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 MICROHABITAT PREFERENCES OF COASTAL
 COMBTOOTH BLENNIES FAUNA (BLENNIIDAE)
 IN THE GULF OF TRIESTE

Ph.D. Thesis

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The combtooth blennies (family Blenniidae) are benthic bottom-dwellers which occur in coastal waters. Nowhere else are these blennies as abundant and diverse as in the Mediterranean Sea, where researchers have found 19 species. Fifteen of them live in the Slovenian sea. Since they are without economic value and they inhabit hard bottoms where the collecting of samples with trawls is impossible, they were poorly known just thirty years ago. Knowledge about these benthic species has increased during the last decades by the use of non-destructive visual census methods, aided by SCUBA. However, the ecological factors affecting their distribution in the environment and their microhabitat preferences remain poorly understood.

Combtooth blennies exhibit male parental care, with territorial males preparing nests in the spring-summer period. They then invite females inside in order to lay eggs. After fertilization the males guard and defend the eggs against predators, until they hatch. Males can be distinguished from females in most species, because they exhibit distinctive colors during the breeding season. Many blennies utilize endolithic holes, which are bored by etching bivalves, like the date mussel *Lithophaga lithopaga* and the smallest *Gastrochaena dubia*. Bigger blennies species prepare their nests in cracks and crevices among boulders.

The aim of the study was to find out differences among blennies species in microhabitat preferences and in the utilization of different endolithic holes as nests during the breeding season. During the period 1998–2005, the blennioid assemblage was studied along the Slovenian coastal sea. The total number of surveys was 286, all conducted diurnally. The methods used were: vertical transects, horizontal transects, all-occurrence sampling, linear cinetransects and the square method. During diving the microhabitat variables of the site where the blenny was found were annotated, in order to understand the ecological demands of blennies during the breeding season. The importance of biotic (vegetation, benthic fauna, floral cover) and abiotic (depth distribution, illumination, bottom type) factors were supposed to be very important in the microhabitat choice. For endolithic species of blennies we searched for possible correlations between a single species and the endolithic hole's parameters (type of shell, width, length, position and inclination of the hole). These observations

were carried out in the natural environment and during laboratory experiments, as well.

During the study period a total of 14 blennies species were recorded in the Slovenian coastal sea, with the use of the above mentioned non-destructive methods. All 14 species were detected using the all-occurrence sampling method, which showed the best results with the lowest sampling effort in terms of number of surveys. The presence of the cryptobenthic species *P. zvonimiri* was confirmed only with the all-occurrence sampling method. The highest number of species was recorded in the first meter of depth, where 10 species were present. The number of species then decreased toward deeper waters.

Four species of blennies showed themselves to be indiscriminate in their microhabitat choice, as they were found in 25 to 50% of the inspected microhabitats. These species include *P. incognitus*, *L. dalmatinus*, *A. sphynx* in *P. rouxi*. Ten species were classified as infrequent, as they were recorded in less than 25% of the microhabitats.

A Canonical Correspondence Analysis (CCA) was carried out in order to determine the distribution and codependence of 13 blennies species with 13 environmental variables. The results show that the structure of the blennies assemblage in the Slovenian sea is affected by a large number of interplaying factors. The bottom composition, depth, benthic flora and fauna, incorporating both biotic and abiotic variables, are some of the factors responsible for the distribution of coastal blennies. Species inhabiting shallow waters showed a high positive correlation with boulders bigger than 2 m, the presence of mussels and cirripeds, and the presence of empty holes excavated by the date mussel. Species inhabiting deeper waters showed a high correlation with precoralligenous formations, which are frequent on a hard bottom below 4 m depth.

During the surveys, 203 individuals from 10 blennies species were recorded in dwelling places, which are mostly used as nests. Small benthic fish are nesting in holes and crevices in order to defend themselves and the fertilized eggs from predators. Holes, which have narrower entrances than crevices, enable the territorial male that is inside to prevent the entry of other males, and thus to defend its hole. Species found in crevices or in places among boulders were only rarely recorded in nests. Species-specific differences in the utilization of holes were found for species that nest in endolithic holes. The results show that some species (*L. dalmatinus* and *A. sphynx*) choose holes that are little larger than their heads, which prevent small males from being dislodged by bigger ones. *L. dalmatinus* was recorded in date mussels' holes, in holes bored by *G. dubia*, and it was the only species also found in holes made by the yellow boring sponge *Clione celata*, which enable the smallest Adriatic blenny to avoid interspecific competition for the hole. Two bigger species (*P. rouxi* and *P. zvonimiri*), which are probably

less exposed to intraspecific competition for holes, also choose holes with an entrance diameter twice as big as their head diameter. *P. zvonimiri* also occupies holes much longer than its body, while the majority of smaller endolithic species dwell in holes that are approximately as long as 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*, which lives in the mediolittoral belt, avoids competition with other species by choosing vertical holes, while other species mostly occupy horizontal holes.

Six males of *P. zvonimiri* and 6 males of *P. incognitus* were used in laboratory experiments. During 6 series of experiments we found a positive correlation between the rank (the dominance) of males and the access to the hole. Our findings confirm the thesis of "wide territoriality", in which a male defends a net of holes and not just one hole in his territory. *P. zvonimiri* predominates over *P. incognitus*. In the natural environment *P. zvonimiri* dwells in a smaller number of microhabitats than *P. incognitus*. For this reason, it has to defend more successfully its own territory and nests than *P. incognitus*, which is a widely distributed species and could find nesting holes in very different microhabitats.

The results of single-species experiments confirm our field observations, in which *P. zvonimiri* and *P. incognitus* males choose the widest available holes. Both species dwell in holes that are longer than their bodies. In two-species experiments, males of *P. incognitus* were forced to occupy the shortest holes, which confirms the dominance of *P. zvonimiri*. During the series of experiments with differently inclined holes in the aquarium, the two dominant males mostly occupied horizontal holes and holes with 135° of inclination.

The dissertation gives new knowledge on the ecology of blennies and also useful information about the ecological conditions of hard bottom microhabitats in the marine coastal area. These kinds of habitats are very important not only for blennies, but also for the whole fish assemblage and benthic flora and fauna.

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THE ECOLOGICAL CHARACTERISTICS
OF PLANKTONIC DINOFLAGELLATES (Dinophyceae)
IN THE GULF OF TRIESTE WITH AN EMPHASIS
ON TOXIC SPECIES

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Dinoflagellates are important members of the phyto-

plankton community in the coastal sea. In temperate regions, dinoflagellates achieve maximal abundance in the late spring and summer period. They are generally well adapted to environments with low turbulence and low nutrient concentrations. Their directional swimming ability is one of adaptation strategies to overcome scarce nutrient availability. Diel vertical migrations through the water column allow dinoflagellates an adequate nutrient uptake, as well as to avoid grazing and high light intensities. The ability to actively choose their depth is an important contributing factor to dinoflagellate bloom formation, but differs among different species and under different environmental conditions. Dinoflagellate blooms may sometimes have harmful consequences on marine ecosystem and humans. Among various types of intoxication, two are of major importance in the Gulf of Trieste, as their causative organisms are commonly found in the northern Adriatic. First is diarrhetic shellfish poisoning (DSP), which is caused mainly by various species of the genus *Dinophysis*. Second, and more dangerous, is paralytic shellfish poisoning (PSP) caused by some species of the genus *Alexandrium*. At shellfish farms on the Slovenian coast, DSP occurs almost every year, and results in the ban on shellfish sale. PSP, however, has not been observed in this area thus far, despite the persistent occurrence of *Alexandrium* in water samples. Recently, there has been a growing interest in new toxin types, such as the yessotoxins. They are produced by *Lingulodinium polyedrum* and *Protoceratium reticulatum* regularly found in the phytoplankton community of the Gulf of Trieste.

The aim of the study was to advance the knowledge of dinoflagellate ecology in two ways. In the first place, long-term data were analyzed in order to uncover seasonal occurrence patterns of toxic dinoflagellates and to determine the predictability of succession of most recurrent *Dinophysis* species. Sea water was sampled from 1995 to 2003 and examined with the use of an inverted microscope. Data from different depths at two sampling sites in the vicinity of shellfish farms were compared. Correlations between some environmental parameters and the abundance of toxic species for the year 1997 were investigated as well. The occurrence pattern of most frequent and abundant *Dinophysis* species was analyzed using the STATIS multivariate analysis. Secondly, to assess the ability of the dinoflagellate species to perform active vertical movements, two 24-hour samplings of the water column were carried out. In order to include the entire set of environmental stresses that dictate the organisms' response, sampling in the natural water environment was chosen. Samples were taken at 4-hour intervals at different depths. The first 24-hour sampling was performed in November during a period of a mixed water column, while the second was performed under stratified water column conditions in June. Combining the findings from the two parts of this study, improvements were suggested