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Biometric differences between date mussels *Lithophaga lithophaga* colonizing artificial and natural structures

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Shell width and body live weight related to shell length of the endolithic bivalve Lithophaga lithophaga *(date mussel) colonizing a specific habitat (vaults under boulders) formed by artificial and natural structures were examined. Artificial structures consisted of limestone boulders of a breakwater (Marina Rovinj, northern Adriatic Sea, Croatia) constructed 19 years before sampling of the date mussel. Date mussels' density (around 80 individuals per 0.1 m^2) did not differ between the two types of structure. However, the length frequency distribution in artificial structures (25th percentile = 3.20, median = 4.30 and 75th percentile = 5.10 cm) differed from that in natural structures (25th percentile = 3.66, median = 5.15 and 75th percentile = 6.20 cm) leading to a substantial difference in total biomass (0.3 and 0.8 kg per 0.1 m^2 for artificial and natural structures, respectively). Parameter estimates of regression functions for width against length (linear function) and for live weight against length (allometric function) also significantly differed, indicating variations in date mussels' morphometry between the two types of structure. Analyses of variance did not detect differences in width or weight for date mussels in the length range from 3 to 3.5 cm. However, width (average ± s.d., n = 18) of individuals in the range from 5.5 to 6 cm was significantly lower in artificial structures (1.46 ± 0.13 cm) than in natural structures (1.66 ± 0.10 cm). Consistent with this, live weight in artificial structures (8.36 ± 1.17 g) was significantly lower than that in natural structures (12.33 ± 1.48 g). It is suggested that these patterns reflect a growth rate of the date mussel that is higher in artificial than in natural structures. Information about date mussels' biometric patterns in different habitats is important in planning studies assessing the resilience capability of natural populations after illegal destructive harvesting, particularly as,such studies are lacking.*

Key words: *Lithophaga lithophaga*, artificial structures, rocky bottom, growth, morphometry, Adriatic

INTRODUCTION

The date mussel (*Lithophaga lithophaga* L., 1758; Bivalvia: Mytilidae) is an endolithic bivalve which bores calcareous substrata by glandular secretion (MORTON & SCOTT, 1980). It is widespread in the infralittoral, usually at shallow depths, of the Mediterranean, of the east Atlantic from Portugal to Morocco and in the Red Sea (FISCHER *et al.*, 1987). To collect these bivalves, SCUBA divers break the rocky substratum with special sledgehammers, with a detrimental effect on organisms living on the surface and within the substratum (FANELLI *et al.*, 1994; FRASCHETTI *et al.*, 2001; GUIDETTI & BOERO, 2004; GUIDETTI *et al.*, 2004). Date mussel harvesting is illegal in the majority of Mediterranean countries. However, due to the extremely high price and demand for the mollusc, shallow rocky habitats are heavily threatened by this human activity which leads to the desertification of tens of kilometres of the Mediterranean rocky coast each year (FANELLI *et al*., 1994; FRASCHETTI *et al*., 2001). Hence, information about date mussels' biometric patterns is important in planning studies assessing the resilience capability of natural populations after illegal destructive harvesting, particularly as such studies are lacking.

Date mussels grow very slowly. In natural populations, date mussels of length 1 cm are approximately 3 years old (KLEEMANN, 1973a; GALINOU-MITSOUDI & SINIS, 1995). The age of larger individuals varies substantially. GALINOU-MITSOUDI & SINIS (1995) found that date mussels of 5.0 ± 0.2 cm can range in age from 18 to 36 years; the length of the oldest date mussel (54 years) was 8.16 cm while the largest (9.00 cm) was aged 40 years. Date mussels also colonize limestone artificial structures. For example, in the Tyrrhenian Sea, date mussels of 5 to 6 cm were found in blocks which had been in the sea for 25 years (PIEROTTI *et al*., 1966) and, in the central Adriatic Sea, date mussels up to 7.8 cm were found on limestone boulders of a breakwater constructed 35 years before sampling (ŠIMUNOVIĆ & GRUBELIĆ, 1992). Furthermore, GRUBELIĆ *et al*. (2004) ascertained that along the east Adriatic coast date mussels inhabiting rocks which had been in the sea for 24 – 35 years had the characteristics of a healthy population, while in rocks immersed for 51 years the population showed signs of decay and absence of renewal. In the northern Adriatic, 19 years after the placement of limestone boulders in the sea, date mussels of length from 5 to 7 cm represented from 3 to 35% of the total number, depending on the topographic conformation of artificial structures (DEVESCOVI & IVEŠA, 2008).

Limestone artificial structures can be colonized by juveniles within a year (GALINOU-MITSOUDI & SINIS, 1995, 1997). In other cases, the substratum must firstly be eroded by other endolithic species as, for example, the boring sponge *Cliona celata* Grant, 1826, and 5 to 10 years may pass before the settlement of date mussels (PIEROTTI *et al*., 1966; ŠIMUNOVIĆ & GRUBELIĆ, 1992). Independently of the time required for the beginning of the colonization, growth seems to be higher in artificial than in natural structures. As, in molluscs, the morphometry of the shell could reflect levels of growth rate (ALUNNO-BRUSCIA *et al*., 2001), it can be assumed that date mussels growing in artificial and natural structures differ both in shell shape and live weight. The principal targets of this study are; (1) to assess the length frequency distribution of date mussel populations living in artificial and natural structures of a particular topographic conformation, (2) to compare width against length and live weight against length relationships between the two structures and (3) to test for differences in width and live weight between date mussels of similar length colonizing the two structures.

MATERIALS AND METHODS

Artificial structures consisted of boulders of the breakwater of the marina of Rovinj (45.08338º N, 13.63114º E; northern Adriatic Sea, Croatia) which was constructed 19 years before sampling of the date mussel. Samples were collected from a sheltered habitat of specific topographic conformation, i.e. vaults of crevices under boulders open at both sides to the water body at 4 m depth. Detailed observations revealed that this habitat was intensively colonized by date mussels (DEVESCOVI & IVEŠA, 2008). Samples of the natural population were collected from structures of the same topographic conformation as for artificial structures. Both structures were composed of limestone. Three samples were collected per each type of structure during autumn 2003 by SCUBA diving. An orthogonal projection of 0.1 m^2 on the substratum was demolished using hammer and chisel and all date mussels were collected and placed in a plastic bag for biometric measurements. Natural structures were sampled at locations spaced approximately 10 km apart. For artificial structures, samples were collected along the breakwater at positions spaced approximately 100 m apart.

In the laboratory, measurements of the shell length (maximum distance along the anterior

– posterior axis) and width (maximum lateral axis) were made to the nearest 0.01 cm with a calliper. Date mussels were weighed on a top loading digital balance with a precision of 0.01 g after drying the shell surface with filter paper (live weight).

Date mussels' length frequency distributions were compared using the Kolgomorov-Smirnov (K-S) two sample test which examines differences in both shape (for example skewness and kurtosis) and location of two distributions (SOKAL & ROHLF, 1995). The K-S two sample test was used at first in a series of comparisons for the three samples within each type of structure and then, after pooling of samples, to compare length frequency distributions between the two types of structures. Distributions were also tested for skewness and kurtosis (SOKAL & ROHLF, 1995).

Data for the two types of structures were also submitted to regression analysis. Parameter estimates of regression functions for relationships of width against length (linear regression) and live weight against length (nonlinear regression using the allometric function $y = A \cdot x^B$) were compared using 95% confidence intervals (CI). The allometric function was fitted using the Gauss-Newton method. No starting values were provided. The algorithm estimated parameters by iteration along with their 95% Wald CI.

Nested analyses of variance (ANOVA) were used to test for the effect of structure (fixed factor, 2 levels: artificial and natural) on width and live weight of date mussels in the length ranges from 3.00 to 3.50 and from 5.50 to 6.00 cm. Six date mussels were randomly chosen for each length range within each sample, so samples became levels of a nested factor (3 random levels). Prior to analysis, variances were tested for homogeneity using the Cochran's *C*-test. Statistical analyses were performed using the software package SYSTAT (Version 10, SPSS Inc.).

RESULTS

Density and total biomass of date mussels for the three samples from artificial structures were 78.7 ± 5.9 individuals per 0.1 m^2 and 310.12 ± 16.70 g per 0.1 m^2, respectively (mean ± *s.d.*). For natural structures, density and total weight were 93.0 ± 13.1 individuals per 0.1 m^2 and 834.57 ± 103.45 g per 0.1 m^2, respectively. The density of date mussels colonizing artificial structures was similar to that for natural structures (t = 1.733, $d.f.$ = 4, P = 0.158) while the total biomass was higher in natural than in artificial structures (t-test assuming unequal variances, t = 8.669, $d.f.$ = 2, P = 0.013).

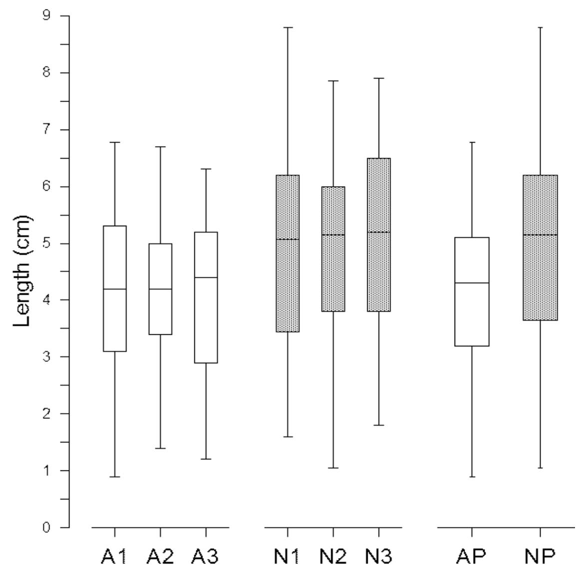

Fig. 1. Box-plots for the length of the date mussel Lithophaga lithophaga. *A1, A2 and A3 are samples from artificial structures; N1, N2 and N3 are samples from natural structures. AP and NP are pooled samples for artificial and natural structures, respectively*

Box-plots of date mussels' length for each sample and for pooled samples within each type of structure are shown in Fig. 1. Comparisons of the distributions (K-S two sample test) between samples within each structure did not detect significant differences. However, the K-S two sample test revealed that date mussels' length frequency distributions significantly differed between the two types of structure (Table 1). In both structures, date mussels' length was not normally distributed. For artificial structures, the distribution was skewed to the left (g_1 = -0.333, SE = 0.158, n = 236, P < 0.05) while kurtosis was not significant (g_2 = -0.530, SE = 0.316, P > 0.05). The 25th percentile was 3.20 cm, the median was 4.30 cm and the 75th percen-

Table 1. Kolgomorov-Smirnov (K-S) two sample tests comparing frequency distributions of the length of date mussels Lithophaga lithophaga *between samples and types of structures*

K-S test	Artificial structures		Natural structures	
	D_{max}	P	D_{max}	P
Sample 1 *vs.* Sample 2	0.119	0.657	0.078	0.931
Sample 1 *vs.* Sample 3	0.089	0.927	0.116	0.547
Sample 2 *vs.* Sample 3	0.118	0.639	0.149	0.312
Artificial *vs.* natural structures (after pooling of samples)				
D_{max} = 0.280; P < 0.001				

The number of date mussels in each sample was n_1 = 72, n_2 = 81 and n_3 = 83 for artificial structures and n_1 = 108, n_2 = 87 and n_3 = 84 for natural structures

tile was 5.10 cm. Date mussels ranged in length from 0.90 to 6.77 cm. For natural structures, skewness was not significant (g_1 = -0.208, SE = 0.146, n = 279, P > 0.05); however, the distribution was platykurtic (g_2 = -0.648, SE = 0.291, P < 0.05) showing that the frequency of length classes around the median was particularly high (clumped distribution). The 25th percentile, the median and the 75th percentile of the length distribution were 3.66, 5.15 and 6.20 cm, respectively. Lengths of date mussels were in the range from 1.05 to 8.80 cm.

Date mussels' width was linearly related to length, and scatter-plots of width against length with linear regression lines fitted for artificial structures (r^2 = 0.957, n = 236, P < 0.001) and natural structures (r^2 = 0.879, n = 279, P < 0.001) are shown in Fig. 2 A and B, respectively. The intercept of both linear regression lines was not significantly different from zero (artificial: 95% CI = -0.029 and 0.028; natural: 95% CI = -0.060 and 0.064). However, slopes of fitted lines differed (artificial: 95% CI = 0.236 and 0.249; natural: 95% CI = 0.261 and 0.285).

Scatter-plots of weight against length with nonlinear ($y = A \cdot x^B$) regression lines fitted for artificial (r^2 = 0.989, n = 236, P < 0.001) and natural structures (r^2 = 0.972 n = 279, P < 0.001) are shown in Fig 2 C and D, respectively. Values of the coefficient A differed between the two types of structure (artificial: 95% Wald CI = 0.041 and 0.055; natural: 95% Wald CI = 0.094 and 0.147) as well as values of the exponent B (artificial: 95% Wald CI = 2.841 and 3.016; natural: 95% Wald CI = 2.471 and 2.708).

ANOVA-s did not detect differences in average width or average live weight between the two types of structure for date mussels of the length ranging from 3.00 to 3.50 cm. There was no significant variation among samples (levels of the nested factor) within each type of structure (Table 2). On the contrary, for date mussels

Table 2. Analyses of variance to test for the effect of the type of structure on shell width and live weight of date mussels Lithophaga lithophaga *ranging in length from 3.00 to 3.50 cm*

Source of variation	*d.f.*	Date mussels' shell width			Date mussels' live weight		
		MS	F	P	MS	F	P
Structure	1	0.055	4.231	0.109	0.451	2.179	0.214
Sample (Structure)	4	0.013	1.857	0.144	0.207	1.005	0.420
Residual	30	0.007			0.206		
Cochran's C-test		C = 0.383; P = 0.094			C = 0.472; P = 0.027		
Means ± *s.d.* (n = 18) of pooled data							
Artificial structure		0.773 ± 0.056			1.637 ± 0.256		
Natural structure		0.852 ± 0.110			1.861 ± 0.589		

Six date mussels (n = 6) were randomly chosen within each of 3 random samples (0.1 m^2) which were nested within each of the 2 levels (artificial and natural) of the fixed factor Structure; N = 32. Data were not transformed

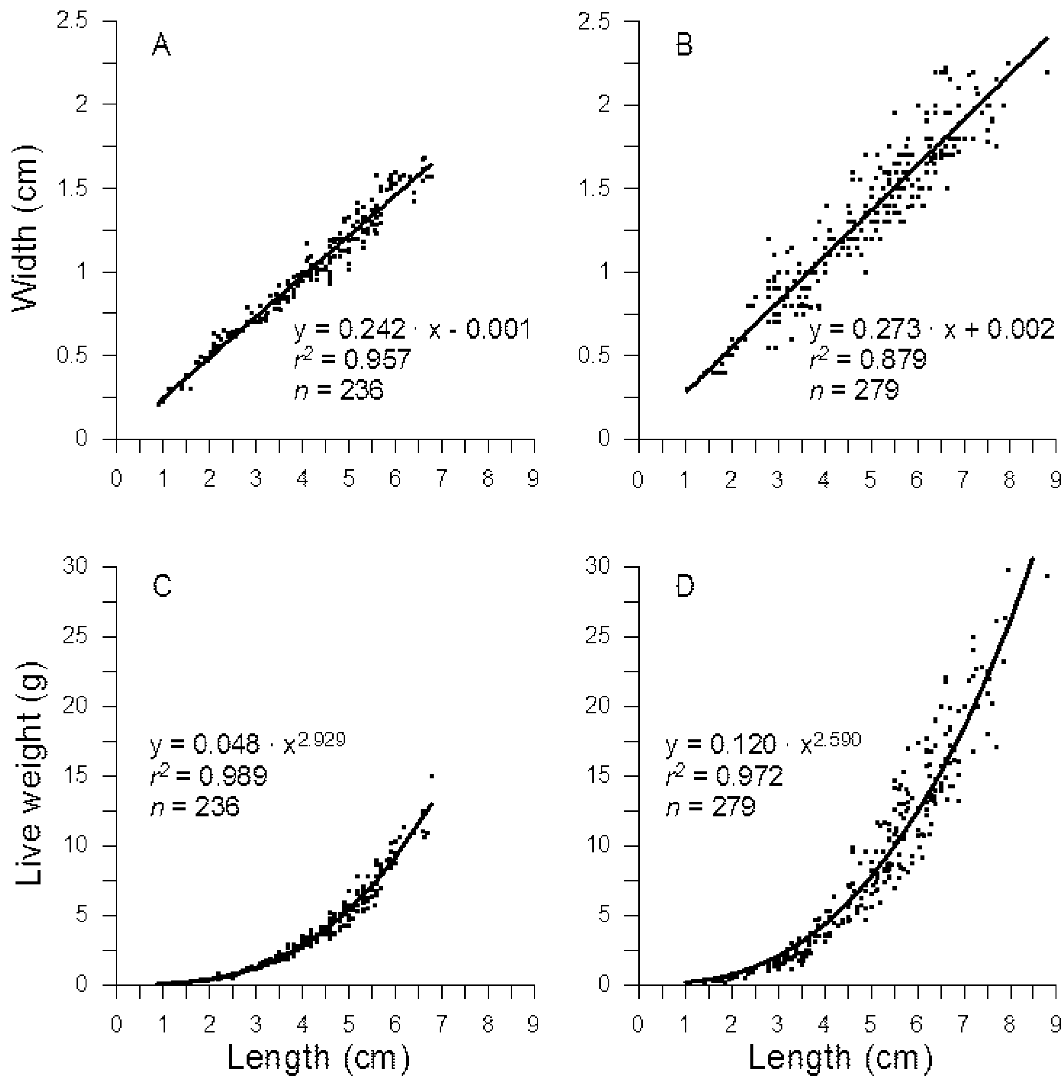

Fig. 2. Scatter-plots of width against length with linear regression line fitted for artificial (A) and natural structures (B), and of live weight against length with non-linear (allometric) regression line fitted for artificial (C) and natural structures (D) for date mussels Lithophaga lithophaga

Table 3. Analyses of variance to test for the effect of the type of stucture on shell width and live weight of date mussels Lithophaga lithophaga *ranging in length from 5.50 to 6.00 cm*

		Date mussels' shell width			Date mussels' live weight		
Source of variation	*d.f.*	MS	*F*	*P*	MS	*F*	*P*
Structure	1	0.374	46.750	0.002	164.139	104.881	< 0.001
Sample (Structure)	4	0.008	0.571	0.686	1.565	0.868	0.495
Residual	30	0.014			1.803		
Cochran's *C*-test		C = 0.337; P = 0.071			C = 0.314; P = 0.511		
Means ± *s.d.* (n = 18) of pooled data							
Artificial structure		1.458 ± 0.125			8.360 ± 1.172		
Natural structure		1.662 ± 0.101			12.331 ± 1.475		

Factors and number of replicates are as in Table 2. Data were not transformed ranging in length from 5.50 to 6.00 cm, average width and live weight were significantly lower in artificial than in natural structures. Significant variation among samples within each type of structure was also not detected (Table 3). Variances were homogeneous for all analyses, except for live weight of date mussels in the range from 3.00 to 3.50 cm (Tables 2 and 3). In spite of the heterogeneity of variances, results of this analysis were sound as they were non-significant (heterogeneous variances lead to excessive Type I error).

DISCUSSION

Over 19 years, date mussels intensively colonized a particular artificial habitat, i.e. vaults of crevices under breakwater limestone boulders, attaining a density (approximately 80 individuals per 0.1 m^2) similar to that of the population colonizing a natural habitat of the same topographic conformation. There was no difference in date mussels' length frequency distribution between samples within both artificial and natural structures. However, larger date mussels were less frequent in artificial than in natural structures leading to substantial differences in total date mussels' biomass (around 0.3 and 0.8 kg per 0.1 m^2 for artificial and natural structures, respectively). Parameters of the fitted regression line for date mussels' width against length (linear), as well as of live weight against length (allometric), differed between the two types of structures.

A preliminary comparison of plotted lines indicated that there were interesting patterns of difference between artificial and natural structures. There appeared to be no differences in width or weight for shorter date mussels while for longer ones, both values of width and weight tended to differ between the two types of structure. Further explorations of data using analysis of variance confirmed these patterns. While for the length range from 3.0 to 3.5 cm no differences were detected, for the length range from 5.5 to 6 cm, date mussels growing in natural structures were significantly wider and heavier than those from artificial structures.

Based on these results, a growth in weight lower than that in natural structures could be supposed for date mussels colonizing artificial structures. However, according to the equation for natural structures (Fig. 2D), the estimate of the length of a date mussel of 8.36 g (average for the range from 5.5 to 6.00 cm in natural structures) is 5.15 cm. In natural populations, date mussels of such length may be from 18 to 36 years old (GALINOU-MITSOUDI & SINIS, 1995). Estimations for the northern Adriatic suggest an age of 35 years (KLEEMANN, 1973a). As even larger date mussels were found in artificial structures, both growth in length and growth in weight may be considered higher than those expected in natural populations.

Various abiotic and biotic factors can affect date mussels' growth rate, among them particularly important are: (1) the composition of the substratum, (2) hydrodynamic conditions, (3) habitat physical features, (4) food concentration and (5) intra-species competition for food and space (KLEEMANN, 1973a,b, 1974; VALLI *et al*., 1986; GALINOU-MITSOUDI & SINIS, 1995, 1997). Very likely, in the present study, factors (1) to (3) did not produce differences in growth patterns. Both examined substrata were composed of limestone. Moreover, hydrodynamic conditions, the topographic conformation of the rocky bottom and the depth were similar at each sampling position. In the dolomitic limestone substratum, both density and growth rate of the date mussel are very low (KLEEMANN, 1973a). Attention had been paid to not sample this kind of natural substratum, which is very widespread along the west Istrian coast (KLEEMANN, 1973a; DEVESCOVI *et al*., 2005).

The most important factor in determining growth rate of molluscs is probably the food supply, since if food is scarce growth will be retarded regardless of all other conditions (SEED, 1976). For example, in northern Norway blue mussels *Mytilus edulis* (L., 1758) close to a fish-farming station could maintain their summer growth rates throughout the winter despite very low water temperatures by feeding on microscopic particles of fish food (WALLACE, 1980). Accordingly, high growth rates of the date mussel in artificial substrata could be due to an increased concentration of food due to eutrophication or pollution levels that usually characterize urbanized areas where artificial structures are constructed.

However, owing to the substantial difference in date mussels' total biomass between artificial and natural structures, the intensity of intra-species competition for food and space could also play an important role. In molluscs, population density influences both growth and morphometry of the shell through either food regulation, physical interference, or their interaction (ALUNNO-BRUSCIA *et al.*, 2001). For instance, parameters of the allometric relationship of body mass against length for blue mussels reared in high-density situations significantly differ from expected values as a reflection of competition for food and space (FRÉCHETTE *et al.*, 1992). In contrast to the blue mussel which increases in width when growing at high densities and in conditions of food shortage (ALUNNO-BRUSCIA *et al.*, 2001), the width of larger date mussels growing in artificial structures was lower than that of individuals of similar length sampled in natural structures where competition was probably greater. It seems that, under optimal growth conditions, the endolithic date mussel tends to monopolize the substratum in depth leading to an elongated shape of the shell.

Data reported in this study are related to a habitat of particular architectural conformation, i.e. vaults of crevices under boulders, where recruitment and growth are particularly high in artificial structures. The natural habitat of this architectural conformation is usually destroyed because of hammering during illegal date mussel harvesting. Less topographic changes are expected for inclined and vertical walls which, along the west Istrian coast, are very widespread and abundantly colonized by date mussels (DEVESCOVI *et al.*, 2005). However, this habitat seems not to be adequate for rapid repopulation which may require significantly longer periods than for artificial vaults (DEVESCOVI & IVEŠA, 2008). Although the shallow rocky bottom is damaged to a significant degree, this usually does not cause local extinction of the date mussel. In heavily exploited areas, date mussels are present in places where they are not abundant enough to be collected as, for example, small indentations of the nearly horizontal rocky bottom. However, harvesting provokes long lasting changes in the structure and functions of benthic assemblages where previously the date mussel was abundant (PARRAVICINI *et al.,* 2008, 2009; ROVERE *et al.,* 2009).

The mariculture of the date mussel seems not to be an adequate implement to mitigate impacts on the shallow rocky bottom. In spite of the use of artificial structures for the mariculture of the date mussel already being suggested (ALBERTELLI *et al.*, 1995), the assessment of optimal biotic and abiotic environmental conditions, allowing intense recruitment and rapid growth, needs further investigation. Moreover, the introduction of such mariculture may be risky for conservation purposes. Date mussels illegally collected from natural habitats might be commercialized as cultivated, threatening the Mediterranean subtidal ecosystem further.

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Biometrijske razlike između prstaca *Lithophaga lithophaga* koji koloniziraju umjetne i prirodne strukture

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SAŽETAK

Istražene su širina ljušture i težina živog organizma u odnosu na dužinu ljušture endolitskog školjkaša *Lithophaga lithophaga* (prstac) u specifičnom staništu (svodovi ispod blokova) sastavljenom od umjetnih i prirodnih struktura. Umjetne strukture su bile vapnenački blokovi lukobrana (Marina Rovinj, sjeverni Jadran, Hrvatska), izgrađenog 19 godina prije uzorkovanja prstaca. Brojnost prstaca (oko 80 jedinki po 0.1 m^2) nije se razlikovala između tipova struktura. Međutim, raspodjela frekvencije dužina u umjetnim strukturama (25. percentil = 3,20, medijan = 4,30 i 75. percentil = 5,10 cm) razlikovala se od one u prirodnim strukturama (25. percentil = 3,66, medijan = 5,15 i 75. percentil = 6,20 cm) što je dovelo do značajne razlike u ukupnoj biomasi (0,3 kg po 0.1 m^2 za umjetne i 0,8 kg po 0.1 m^2 za prirodne strukture). Nadalje, procijenjeni parametri regresijskih funkcija širine u odnosu na dužinu (linearna funkcija) i težine u odnosu na dužinu (alometrijska funkcija) značajno su se razlikovali, što upućuje na morfometrijske razlike prstaca između tipova struktura. Analizom varijance nije ustanovljena razlika u širini ili težini prstaca u rasponu dužine od 3 do 3,5 cm. Međutim, širina (aritmetička sredina ± *s.d.*, n = 18) jedinki u rasponu od 5,5 do 6 cm bila je značajno manja u umjetnim (1,46 ± 0,13 cm) nego u prirodnim strukturama (1,66 ± 0,10 cm). U skladu s time, i težina jedinki u umjetnim strukturama (8,36 ± 1,17 g) je bila manja nego u prirodnim strukturama (12,33 ± 1,48 g). Pretpostavlja se da su navedene razlike bile posljedica veće brzine rasta prstaca u umjetnim nego u prirodnim strukturama. Informacije o biometrijskim karakteristikama prstaca u različitim staništima su važne za planiranje studija o obnovi prirodne populacije nakon nezakonitog destruktivnog sakupljanja, te takve studije zasada nedostaju.

Ključne riječi: *Lithophaga lithophaga*, umjetne strukture, kamenito dno, rast, morfometrija, Jadransko more