

Ecological survey of endolithic blennies spawning in a sandstone habitat in the Gulf of Trieste

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*The present research aimed at evaluating the importance of endolithic holes for the spawning of blennies and at studying the selection and utilization of holes by blennies in the Gulf of Trieste. The study was conducted using the all-occurrence sampling method, a non-destructive visual census method, aided by SCUBA. Resident, egg-guarding males of *Lipophrys dalmatinus*, *Lipophrys canevae*, *Aidablennius sphynx*, *Parablennius incognitus*, *Parablennius zvonimiri* and *Parablennius rouxi* were caught. Species-specific differences in the utilization of holes were found for species that nest in endolithic holes. Smaller species (e.g. *Lipophrys dalmatinus* and *Lipophrys canevae*) choose holes that are little larger than their heads and approximately as long as their body, which prevent small males from being dislodged by bigger ones. Bigger species that are probably less exposed to interspecific competition for holes, choose mostly holes with an entrance diameter twice as big as their head diameter and much longer than their body. Species living in shallow waters (e.g. *Aidablennius sphynx*) prefer hole positions exposed to daylight, while species living in deeper waters were mostly found in the shade of boulders and rocks.*

Key words: Adriatic Sea, combtooth blennies, sandstone boulders, endolithic holes, species-specific differences

INTRODUCTION

The combtooth blennies (family Blenniidae) are benthic bottom-dwellers found in the rocky intertidal and shallow subtidal habitats. For the Mediterranean Sea 19 species have been confirmed (ALMADA *et al.*, 2001) and 15 of them live in the Gulf of Trieste (LIPEJ & RICHTER, 1999; LIPEJ *et al.*, 2005). Since they are without economic value and the collecting of samples with trawls is impossible on hard bottoms, the knowledge on these benthic species has mainly been gained by the use of non-destructive visual census methods, aided by SCUBA (KOPPEL, 1988; KOTRSCHAL, 1988; PALLAORO, 1989; KOVAČIĆ,

2002; LIPEJ & ORLANDO-BONACA, 2006; ORLANDO-BONACA & LIPEJ, 2007).

Combtooth blennies exhibit male parental care, with territorial males preparing nests mostly in the spring-summer period (depending on latitude). Males invite females to visit the nests in order to lay eggs. After fertilization the males guard and defend the eggs against predators, until they hatch (GIBSON, 1969, 1982; KOTRSCHAL, 1988; NEAT & LOCATELLO, 2002; FARIA *et al.*, 2005). Males can be distinguished from females in most species, because they exhibit distinctive colours during the breeding season. Many blennies utilize endolithic holes, which are bored by etching bivalves, like *Lithophaga lithopaga* and

Gastrochaena dubia (KOPPEL, 1988; KOTRSCHAL, 1988; ILLICH & KOTRSCHAL, 1990). Bigger blennies species prepare their nests in cracks and crevices among boulders (SANTOS *et al.*, 1995; OLIVEIRA *et al.*, 2002; FARIA *et al.*, 2005).

Most blennioid species show a resource-based, promiscuous mating system, where males and females mate with multiple partners, associated with strong intrasexual competition (NEAT & LOCATELLO, 2002). Bigger males receive more female visits and more female courtship acts, which results in more spawning (OLIVEIRA *et al.*, 2000). Moreover, females prefer a male with eggs since such males are more likely to care for a large brood than a small brood, or because spawning in a large brood dilutes the probability of egg cannibalism (NEAT & LOCATELLO, 2002). For species that are not nesting in endolithic holes, like *Parablennius sanguinolentus parvicornis*, SANTOS & ALMADA (1988), SANTOS *et al.* (1996) and OLIVEIRA *et al.* (2001) noted that larger and older males establish breeding territories and smaller males act as satellites on these territories, trying to opportunistically participate in fertilization acts. Obligatory hole-nesting blennies, like

Aidablennius sphynx, appear not to use »sneaking« mating tactics (NEAT & LOCATELLO, 2002).

The coastal Adriatic reefs consist mainly of limestone, which is substituted by sandstone in Slovenian coastal waters, containing high densities of the etching endolithic bivalves. The date mussel *Lithophaga lithophaga* inhabits shallow waters, reaching its maximum densities (up to 300 ind. m⁻²) in the rocks within 5 m depth (HRS-BRENKO *et al.*, 1991). The growth of the date mussel is extremely slow reaching its minimal commercial size with 5 cm in 15 to 20 years (FANELLI *et al.*, 1994). The collection of the date mussel is a strong source of disturbance, since harvesting is carried out by demolition of the hard substrate and causes the disappearance of benthic communities' coverage, as exploited rocks remain completely bare (FANELLI *et al.*, 1994; FRASCHETTI *et al.*, 2001). According to a recent study (GUIDETTI *et al.*, 2004) the demolition of hard substrata has also a negative impact on coastal fish populations.

The present research aimed at evaluating the importance of endolithic holes as spawning habitat of blennies and at studying the selection and utilization of these holes by blennies in the

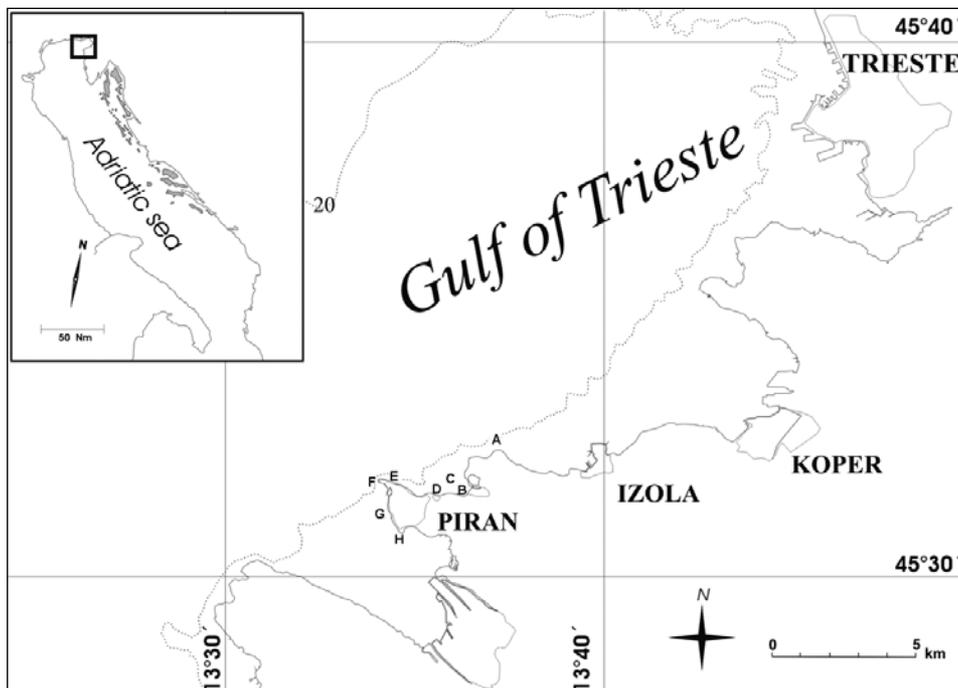


Fig. 1. The map of the study area with survey sites (A–H). Site A: Cape Roněk in the Strunjan Nature Reserve; B: Salinera; C: Pacug; D: Fiesa; E: under the main church of the Piran town; F: Cape Madonna Nature Monument; G: in front of Marine Biology Station; H: Bernardin

Gulf of Trieste. Bivalve's holes vary in size but not in shape, which allowed to compare patterns of utilization of these holes by different blenny species, searching for possible correlations between a single blenniid species and the endolithic hole's parameters (width, length, light exposition and inclination).

MATERIAL AND METHODS

Study area

The study area comprises Slovenian coastal waters which are considered an ecological unit within the Gulf of Trieste (Fig. 1), the northernmost part of both the Adriatic and the Mediterranean seas. The surface area of the Gulf is approximately 600 km², with a water volume of about 9.5 km³. It is a shallow semi-enclosed gulf (with a maximum depth of 33 m off Piran) influenced by freshwater inflow, bottom sediment resuspensions and different sources of pollution. The Slovenian coastline is approximately 46 km long and was once completely sandstone (flysch), which is the major source of detrital material. Nowadays only 18% of the coastline is still in its natural state (TURK, 1999).

The blenniid fauna was studied in Slovenian coastal waters during the spring-summer period (from May to the end of September) of the years 2004-2005. The research was conducted at shallow sites, at a depth ranging from 0 to 10 meters. Eight survey sites (A to H in Fig. 1) were selected, that included a broad variety of biotopes, with two of them being located in marine protected areas (A and F).

Fieldwork

The study was conducted using the all-occurrence sampling method (SYMS, 1995), a non-destructive visual census method, aided by SCUBA. During each all-occurrence survey, two divers swam slowly (approximately 2 m min⁻¹) and at random over the selected area, along depth strata, and recorded all blennies encountered in shelters (holes, crevices, shells, etc). In the sampling period, 46 all-occurrence

surveys were conducted, over a total of 2715 minutes. The duration of a single survey was about 60 min. At each survey site the assessment was repeated at least three times. All surveys were carried out between 8 and 12 a.m.

Resident, egg-guarding males in holes were caught with the method proposed by KOTRSCHAL (1988). The opening of a plastic bag was held tightly around a selected hole. The male was then disturbed by stirring a tight stick contained in the bag. The fish darted out of its hole and it was confined in the bag. Total length of the fish as well as head width and height were measured, while it was gently confined within the bag. The entrance width and height as well as the length (depth) of the hole were also recorded (Fig. 2). Measurement (to the nearest mm) of holes and fish were performed with sliding callipers. As the cross section areas of both blenny heads as well as hole entrances are oval, the calculation of the diameter of a circle was approximated according to KOTRSCHAL (1988): EnDi (entrance diameter) = [(entrance width + entrance height)/2] and HeDi (head diameter) = [(head width + head

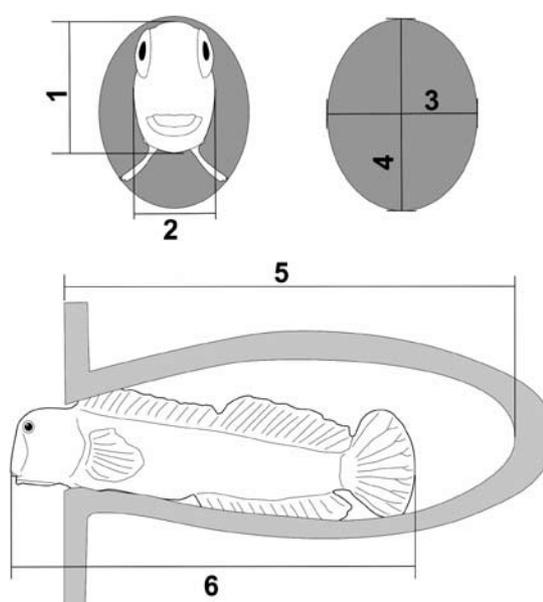


Fig. 2. Schematic sketch to delineate measurement points chosen for fish and spawning site during the fieldwork. 1 = height of the head; 2 = width of the head; 3 = entrance width; 4 = entrance height; 5 = hole length; 6 = total length of the fish (drawing: T. MAKOVEC)

Table 1. Records of male blennies in different nests. The measurements are incomplete for those records where the fish escaped before total length and/or head height and width were measured

Species	<i>L. lithophaga</i> holes	<i>G. dubia</i> holes	<i>C. celata</i> holes	<i>P. nobilis</i> shells	<i>O. edulis</i> shells	Crevices	Plastic tubes	Bricks	Total	Incomplete measurements
<i>Aidablennius sphyrx</i> (Valenciennes, 1836)	39	-	-	-	-	-	-	-	39	5
<i>Coryphoblennius galerita</i> (Linnaeus, 1758)	1	-	-	-	-	-	-	-	1	1
<i>Lipophrys canevae</i> (Vinciguerra, 1880)	10	1	-	-	-	-	-	-	11	1
<i>Lipophrys dalmatinus</i> (Steindachner & Kolombatovic, 1883)	18	17	3	-	-	-	-	-	38	15
<i>Parablennius incognitus</i> Bath (1968)	82	3	-	-	1	1	1	-	88	11
<i>Parablennius rouxi</i> Cocco (1833)	7	-	-	-	-	-	-	-	7	3
<i>Parablennius tentacularis</i> (Brünnich, 1768)	-	-	-	2	1	-	-	1	4	1
<i>Parablennius zvonimiri</i> (Kolombatovic, 1892)	8	-	-	-	-	-	-	-	8	2
<i>Paralipophrys trisuloides</i> (Valenciennes, 1836)	1	-	-	-	-	2	-	-	3	1
<i>Salarias pavo</i> (Risso, 1810)	1	-	-	-	-	3	-	-	4	4
Total	167	21	3	2	2	6	1	1	203	44

height)/2]. After measurements all the fish were released unharmed near the nest. In most cases the exposition of the hole (sunny or shady) and the inclination of the hole within the rock were also annotated. The inclination of the hole was defined according to KOPPEL (1988): vertical hole - 0°, horizontal hole - 90°, downwards hole - 45° and upwards hole - 135°.

Data analysis

Linear regression analysis was used to determine possible relations between the length of holes as the dependent variable and the total body length as the independent variable as well as between hole diameter and head diameter. The Pearson's Correlation Coefficient (r) was used to evaluate the relationship between the two variables.

The G-test on homogeneity of replicates (tested for goodness of fit) was used to compare differences among species in the choice of the hole inclination. The critical significance level was set at 5 %.

RESULTS

Types of shelters utilized for nesting

During the surveys, 203 males of 10 blenniid species were recorded in dwelling places, which are mostly used as nest sites (Table 1). The measurements are incomplete for those records where the fish escaped before their total length or head height and width could be measured (44 individuals).

Four species, *Lipophrys adriaticus*, *L. nigriceps*, *Parablennius gattorugine* and *P. sanguinolentus*, were never found in nests. The preferences of the other species for different types of nesting sites are listed in Table 1. The majority of blennies (82.2%) were utilizing *L. lithophaga* holes, while 10.3% of males were recorded in *G. dubia* holes. Among 10 blenniid species, seven were predominantly using the date mussel holes; only males of *P. tentacularis* were never observed in such holes. *Lipophrys dalmatinus* was almost equally using date mussel holes (47.3%) and *G. dubia* holes (44.7%),

Table 2. Different measurements of the resident fish and occupied holes (n = number of observations; sd = standard deviation; TL = total length of the fish; $HoLe$ = hole length; $m(TL/HoLe)$ = mean ratio between the total length of the fish and the hole length; $HeDi$ = head diameter [(head width + head height)/2]; $EnDi$ = entrance diameter of the hole [(entrance width + entrance height)/2]; $m(HeDi/EnDi)$ = mean ratio between the head diameter and the entrance diameter of the hole)

species (n)	TL (mm)	HoLe (mm)	$m(TL/HoLe)$	HeDi (mm)	EnDi (mm)	$m(HeDi/EnDi)$
<i>A. sphynx</i> (34)						
mean	53.3	55.6	1.0	6.8	10.2	0.7
sd	7.1	1.7	0.2	1.0	2.4	0.1
<i>L. canevae</i> (10)						
mean	35.8	38.5	1.0	4.7	9.1	0.6
sd	8.5	8.4	0.2	1.1	2.4	0.2
<i>L. dalmatinus</i> (23)						
mean	28.9	32.9	1.0	3.5	5.6	0.7
sd	6.3	12.8	0.3	0.5	1.8	0.2
<i>P. incognitus</i> (77)						
mean	39.7	41.5	1.1	5.7	9.8	0.6
sd	8.8	17.4	0.4	1.2	3.3	0.2
<i>P. rouxi</i> (4)						
mean	55.5	61.8	0.9	7.5	15.4	0.6
sd	10.3	14.6	0.1	0.6	6.7	0.2
<i>P. zvonimiri</i> (6)						
mean	49.8	76.7	0.7	6.9	18.0	0.4
sd	12.1	22.9	0.2	1.5	7.5	0.2

and it was the only species also found in holes made by the yellow boring sponge *Clione celata* (7.9%).

Utilization of endolithic holes

A broad range of entrance sizes and hole lengths were utilized by the species that nest in endolithic holes (Table 2). In *L. dalmatinus* and *A. sphynx* heads tightly fitted into these hole entrances, while the ratio between the head diameter and the entrance diameter of the hole slightly decrease for *L. canevae*, *P. incognitus* and *P. rouxi*. *P. zvonimiri* utilizes also holes with an entrance diameter twice as big as their head diameter (Table 2).

A positive correlation between head diameter and hole entrance diameter was found only for *P. incognitus* (Pearson's Correlation Coefficient $r > 0.3$, $p \leq 0.05$) (Fig. 3). For *L. dalmatinus*, *L.*

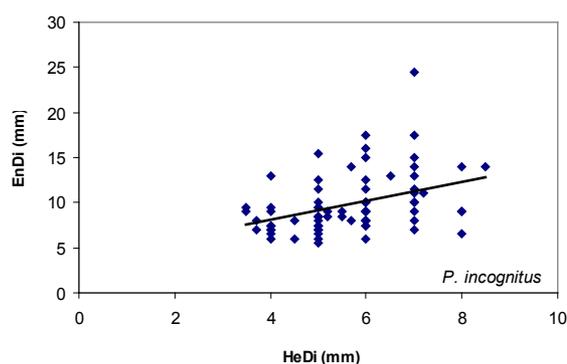


Fig. 3. Linear regression between hole entrance diameter ($EnDi$) and head diameter ($HeDi$) for *P. incognitus* males guarding egg batches ($n = 77$; $r = 0.39$; $p < 0.001$)

canevae, *A. sphynx* ad *P. zvonimiri* the correlation was statistically not significant.

A. sphynx, *L. canevae* and *L. dalmatinus* inhabited holes whose length approximated body length (Tab. 2). *P. incognitus* was found to

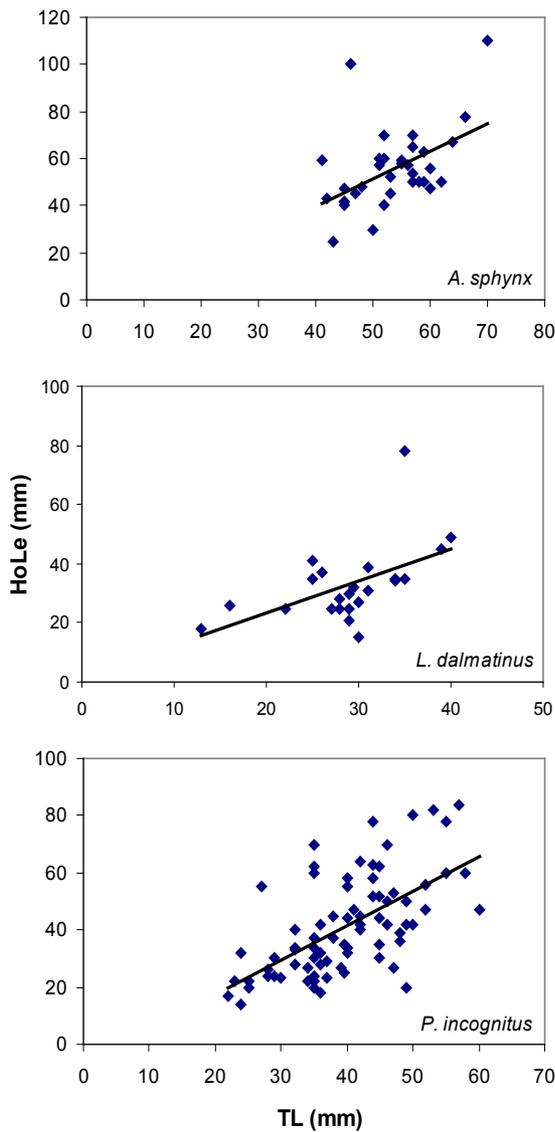


Fig. 4. Linear regression between hole length (HoLe) and total length of the fish (TL). *A. sphynx* ($n = 34$; $r = 0.49$; $p < 0.01$). *L. dalmatinus* ($n = 23$; $r = 0.53$; $p < 0.01$). *P. incognitus* ($n = 77$; $r = 0.62$; $p < 0.0001$)

occupy also shorter holes and consequently the ratio between total length of the fish and the hole length slightly increased (Tab. 2). For *P. rouxi* and especially for *P. zvonimiri* mean total lengths were less than mean hole lengths, indicating preferences for relatively long holes (Tab. 2).

A positive correlation existed between body length and hole length (Pearson's Correlation Coefficient $r > 0.3$, $p \leq 0.01$) for *A. sphynx*, *L. dalmatinus* and *P. incognitus* (Fig. 4). The cor-

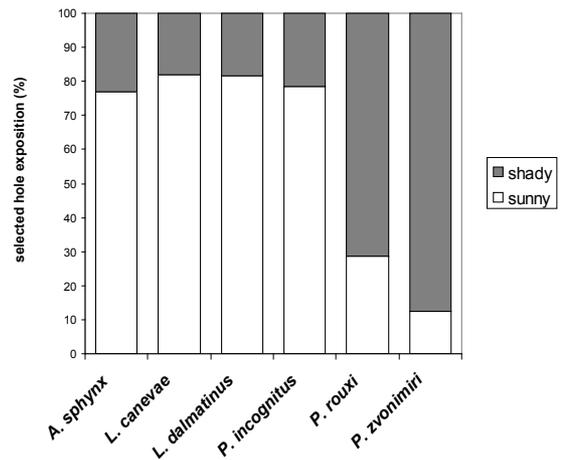


Fig. 5. Selection of holes in relation to daylight exposition (*A. sphynx* $n = 39$; *L. canevae* $n = 11$; *L. dalmatinus* $n = 38$; *P. incognitus* $n = 88$; *P. rouxi* $n = 7$; *P. zvonimiri* $n = 8$)

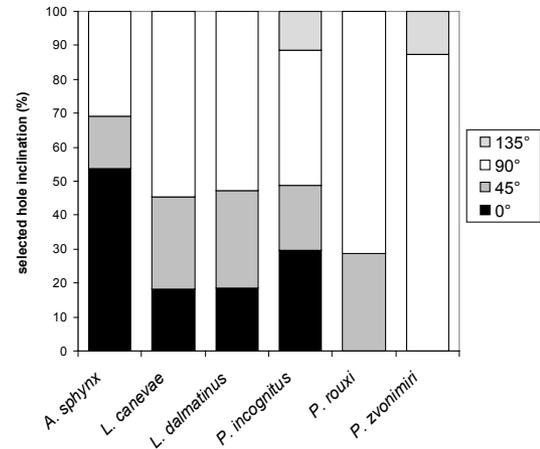


Fig. 6. Selection of holes in relation to inclination in the boulder (*A. sphynx* $n = 39$; *L. canevae* $n = 11$; *L. dalmatinus* $n = 38$; *P. incognitus* $n = 88$; *P. rouxi* $n = 7$; *P. zvonimiri* $n = 8$)

relations were statistically not significant for *L. canevae* and *P. zvonimiri*.

Four species, *A. sphynx*, *L. canevae*, *L. dalmatinus* and *P. incognitus*, showed a preference for holes exposed towards the direction of sunshine (Fig. 5), while *P. rouxi* in *P. zvonimiri* mostly inhabited holes in shady parts of boulders. Observations on the inclination of occupied holes indicated that *A. sphynx* is the only species which was nesting mostly in vertical holes (inclination

0°), while other species showed a preference for horizontal holes (inclination 90°) (Fig. 6). *P. incognitus* was the only species found in holes at all inclinations. Four species, *A. sphynx*, *L. canevasae*, *L. dalmatinus* and *P. rouxi*, were never found in upward oriented holes (inclination 135°). *P. rouxi* was noted only in horizontal and downward pointing holes (inclination 45°), while *P. zvonimiri* was found only in horizontal and upwards holes (inclination 135°).

Differences among species in the choice of hole inclination were not statistically significant among *P. incognitus*, *L. canevasae* and *L. dalmatinus* ($G_H = 12.62$, $P = 0.63$) and between *P. rouxi* and *P. zvonimiri* ($G_H = 4.22$, $P = 0.99$).

DISCUSSION

Small benthic fish are nesting in holes and crevices in order to protect themselves and the fertilized eggs from predators (LINDQUIST, 1985). Holes, which have narrower entrances than crevices, enable the territorial male to prevent entry of other males, and thus to defend its hole. This is probably one of the reasons why endolithic blenniid species have not adopted alternative mating strategies (TABORSKY, 1998). Endolithic bivalves bore holes that provide optimal shelters for breeding, with relatively small entrances and wide enough interiors for spawning and guarding eggs (KOTRSCHAL, 1988). ALMADA & SANTOS (1995) noted that in most of the fish species inhabiting rocky littoral habitats parental care is well developed.

The findings of the present study were in many aspects different from the results presented by KOTRSCHAL (1988). KOTRSCHAL (1988) defined *P. tentacularis* as a species facultatively using holes of endolithic bivalves. Mature males of this species can reach 15 cm in length (ZANDER, 1986), while *L. lithophaga* holes are much smaller. Boulders with old, deep and eroded holes were noted only in the Cape Madona Nature Monument (survey site F, depth range 5-10 m), where *P. tentacularis* was rarely found. The species was mostly recorded on single hard substrata in *Cymodocea nodosa* seagrass meadows.

P. trigloides and *S. pavo*, defined by KOTRSCHAL (1988) as species facultatively using holes of endolithic bivalves, were only exceptionally found in *L. lithophaga* holes in Slovenian waters, as mature males of both species may reach 13 cm in length (ZANDER, 1986). Moreover, KOTRSCHAL defined *L. dalmatinus* as an obligatory user of *G. dubia* holes, while during the present research mature males of the species were also found to nest in date mussels' holes and exceptionally in holes made by *C. celata*. *L. canevasae* and *P. incognitus* were also exceptionally found in *G. dubia* holes. Mature males of *P. incognitus* were noted to nest predominantly in *L. lithophaga* holes, but since the species was found to occupy also other types of holes (crevices, Native oyster shells and artificial holes), we do not consider it as an obligatory user of date mussels' holes, as KOTRSCHAL (1988) concluded.

In Slovenian waters *A. sphynx*, *P. rouxi* and *P. zvonimiri* were found nesting only in holes of the date mussel. NEAT & LOCATELLO (2002) noted in the Bay of Calvi (Corsica) that *A. sphynx* occupied also artificial holes, because fertilized eggs were found in such holes. Since *A. sphynx* was never found nesting in artificial holes in Slovenian waters, we can assume that in this environment the number of date mussel holes is sufficiently high so that competition for sites is minimized and a successful spawning of all mature males is assured.

Species-specific differences in the utilization of holes were found for species that nested in endolithic holes. The results showed that *L. dalmatinus* and *A. sphynx* choose holes that are little larger than their heads, thereby preventing small males from being dislodged by bigger ones. Therefore, bigger males are forced to divide the breeding territory with smaller ones of the same species (NURSALL, 1977). Especially for *A. sphynx* many males were found nesting in holes on the same boulder (own observations). By occupying narrow and short holes, as those made by *G. dubia* and *C. celata*, *L. dalmatinus* (the smallest Adriatic blenny) avoided also interspecific competition for the nesting place. A high intraspecific and

interspecific competition for holes is present also in other smaller species, which are living in shallow waters. For example, males of *L. canevae* have to defend their territory also from males of the opportunistic species *P. incognitus*, although they have different feeding habits (GOLDSCHMID & KOTRSCHAL, 1981; KOPPEL, 1988). Since the ratio between head diameter and entrance diameter of the hole is lower for bigger species, they are probably less exposed to interspecific competition for holes, which can be the case for the cryptobenthic species *P. zvonimiri*. This species, living in deeper waters (PATZNER, 1999; ORLANDO-BONACA & LIPEJ, 2007) occupies also holes much longer than its body, while the majority of smaller endolithic species dwell in holes that are approximately as long as their body. Moreover, species living in shallow waters show a preference for holes positioned towards full daylight, while *P. rouxi* and *P. zvonimiri* were mostly found in the shaded areas of boulders and rocks. *A. sphynx* seems to avoid competition with other shallow waters species also by choosing vertical holes for nesting, while other species mostly occupy horizontal holes. As previously reported (ORLANDO-BONACA & LIPEJ, 2007), ten blenniid species were found to be constant dwellers in the first meter of depth. The variety of blennies then decreases in deeper waters and the number of species remains constant at a value of 4 between 6 and 10 m depth.

Although these results indicate that at present the number of date mussels' holes seems to be high enough in the North Adriatic to assure a successful spawning of these blennies that obligatory or facultatively use holes, for the whole Adriatic area the requirement for such substrates in the recruitment of these species is essential and therefore the enforcement of a respective law prohibiting date mussels illegal fishing is an urgent matter. Unlawful collection of date mussels, carried out by demolition of the rocky substratum, in some areas has already caused the disappearance of substrate typical benthic communities (FANELLI *et al.*, 1994; FRASCHETTI *et al.*, 2001; GUIDETTI *et al.*, 2004). In the empty holes bored by date mussels dwell not only blennies, but also other benthic fishes, sponges, molluscs,

decapods and other invertebrates (FABI *et al.*, 2001), which increase the importance of endolithic holes for the biodiversity of this habitat type. There is certainly enough evidence available to consider spawning habitat for blenniid communities as an integral part of community structure under the EU habitat and Water Framework Directives.

CONCLUSIONS

Species-specific differences in the utilization of holes were found for species that nest in endolithic holes. *L. dalmatinus* was recorded in date mussel holes, in holes bored by *G. dubia*, and it was the only species found also in holes made by *C. celata*. Smaller species, like *L. dalmatinus* and *A. sphynx*, choose holes that are little larger than their heads and approximately as long as their body, which prevent small males from being dislodged by bigger ones. Bigger species, like *P. zvonimiri*, probably less exposed to interspecific competition for nests, mostly choose holes with an entrance diameter twice as big as their head diameter and much longer than their body. Species living in shallow waters prefer sunny hole positions, while *P. rouxi* and *P. zvonimiri* were mostly found in the shade of boulders and rocks. *A. sphynx* avoids competition with other species by choosing vertical holes, while other species mostly occupy horizontal holes. At present, in the North Adriatic the number of date mussels' holes seems to be high enough to assure a successful spawning of blennies that are using these holes obligatory or on facultative bases.

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One of the most important ichthyologists in the Adriatic Sea, Juraj Kolombatović, studied and described some new species, even among blennies, such as *Parablennius zvonimiri*. Since this species was also object of the present study, the authors would like to take this opportunity to dedicate this paper to the memory of this important and renowned pioneer in the Adriatic ichthyologic research.

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Ekološka studija o razmnožavanju endolitskih babica na pješčenjaku tršćanskog zaljeva

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SAŽETAK

Cilj ovog rada je istaknuti značaj endolitskih rupa za razmnožavanje endolitskih babica i proučavanje načina odabira i uporabe istih u tršćanskom zaljevu. U radu su primjenjene metode neselektivnog uzorkovanja i vizualna metoda potpomognuta SCUBA promatranjem. Uzorkovani su primjerci mužjaka koji su čuvali polegnuta jaja od grabežljivaca. Zamjećena je različita uporaba rupa ovisno o vrsti koja se razmnožava u endolitskim rupama. Manji primjerci odabiru rupe koje su nešto veće od njihove glave i približno njihove duljine tijela što sprječava mogućnost da manji mužjaci budu istjerani iz rupe od strane većih primjeraka mužjaka. Veći primjerci koji su manje izloženi međusobnom natjecanju za rupu, pretežito biraju rupe sa ulazom čiji je dijametar otvora dvostruko veći od dijametra njihovih glava i veći je od duljine njihovih tijela. Primjerci koji žive u plitkim vodama imaju sklonosti prema rupama izloženim sunčevoj svjetlosti, dok su primjerci iz dubljeg mora pronađeni u sjenovitim mjestima ispod velikih morskih stijena.

Ključne riječi: Jadransko more, češljouste babice, pješčenjak, endolitske rupe, značajke vrste

