, vaults, and whole stones. The abundance and biomass of* L. lithophaga *at the Marina were compared with those in natural control locations.*

Results: *At the Marina, no date mussels were found on the horizontal and inclined sides of the boulders, and very few date mussels were found on the vertical side. On the contrary, sheltered and vault sides of boulders and whole stones were intensively colonized by* L. lithophaga. *In vaults, the abundance was similar to that in nature. In these habitats,* L. lithophaga *biomass was generally lower than in nature. However, large date mussels, of lengths from 50 to 70 mm, were already present on boulders of the Marina. They amounted to 35% in the sheltered habitat, 24% in vaults, and 3% of the total number in whole stones.*

Conclusions: *An unexpected high colonization rate and growth of date mussels was detected in certain unexposed rocky habitats at the boulders of the Marina. However, on the natural rocky Istrian coast, the date mussel was mostly collected on exposed inclined and vertical rocky surfaces where repopulation after harvesting may require very long periods.*

INTRODUCTION

The European date mussel *Lithophaga lithophaga* (L., 1758) is a rock-boring bivalve mollusc widespread along the whole Mediterranean rocky coast *(1)*. It lives inside galleries bored in calcareous rocks by glandular secretions *(2)*. Date mussel distribution is constrained by the nature of the substratum (limestone or dolomitic limestone), inclination of the substratum, general architecture of the rocky bottom, and by depth *(3-6)*. Along the western Istrian coast, the date mussel is most abundant, and thus harvested, on inclined and approximately vertical surfaces. Patches intensively colonized by the date mussel can be found up to 6 m depth on all types of rocky profiles. Up to 14 m depth, it is abundant only in ridged shallows, a particular type of rocky bottom *(5)*. However, it can be found sporadically up to the 25 m depth (Massimo Devescovi, personal observation).

Due to extremely high price and demand for the date mussel in the Mediterranean area, *L. lithophaga* is intensively collected by SCUBA divers who break the rock by hammers to extract the date mussel in spite of the fact that the law usually prohibits its collection and sale. This activity has a great impact on the benthic communities covering the rocks colonized by the date mussel. All sessile organisms are eradicated so that exploited rocks remain completely bare, leading to long-term structural degradation and impoverishment of infralittoral epibenthic communities, both locally and on a landscape scale *(5-7)*. Additionally, in affected areas, there are likely to be severe impacts on the ichthyofauna *(8-10)* with a concomitant increase of sea urchin populations *(6, 11)*.

Since divers break the rock stratum with hammers to expose and collect the date mussel, the surface geometrical arrangement of the hard substratum changes, resulting in a decrease of the rocky bottom structural complexity *(5, 12)*. The physical features of the substratum may exert strong effects on species. Marked settlement preferences related to surface complexity have been recorded for demersal fish and invertebrates *(13, 14)*. Surface complexity may influence the movement patterns of organisms, is often concomitant with the availability of food and shelter, and may influence processes such as recruitment, competition, and predation *(13, 14)*. Thus, date mussel collection can permanently affect rocky shallow water communities *(5, 12)*.

Detailed studies of the recovery of the exploited stocks are lacking, but it is highly probable that the process of recolonization of the bare substratum by *L. lithophaga* is rather slow *(6)*. Thus, studies of the growth and abundance of date mussels in different habitats, including different architectural types of rocks, and at different spatial scales are needed. The scope of this study is to assess the distribution of date mussels on limestone boulders placed in the sea for 19 years. Particular attention was paid to the effects of inclination and of the physical arrangement of the substratum on the colonization patterns of date mussel.

MATERIALS AND METHODS

In 1984, the external part of the dike of the Marina of Rovinj (northern Adriatic, Istrian peninsula, Croatia) was reinforced by large limestone breakwater boulders. In the summer of 2003, sampling was carried out by SCUBA diving to assess colonization patterns of *Lithophaga lithophaga* in this limestone substratum. Six distinct habitats were sampled: (1) nearly horizontal surfaces, (2) inclined surfaces exposed to light, (3) nearly vertical substratum, (4) sheltered substratum immediately under boulders open on one side to the water body, (5) vaults under boulders open on both sides to the water body, and (6) small stones of approximately 30 x 30 x 20 cm in size with the lower side forming a small vault over the bottom (Fig. 1).

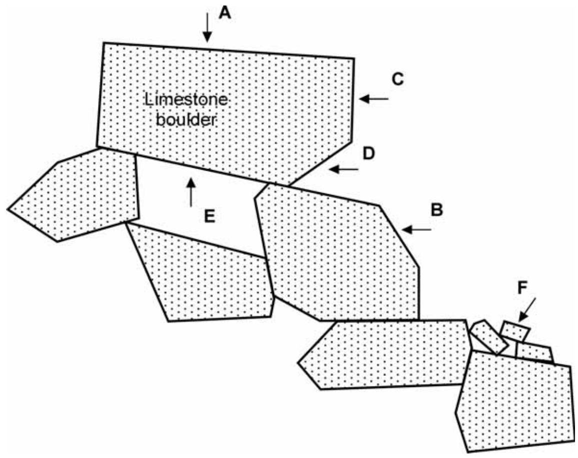

Figure 1. *Schematic representation of habitats sampled on limestone breakwater boulders of the Marina of Rovinj. A: Approximately horizontal surfaces; B: Inclined surfaces; C: Approximately vertical surfaces; D: Sheltered habitat open to the water body on one side; E: Vaults open to the water body on both sides; F: Stones of approximately 30 x 30 x 20 cm with the lower side forming a small vault over the sea bottom.*

All habitats were sampled between 3 and 5 m depth but the stones were sampled between 7 and 9 m depth. Two methods of sampling were used: the visual count of date mussels in 30 x 20 quadrates *(5)* and the destructive method, collecting all date mussels in 20 x 20 cm quadrates using hammer and chisel. For the stones, the number of date mussels was estimated visually underwater for the whole stone or the stone was sampled and broken in the laboratory to collect all date mussels. Results for the stones were recalculated considering the whole stone surface. Thus, stones included all 5 habitat types: the horizontal at the top, inclined and vertical at the sides, sheltered and vault under the stone. Two natural control locations were randomly chosen, one 5 km northward, the other 10 km southward from the Marina. The visual method of counting was applied primarily to minimize the impact (i.e. the number of replicates) on the natural substratum.

Visual counting

Date mussels were counted at the Marina in 5 replicates per each of the 3 habitats (sheltered, vault, and stones) at 2 sites about 100 m apart. The same sampling procedure was performed also at the two control locations (each with 2 sites). The total number of replicates was 90 (30 at the Marina, 60 on the natural substratum).

TABLE 1

Asymmetrical ANOVA to compare the abundance of Lithophaga lithophaga (visual count) on limestone breakwater boulders of the Marina of Rovinj (M) 19 years after placement at sea with two natural control locations (Cs) and a priori contrasts comparing the abundance between M and the average of Cs at three habitats (Sheltered, Vault, and Stones).

Dependent variable: number of *L. lithophaga* per 600 cm^2; N = 90; n = 5					
Cochran's *C*-test: $C = 0.101$; $p = 0.810$; transformation: None					
Source of variation	*Df*	MS	*F*	*p*	Tested over
Habitat = H	2	1148.700			
M *versus* Cs	1	1843.199			
Cs	1	41.667	0.510	0.549	S(Cs)
H x M *versus* Cs	2	480.199	12.157	0.008	H x S(L) [(a)]
H x Cs	2	26.867	0.482	0.649	H x S(Cs)
Site(Location) = S(L)	3	55.833	1.406	0.248	Residual
S(Cs)	2	81.733	2.058	0.135	Residual
S(M)	1	0.133	0.003	0.956	Residual
H x S(L)	6	39.500	0.994	0.436	Residual
H x S(Cs)	4	55.733	1.403	0.242	Residual
H x S(M)	2	74.533	1.876	0.161	Residual
Residual	72	39.722			
A priori contrasts:					
Sheltered	1	1926.667	48.776	<0.001	H x S(L)
Vault	1	1.667	0.042	0.844	H x S(L)
Stones	1	806.667	20.422	0.004	H x S(L)

Factors are: Habitat (fixed, 3 levels: Sheltered, Vault, Stones), Location (random, 3 levels: the Marina of Rovinj and 2 natural locations) and Site (random nested in Location, 2 levels).
[(a)] *F*-ratio obtained after elimination of the H x Cs interaction from the model ($p > 0.25$)

Destructive sampling

Three random replicates for each of the 6 habitats were collected at the Marina (18 replicates) and on the natural substratum (18 replicates). At the laboratory, the weight and the length of each collected date mussel were determined.

Data analysis

To compare the abundance of *L. lithophaga* at the Marina with the two control locations, an asymmetrical ANOVA design was used *(15, 16)*. The factors were: Habitat (fixed, 3 levels: sheltered, vault, and stones), Location (random, 3 levels: the Marina and 2 control locations), and Site (random, nested in Location, 2 levels). The analysis included *a priori* contrasts for the Habitat x Marina *versus* Controls interaction *(16, 17)*.

A two-way ANOVA was used to analyze data of destructive sampling (*L. lithophaga* number and biomass) using these factors: Substratum (fixed, 2 levels: Marina and natural) and Habitat (fixed, 3 levels: sheltered, vault, and stones). The analyses were followed by Student-Newman-Keuls tests (SNK-tests) at the level of significance $\alpha = 0.05$ *(17)*.

Prior to ANOVA, all data were analyzed for homogeneity of variance using Cochran's *C*-test. Data showing heterogeneity of variance were transformed to meet homogeneity. The sums of squares were obtained using SYSTAT, Version 10 SPSS Inc.

RESULTS

Visual counting

A priori contrasts revealed that on vaults, the abundance of *Lithophaga lithophaga* at the Marina was similar to the mean value at control locations. For the other two habitats (sheltered and stones), the abundance significantly differed between the Marina and control locations (Table 1). Asymmetrical ANOVA (Table 1) showed that at each level of habitat there was no significant difference between sites at the same location (Habitat x Site(Location) interaction, $p = 0.436$) and that there was no significant difference between control locations at each habitat (Habitat x Controls interaction, $p = 0.649$).

Thus, small scale variability (between sites) and large scale variability (between controls) were disregarded in further analyses. This fact greatly reduced the number of destructive samples from the natural population without the risk of spatial confounding *(16)*.

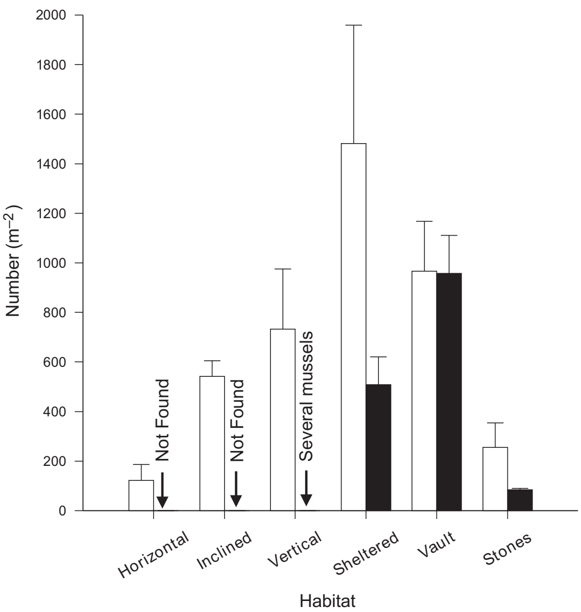

Figure 2. *Abundance of the date mussel* Lithophaga lithophaga *in different habitats. Open bars: Natural substratum; filled bars: Breakwater boulders of the Marina of Rovinj placed at sea for 19 years. Data are means ± SD of 3 samples.*

Destructive sampling

In the natural substratum, the number of mussels was 122 ± 64 m^{-2} for the horizontal, 542 ± 63 m^{-2} for the inclined, and 733 ± 243 m^{-2} for the vertical habitat. At the Marina, no date mussels were found for the horizontal and inclined habitat and only a few mussels were found in samples for the vertical habitat (Figure 2). However, date mussels intensively colonized the other three habitats at the Marina: sheltered, vault, and whole stones. Results for these habitats were compared with data for the natural substratum. ANOVA, with the use of the number of date mussels as the dependent variable, revealed the significance of the interaction between the substratum (Marina and natural) and the habitat type. For the natural substratum, the ranking of habitats was: Stones (225 ± 99 m^{-2}) < Vault (967 ± 201 m^{-2}) < Sheltered (1482 ± 477 m^{-2}); whereas at the Marina the ranking was: Stones (85 ± 5 m^{-2}) < Sheltered (508 ± 113 m^{-2}) < Vault (958 ± 153 m^{-2}). On vaults, the number of date mussels at the Marina was similar to that on the natural substratum. At the other two habitats, the number on the natural substratum was higher than at the Marina (SNK-tests, Table 2).

The total biomass of *L. lithophaga* on the natural substratum was 1.1 ± 0.7 kg m^{-2} (horizontal), 5.6 ± 1.0 kg m^{-2} (inclined), and 8.5 ± 4.6 kg m^{-2} (vertical habitat) (Fig. 3). Results for the other three habitats on natural substratum and at the Marina were compared using ANOVA. Also, the interaction Substratum x Habitat was significant for the biomass. For the natural substratum, the ranking of habitats was: Stones (1.3 ± 0.6 kg m^{-2}) < Vault (8.4 ± 1.1 kg m^{-2}) < Sheltered (14.2 ± 5.2 kg m^{-2}). At the Marina, the ranking was: Stones (0.2 ± 0.1 kg m^{-2}) < Sheltered (2.2 ± 0.4 kg m^{-2}) < Vault (3.7 ± 0.5 kg m^{-2}). The total weight of *L. lithophaga* on the natural substratum was always significantly higher than in corresponding habitats at the Marina (SNK-tests Table 3, Figure 3).

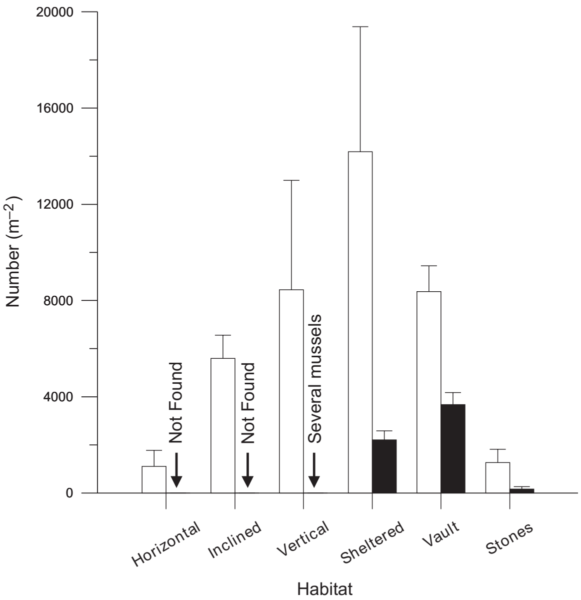

Figure 3. *Total weight of the date mussel* Lithophaga lithophaga *in different habitats. Open bars: Natural substratum; filled bars: Breakwater boulders of the Marina of Rovinj placed at sea for 19 years. Data are means ± SD of 3 samples.*

TABLE 2

Two way analysis of variance to compare the abundance of Lithophaga lithophaga (destructive sampling) growing on breakwater boulders of the Marina of Rovinj (19 years after placing in the sea) and in the natural substratum at three habitats.

Dependent variable: number of *L. lithophaga* per m^2; N = 18, n = 3				
Cochran's *C*-test: $C = 0.5208$, $p = 0.1085$; transformation: square root				
Source of variation	*Df*	MS	*F*	*p*
Substratum	1	249.34		
Habitat	2	659.18		
Substratum x Habitat	2	92.95	8.007	0.006
Residual	12	11.61		
Student-Newman-Keuls test: SE = 1.9672, $df = 12$, $\alpha = 0.05$				
Among Habitats at each level of Substratum	Between Substrata at each level of Habitat			
Natural: Stone < Vault < Sheltered Marina: Stone < Sheltered < Vault	Sheltered: Marina < Natural Vault: Marina = Natural Stone: Marina < Natural			

Factors are: Substratum (fixed, 2 levels: Marina and natural substratum), Habitat (fixed, 3 levels: Sheltered, Vault, and Stones)

The frequency distribution of the length classes of *L. lithophaga* in the natural substratum and in the boulders at the Marina is shown in Figure 4 and 5, respectively. Date mussels of 50 mm or more in length are usually considered to be of commercial value. On the natural substratum, such length classes represented 63% (inclined), 65% (vertical), 56% (sheltered), 49% (vault), and 25% (stones) of the total number of *L. lithophaga*. Larger mussels were in the length classes of 85-90 (vault), 80-85 (vertical and sheltered), and 75-80 mm (inclined and stones) and usually represented several percent of the total number. At the Marina, date mussels of 50 mm or more were 35% (sheltered), 24% (vault), and 3% (stones) of the total number. Larger mussels were in the length classes of 65-70 mm (sheltered and vault) and 55-60 mm (stones). The finding of relatively big date mussels at the Marina of Rovinj was not unexpected. In May 2001, Elvis Zahtila collected a sample of 10 mussels from the Marina; of those with larger openings of the galleries, the length of mussels ranged from 48 to 73 mm.

DISCUSSION

On limestone breakwater boulders, which had been at the sea for 19 years, the abundance of *Lithophaga lithophaga* varied significantly among different types of

TABLE 3

Two way analysis of variance to compare the total biomass of Lithophaga lithophaga (destructive sampling) growing on breakwater boulders of the Marina of Rovinj (19 years after placing in the sea) and in the natural substratum at three habitats.

Dependent variable: total weight of *L. lithophaga* (g m^{-2}); N = 18, n = 3				
Cochran's *C*-test: C = 0.7515, p = 0.0836; transformation: square root				
Source of variation	*Df*	MS	*F*	*p*
Substratum	1	7572.91		
Habitat	2	6299.98		
Substratum x Habitat	2	953.73	9.370	0.004
Residual	12	101.79		
Student-Newman-Keuls test: SE = 5.8249, df = 12, α = 0.05				
Among Habitats at each level of Substratum	Between Substrata at each level of Habitat			
Natural: Stone < Vault < Sheltered Marina: Stone < Sheltered = Vault	Sheltered: Marina < Natural Vault: Marina < Natural Stone: Marina < Natural			

Factors are: Substratum (fixed, 2 levels: Marina and natural substratum), Habitat (fixed, 3 levels: Sheltered, Vault, and Stones)

habitat, i.e. among the different inclinations of the rocky substratum and among the different topographic forms of rocky structures. At the Marina, inclined and vertical rocky surfaces exposed to the water body had practically no colonization by the date mussel. On the contrary, the date mussel achieved high values of total biomass on inclined and vertical natural surfaces, and the majority of date mussels ranged from 50 to 85 mm in length. These types of habitat are the most frequent along the western Istrian rocky coast and are still intensively damaged because of illegal date mussel harvesting *(5)*.

At the Marina, the lack of date mussel colonization of the inclined and vertical habitats is probably due to the fact that the surface of breakwater boulders was of very low structural complexity at the scale of several cm. It is well known that *L. lithophaga* grows by boring the rock in all directions; however, the openings of the galleries must not be exposed to sedimentation *(4)*. Hence, smooth rocky surfaces, even though inclined or vertical, are not suitable for date mussel colonization. Erosion by other members of the endolithion, for example the boring sponge *Cliona celata* Grant, 1826 and the boring mollusc *Gastrochaena dubia* Pennant, 1777, which were abundant in destructive samples from the Marina, will probably lead to an increase of the substratum structural complexity with time, thus enhancing date mussel colonization.

Date mussel harvesting provokes a substantial reduction in the substratum structural complexity *(5, 12)*. Thus, it can be suggested that the recovery of *L. lithophaga* on inclined and vertical rocky surfaces after collection could be very slow. In the Marina habitats not exposed to sedimentation (sheltered, vaults, whole stones), the colonization by the date mussel was very intense, reaching in some cases (vaults) an abundance similar to that of the natural population. Along the Istrian rocky coast, these habitats are becoming more and more scarce due to date mussel harvesting. Hammering transforms sheltered habitats that are inclined or vertical. Vaults are typically of low thickness and collapse because of the hammering. Whole stones are usually completely destroyed.

At the Marina, the total biomass of *L. lithophaga* was always significantly lower than that at corresponding natural habitats. However, a relatively high proportion of date mussels exceeded the length of 50 mm (35% in the sheltered habitat, 24% on vaults). The length of the largest date mussels was between 60 and 70 mm. These results partially contradict the literature data. For the area of Rovinj, it was estimated that the mussels reach the minimal commercial size of 50 mm in 35 years, the size of 70 mm in 63 years, and the maximum length of 80 mm in 80 years *(4)*. Analysis of growth rings revealed that individuals with a length of 50 – 60 mm in the Evoikos Gulf (Greece) ranged in age from 21 to 35 years whereas 80 mm mussels were 54 years old *(18)*. In the Tyrrhenian

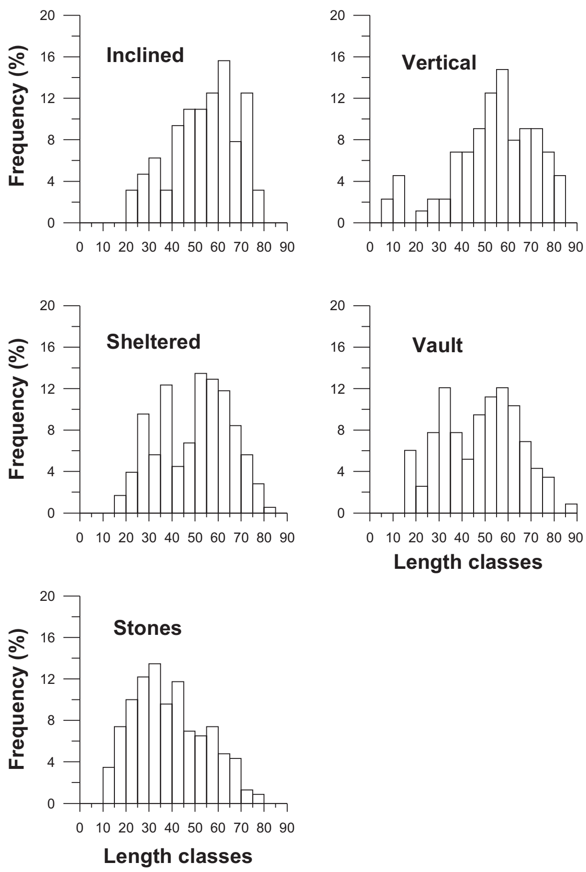

Figure 4. *Frequency distribution of length classes of* Lithophaga lithophaga *in different habitats formed by natural limestone substratum.*

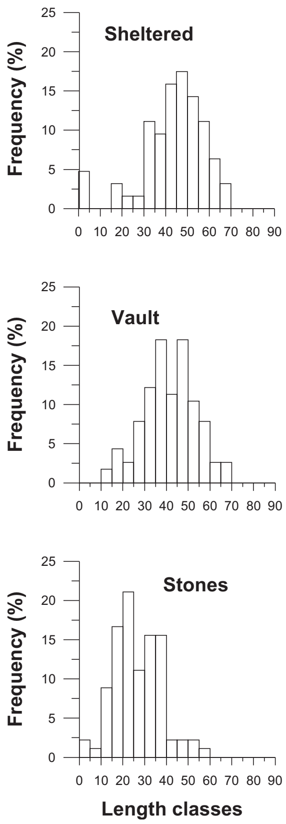

Figure 5. *Frequency distribution of length classes of* Lithophaga lithophaga *in different habitats formed by limestone breakwater boulders of the Marina of Rovinj (which had been at sea for 19 years).*

Sea, date mussels of 50-60 mm were found in rocks which had been in the sea for 25 years *(19)*. The highest growth was reported for the Central Adriatic: date mussels of 78 mm in length were found in rocks which had been in the sea 35 years *(2, 20)*.

There are also contrasting opinions concerning the time necessary for colonization of the substratum to begin. Some researchers have suggested that the substratum must firstly be structurally modified by pioneer species, mainly the boring sponge *Cliona celata*, and that the date mussel begins to inhabit a stone from 5 to 10 years after it has been placed in the sea *(19, 20, 21)*. However, other studies show that juveniles can colonize rocks placed in the sea within a year, reaching a length up to 4 mm and already excavating their galleries *(22)*.

Our results suggest that the architectural structure of the substratum strongly influences the colonization patterns of the date mussel. The action of pioneering organisms could be important for the colonization of habitats that are horizontal, inclined, or vertical. At sheltered habitats, colonization may begin in the same year of placing rocks at sea. Further studies are needed to assess the effects of environmental factors on the growth and biomass of *L. lithophaga*. Such factors could be, for example, the geological type of limestone, the availability of food, and perhaps salinity and temperature. Preliminary data are available only for the effect of the type of substratum. The kind of limestone affects the boring rate of *L. lithophaga (3)*. A mariculture attempt in the Gulf of Trieste (Italy) showed that growth is more rapid in limestone in comparison with sandstone, bricks, and in nets, i.e. outside of the substratum. In limestone, the annual biomass increment of *L. lithophaga* of 24.5 mm in length was approximately 55% *(23)*.

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