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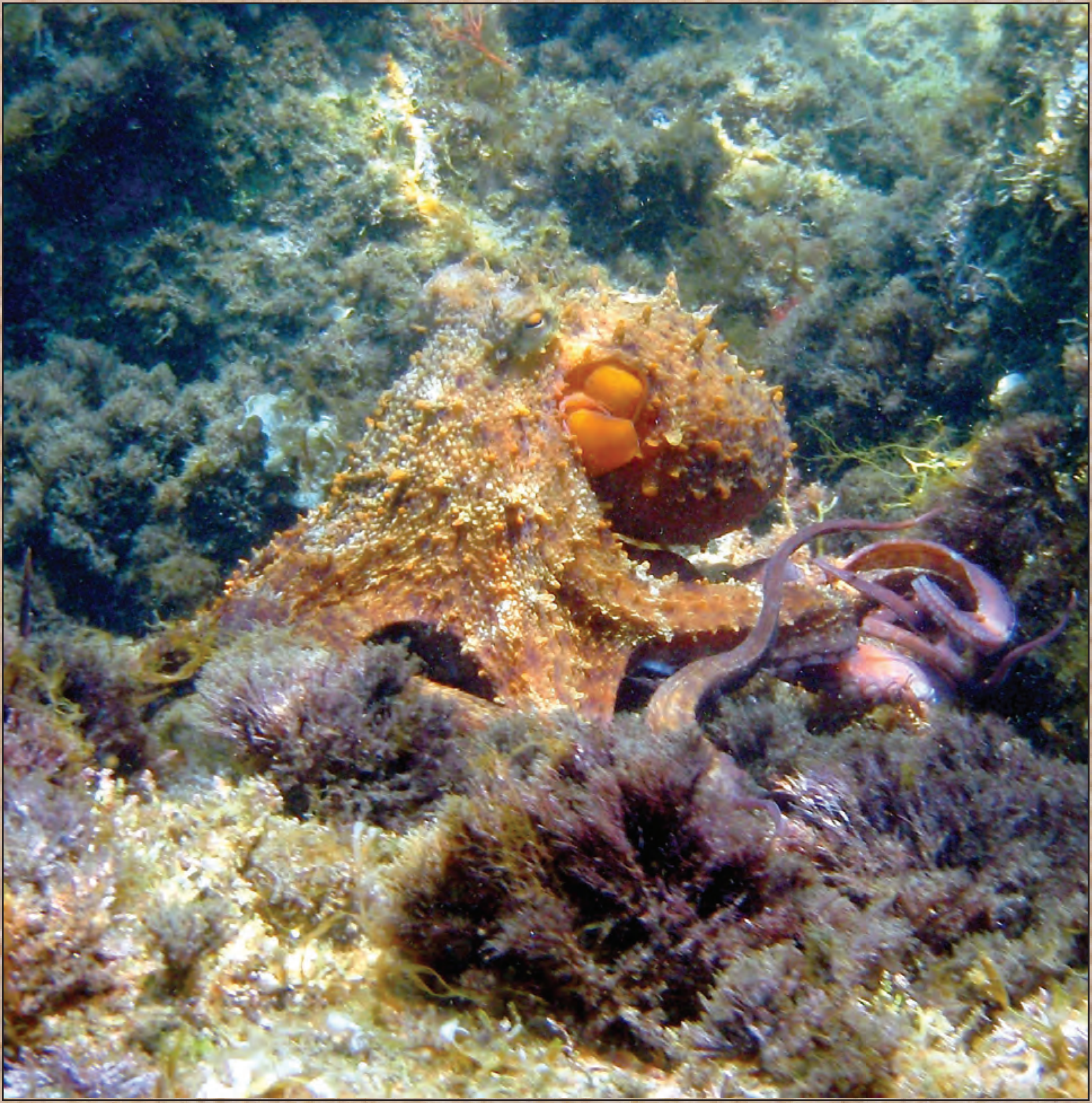

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**FLORA**

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# CORALLINE ALGAE ON BIOGENIC FORMATIONS IN MARINE WATERS OFF SLOVENIA (NORTHERN ADRIATIC SEA)

*Martina ORLANDO-BONACA, Borut MAVRIČ & Lovrenc LIPEJ*
Marine Biology Station, National Institute of Biology, SI-6330 Piran, Fornace 41, Slovenia
E-mail: martina.orlando@nib.si

*Sara KALEB & Annalisa FALACE*
Department of Life Sciences, University of Trieste, I-34127 Trieste, Via L. Giorgieri 10, Italy

## *ABSTRACT*

*Two major biogenic formations, composed mainly by dead corallites of the Mediterranean stony coral (*Cladocora caespitosa*), have been recently studied in Slovenian marine waters. The paper presents new data about the presence of coralline algae on the biogenic formation situated off Cape Ronek and off Cape Debeli rtič. Coralline algae are very important for the creation, development and maintenance of calcareous bio-concretions that offer new niches for many invertebrates and other algae. They are listed as important builders of the coralligenous biocoenosis in the "Draft Lists of coralligenous/maërl populations and of main species to be considered by the inventory and monitoring" of the RAC-SPA, and should be further deeply studied and appropriately protected.*

**Key words:** coralline algae**,** biogenic formations**,** circalittoral, northern Adriatic Sea

# ALGHE CORALLINE DELLE FORMAZIONI BIOGENICHE IN ACQUE MARINE SLOVENE (ADRIATICO SETTENTRIONALE)

## *SINTESI*

*Due formazioni biogeniche, prevalentemente composte da coralliti morti della madrepora a cuscino (*Cladocora caespitosa*), sono state recentemente studiate nelle acque slovene. Nel presente lavoro vengono riportati nuovi dati relativi alle alghe coralline presenti al largo di Punta Ronco e Punta grossa. Le alghe coralline hanno un ruolo importante nello sviluppo e nel mantenimento delle concrezioni biogeniche, offrendo nicchie per invertebrati e altre alghe. Nel documento "Draft Lists of coralligenous/maërl populations and of main species to be considered by the inventory and monitoring" del RAC-SPA, le alghe coralline sono riportate come importanti organismi biocostruttori della biocenosi coralligena, meritevoli di ulteriori studi e di una appropriata protezione.*

**Parole chiave:** alghe coralline, formazioni biogeniche, circalitorale, Adriatico settentrionale

## INTRODUCTION

The shallow northern Adriatic area is dominated by muddy and sandy bottoms (Lipej *et al.,* 2006) and for a long time it was believed that these are the only bottoms existing in this basin. More than 200 years ago Giuseppe Olivi (1792) was the first to mention that in this part of the Adriatic Sea exist also rocky outcrops. Northern Adriatic fishermen have been familiar with this kind of environments before marine scientists discovered them, since they are rich fishing points, called *tegnùe* along the Venetian coast (Casellato *et al.*, 2006), and *trezze* in the Gulf of Trieste. Around 250 such rocky outcrops, calcareous bio-concretions, have been counted in the Italian part of the Gulf of Trieste, derived from the building action of calcareous organisms on hard substrata of diverse geological origins (Falace *et al.*, 2015). Similar formations were recently studied also in Slovenian marine waters (Lipej *et al.*, 2016). Currently, two major biogenic formations are known for the Slovenian Sea, which were sampled within the Interreg project TRECORALA. These biogenic formations are located off Cape Ronek and off Cape Debeli rtič. Both have substantially larger dimensions than *trezze* and are linked to the presence of Mediterranean stony coral (*Cladocora caespitosa*), since they are formed entirely by dead corallites of this species.

During a scientific meeting on the coralligenous environment, which took place at the Marine Biology Station in Piran in March 2011, the Italian, Croatian and Slovenian researchers suggested that the northern Adriatic forms of coralligenous environment, such as trezze, tegnùe, the precoralligenous in the infralittoral belt and biogenic formations of Mediterranean stony coral *C. caespitosa* (Linnaeus, 1767), should be recognized as a specific element within the Mediterranean coralligenous biocoenosis. The expression “biogenic formation” refers to any formations that are the result of limestone loading by some marine organisms, known as bioconstructors, during their lifetime. Among

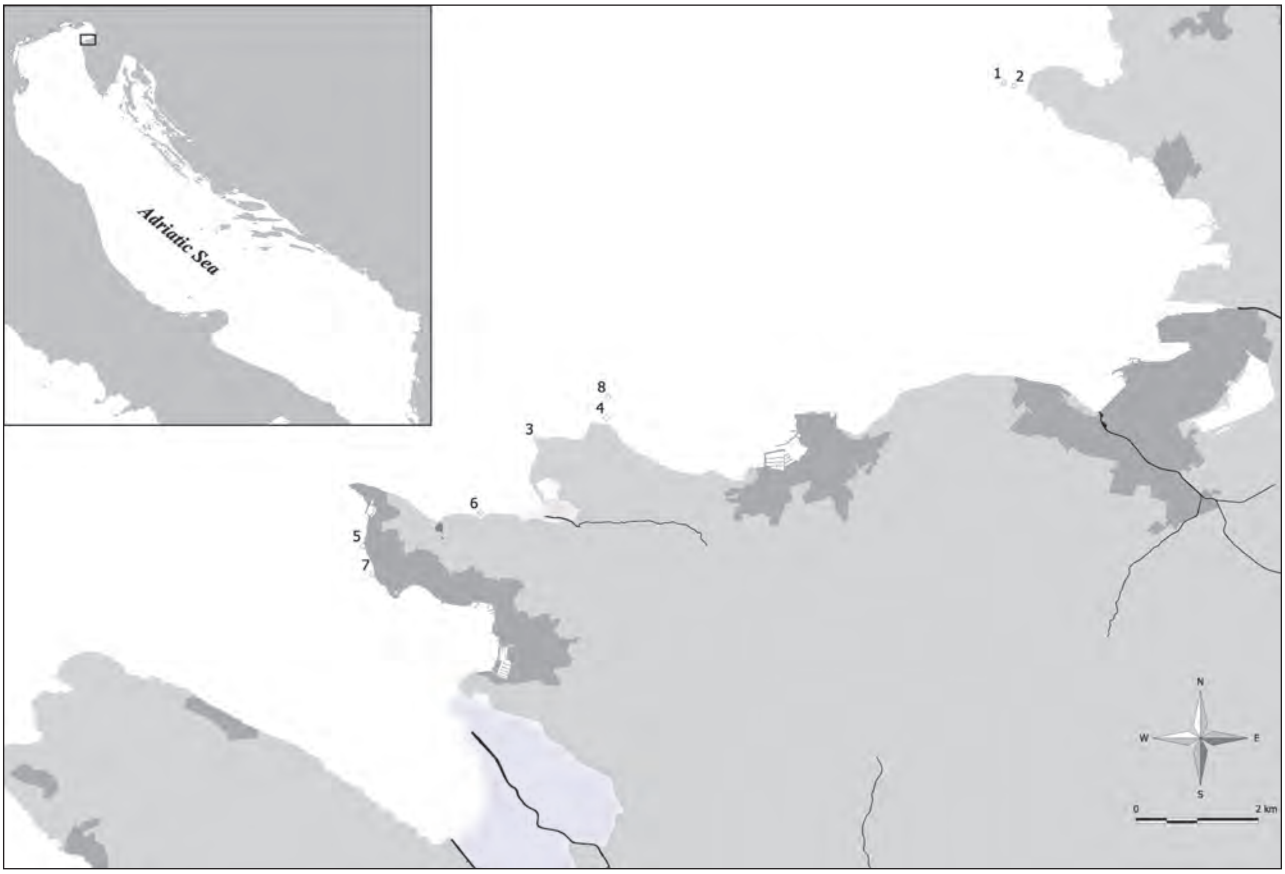

**Fig. 1: Locations with Mediterranean stony coral** *(Cladocora caespitosa)* **colonies in the Slovenian coastal sea. Legend: 1 - Biogenic formation at Debeli rtič; 2 - Cape Debeli rtič; 3 - Cape Strunjan; 4 – Cape Ronek; 5 – Pirančerk; 6 – Pacug; 7 – Bernardin; 8 - Biogenic formation at Ronek.**

**Sl. 1: Lokalitete s kolonijami sredozemske kamene korale** *(Cladocora caespitosa)* **v slovenskem morju. Legenda: 1 – Biogena formacija pred Debelim rtičem; 2 - Debeli rtič; 3 - rt Strunjan; 4 – rt Ronek; 5 – Piranček; 6 – Pacug; 7 – Bernardin; 8 – biogena formacija pred rtom Ronek.**

invertebrates the most well known bioconstructors are corals (Anthozoa), hydrozoans (Hydrozoa) and marine tube worms (Polychaeta Sedentaria) (Lipej *et al*., 2016). However, the precoralligenous and the coralligenous are primarily built by coralline algae (Laborel, 1961; Sartoretto, 1996). Among the eight action plans adopted by the Contracting Parties of the Barcelona Convention, one is devoted to the coralligenous habitat: the Action plan for the conservation of the coralligenous and other calcareous bio-concretions in the Mediterranean Sea, UNEPMAP-RAC/SPA (Ballesteros, 2008). Within this document, the coralligenous formations are considered a typical Mediterranean underwater seascape, comprising coralline algal frameworks that grow in dim light conditions and in relatively calm waters (Ballesteros, 2006).

Coralline algae are very important for the creation, development and maintenance of new niches for many invertebrates and other algae. Their habitat-building capacity is associated with the mechanism of mineralization of the cell wall with calcium and, to a lesser extent, magnesium carbonate. In red algae (Rhodophyta) from the family Corallinaceae, the carbonate is present in the crystalline calcite form mainly, while in red algae from the family Peyssonneliaceae and in green algae (Chlorophyta) from the family Halimedaceae it precipitates as aragonite. The occurrence of calcification helps to balance the carbon dioxide deficit in water, which is due to photosynthesis, and thus contributes to the maintenance of the alkaline potential in sea water (Andreoli *et al*., 2010).

Falace *et al*. (2011) reported on the presence of coralline algae in the Slovenian circalittoral belt, including the biogenic formation off Cape Ronek. The aim of the current paper is to report the first available data about the presence of coralline algal species on the biogenic formation located off cape Debeli rtič, and new data about their occurrence at Cape Ronek.

***Tab. 1: Density of Mediterranean stony coral colonies in various areas of the Slovenian Sea (adopted from Lipej* et al*., 2016).***
***Tab. 1: Gostota sredozemske kamene korale v različnih predelih slovenskega morja (prirejeno po Lipej in sod., 2016).***

| Locality | Density of *C. caespitosa* colonies (n/100m²) |
|---|---|
| Biogenic formation at Debeli rtič | 3 (2-4) |
| Cape Debeli rtič | 83 (70-96) |
| Cape Strunjan | 85 (66-105) |
| Cape Ronek | 108 |
| Piranček | 160 (128-192) |
| Pacug | 186 |
| Bernardin | 285 (263-306) |
| Biogenic formation at Ronek | 652 (498-806) |

## MATERIAL AND METHODS

### Study area

The biogenic formation at Cape Debeli rtič (Figs. 1 and 2) is more or less of triangular shape with a rounded plateau, which looks like a knob. On the reef there is a relatively steep step where the coastal sandy-rocky bottom sweeps into muddy sediment, which happens very quickly in comparison with the near surroundings. The biogenic formation is mostly covered by a thin layer of mud and, therefore, the accurate assessment of its borders was very difficult. It starts at about 10 m of depth and sweeps down to 17.5 m. This biogenic formation is mainly composed of dead Mediterranean stony coral corallites, with only a few living colonies (Lipej *et al*., 2016).

The biogenic formation at Cape Ronek is located outside ~~in~~ the waters off Strunjan Nature Reserve (Fig. 1). Its shape is elliptical, with the longest axis in the west-east direction, and the shortest in the north-south direction (Fig. 3). The highest point of the biogenic formation is at 12.4 m depth, and it extends down to 21 m, where it shifts into a muddy bottom. It is entirely composed of dead, broken corallites of Mediterranean stony coral. This solitary structure is surrounded on all sides by a muddy bottom, significantly less rich in biodiversity (Lipej *et al*., 2016). This formation is not covered by a surface layer of mud, with the density of living colonies of Mediterranean stony coral considerably higher than in other areas of the Slovenian Sea (see Tab. 1).

### Fieldwork and laboratory work

The surveys of coralline algae at biogenic formations were done in July 2013. Algae were randomly manually collected from the sea bottom, scraped when they were found attached, on a transect in a depth range from 10 m to 13 m at Cape Debeli rtič, and from 13 m to 16 m at Cape Ronek (Figs. 2 and 3). Samples were collected in plastic bags and all the material was transported to the laboratory of the Marine Biology Station of the National Institute of Biology. Algal samples were sorted in laboratory and fragments of material were air dried, mounted on aluminium stubs with acrylic adhesive and then analysed by scanning electron microscopy (SEM). Stubs were sonicated with a Vitec sonicator to remove sediments and diatoms and then coated with gold/palladium (with S150 Sputter Coater, Edwards) prior to viewing in a LEICA Steroscan 430i at 20 kV.

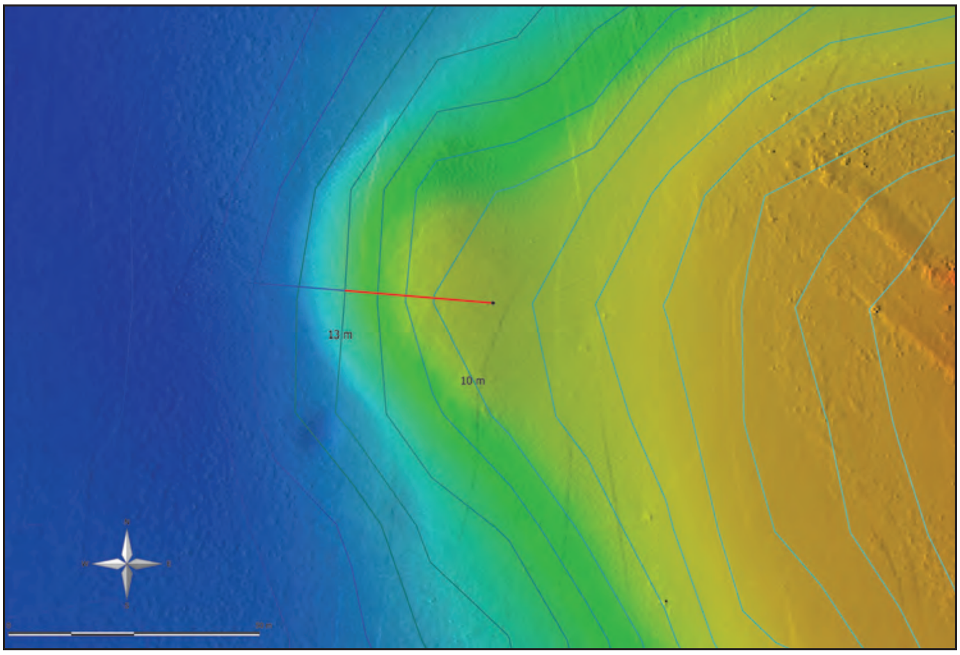

***Fig. 2: Image of biogenic formation at Cape Debeli rtič generated from multi-beam ecosounder data (photo: E. Gordini). Coralline algae were collected along the red transect.***
***Sl. 2: Slika biogene formacije pred Debelim rtičem, narejena na podlagi podatkov iz ehosonderja (avtor E. Gordini). Koraligene alge so bile nabrane vzdolž transekta, označenega z rdečo črto.***

## RESULTS AND DISCUSSION

Over the surveys performed in 2013, six species of coralline algae were found at the biogenic formation of Cape Debeli rtič, while seven were found at Cape Ronek (Table 2). In samples collected in 2010 by Falace *et al.* (2011) at the biogenic formation of Cape Ronek, *Neogoniolithon brassica-florida* (Harvey) Setchell & L.R. Mason and *Titanoderma pustulatum* (J.V. Lamouroux) Nägeli were missing. However, in those samples they recorded *Lithothamnion philippii* Foslie, *Pneophyllum confervicola* (Kützing) Y.M. Chamberlain and *Pneophyllum fragile* Kützing that were not found at Cape Ronek in 2013.

During the present study, encrusting thalli of *Lithothamnion sonderi* Hauck, *N. brassica-florida* and *Phymatolithon lenormandii* (Areschoug) W.H.Adey were collected at both biogenic formations. *P. fragile* was found as an epiphyte on dead Mediterranean stony coral corallites only at Cape Debeli rtič, where also few encrusting thalli of *P. confervicola* were collected. Conversely, *Lithophyllum racemus* (Lamarck) Foslie, *Lithothamnion minervae* Basso and *T. pustulatum* were found only at the biogenic formation of Cape Ronek; the first only as non living sub-globular thalli (rhodoliths), the second both as encrusting form and live rhodoliths, and the third only as encrusting thalli. The term "rhodolith" includes all biogenic excrescences where calcareous red algae represent at least 50% of the nodule, which consists of the coralline alga together with the substrate/core (Bressan & Babbini, 2003). The fact that thalli of *L. minervae* were found alive on all sides of the rhodolith proves that the structure is occasionally rolled by marine currents, representatives of the vagile fauna and/or anthropogenic activities such as fisheries, diving and anchorage.

So far 31 species of coralline algae were reported for the Slovenian sea (Falace *et al.,* 2011). Among the 10 species collected in 2013 (Table 2), four were found for the first time in this coastal area in 2010: *Lithothamnion minervae* Basso, *L. philippii*, *Lithothamnion sonderi* and *N. brassica-florida* (Falace *et al.,* 2011). However, all of them were previously recorded in the Italian part of the Gulf of Trieste. Several species of coralline algae found in Slovenian marine waters are listed as important builders of the coralligenous biocoenosis in the "*Draft Lists of coralligenous/maërl populations and of main species to be considered by the inventory and monitoring*" of the RAC-SPA (UNEP(DEPI)/MED WG.362/3, 2011). In the Mediterranean Sea, the coralligenous biocoenosis comprises at least 315 algal species (Boudouresque, 1973; Ballesteros, 2006). Among them, some species are bioconstructors (coralline algae), others bore holes into hard structures (particularly certain green algae

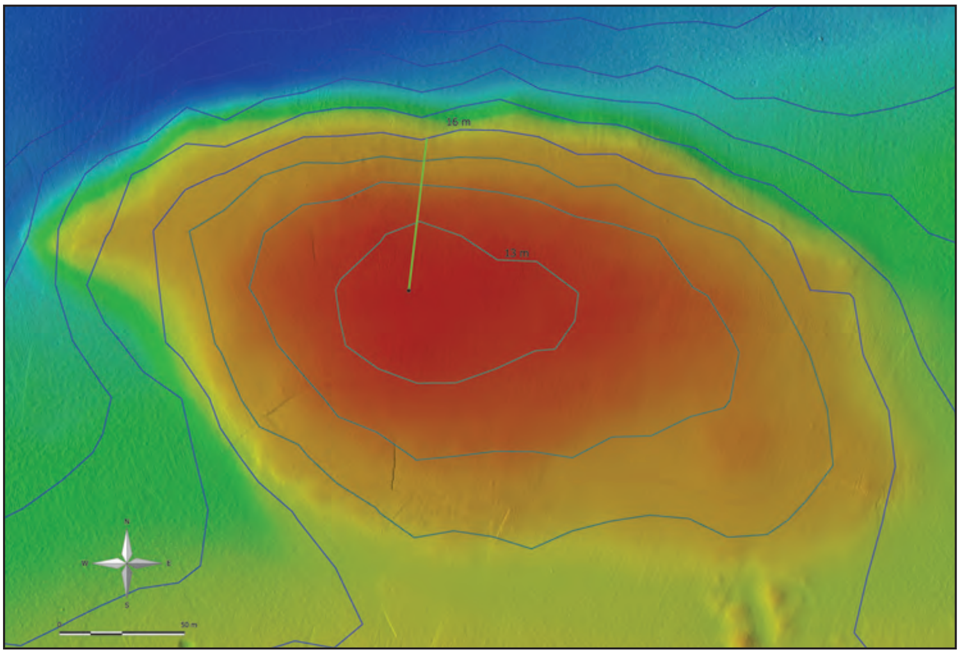

***Fig. 3: Image of biogenic formation at Cape Ronek generated from multi-beam ecosounder data (photo: E. Gordini). Coralline algae were collected along the green transect.***
***Sl. 3: Slika biogene formacije pred rtom Ronek, narejena na podlagi podatkov iz ehosonderja (avtor E. Gordini). Koraligene alge so bile nabrane vzdolž transekta, označenega z zeleno črto.***

and blue-green algae (Cyanobacteria)), and some are accompanying species, which include a number of exotic and invasive taxa (Andreoli *et al.*, 2010). The genus *Lithophyllum* is known to be the most species-diverse genus of coralline algae in the Mediterranean Sea and plays a key role in the formation of several widespread bioconstructions (Falace *et al.*, 2016). These taxa contribute with their growth to the construction of organogenic formations also in shallow northern Adriatic Sea (Bressan & Babbini, 2003; Bressan *et al.*, 2009; Giaccone *et al.*, 2009; Falace *et al.*, 2016). Among the 25 species of coralline algae reported for the northern Adriatic calcareous bio-concretions, *Lithophyllum incrustans* Philippi is one of the most important bioconstructors, in particular at the outcrops located at a depth of 23–25 m and at a distance ≥10 km from the coast (Falace *et al.*, 2015). Even though *L. incrustans* has been reported for the Slovenian area (Falace *et al.*, 2011) it was not observed at the biogenic formations near Cape Debeli rtič and Cape Ronek. Therefore, on the basis of the recent findings of coralline algae on biogenic formations in Slovenian waters, it is reasonable to expect that future researches in the area will reveal new species among algae and benthic invertebrates, as well.

In the light of the current (limited) knowledge about coralline algae, biogenic formations, and other infralittoral and circalittoral coralligenous environments in the Slovenian Sea, some recommendations can be made, according to Ballesteros (2003), for their conservation: a) prohibition of trawling in areas with coralligenous forms and their vicinity, to avoid both the physical damage of trawling and also the indirect effects due to increased turbidity and sedimentation rates; b) prohibition of other anthropogenic activities that lead to increased water turbidity and/or sediment removal (*e.g.* coastline modifications) in the vicinity of coralligenous forms; c) no waste water discharge in these areas; d) implementation of the management of traditional and recreational fisheries in order to prevent stock depletion of target species; e) controlled recreational diving pressures; f) urgent need for a protection law of coralligenous environments; g) further scientific research to increase the knowledge about biology and ecology of taxa inhabiting the coralligenous biocoenosis, to give a more accurate estimation of the coralligenous biodiversity.

## ACKNOWLEDGEMENTS

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***Tab. 2: Coralline algae found at biogenic formations at Cape Debeli rtič and Cape Ronek (* alive thalli, ** dead thalli). Data from 2010 were published in Falace* et al. *(2011).***

***Tab. 2: Koraligene alge, najdene na biogenih formacijah pred Debelim rtičem in rtom Ronek (* žive steljke, ** mrtve steljke). Podatki iz leta 2010 so bili objavljeni v delu Falace in sod. (2011).***

| **Location** | **Biogenic formation Cape Debeli rtič** | **Biogenic formation Cape Ronek** | **Biogenic formation Cape Ronek** |
|---|---|---|---|
| **Taxa/ Year** | **2013** | **2013** | **2010** |
| *Lithophyllum racemus* | | ** | ** |
| *Lithothamnion minervae* | | * | * |
| *Lithothamnion sonderi* | * | * | * |
| *Lithothamnion* sp. | * | * | |
| *Lithothamnion philippii* | | | * |
| *Neogoniolithon brassica-florida* | * | * | |
| *Phymatolithon lenormandii* | * | * | * |
| *Pneophyllum confervicola* | * | | * |
| *Pneophyllum fragile* | * | | * |
| *Titanoderma pustulatum* | | * | |

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# KORALIGENE ALGE NA BIOGENIH FORMACIJAH V SLOVENSKIH MORSKIH VODAH (SEVERNI JADRAN)

*Martina ORLANDO-BONACA, Borut MAVRIČ & Lovrenc LIPEJ*
Morska biološka postaja, Nacionalni Inštitut za biologijo, SI-6330 Piran, Fornace 41, Slovenija
E-mail: martina.orlando@nib.si

*Sara KALEB & Annalisa FALACE*
Department of Life Sciences, University of Trieste, I-34127 Trieste, Via L. Giorgieri 10, Italy

## *POVZETEK*

*Pred kratkim so raziskovalci v slovenskem morju pričeli z raziskavami dveh velikih biogenih formacij, ki jih sestavljajo mrtvi koraliti sredozemske kamene korale (*Cladocora caespitosa*). Avtorji poročajo o novih podatkih o navzočnosti koraligenih alg na biogeni formaciji pri Ronku in biogeni formaciji pred Debelim rtičem. Koraligene alge so zelo pomembne pri ustvarjanju, razvoju in ohranjanju apnenčastih tvorb in nudijo življenjske niše za mnoge nevretenčarje in druge alge. So pomembni gradniki koraligene biocenoze, navedene tudi v seznamu populacij koraligenih/maërl alg, ki jih je potrebno popisati in redno spremljati na podlagi priporočil RAC-SPA, zato bi jih bilo potrebno natančno raziskati in primerno zavarovati.*

**Ključne besede:** koraligene alge**,** biogene formacije**,** cirkalitoral, severni Jadran

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# POTENTIAL DISTRIBUTION OF SILVER FIR (*ABIES ALBA*) IN SOUTH-EASTERN ALPINE AND DINARIC PHYTOGEOGRAPHIC REGIONS OF SLOVENIA AND CROATIA IN THE LIGHT OF CLIMATE CHANGE

*Aljaž KOŽUH*
Pševo 9, Pševo, 4000 Kranj
e-mail: aljazeko@gmail.com

*Mitja KALIGARIČ*
Department of Biology, Faculty of Natural Sciences and Mathematics and Faculty of Agriculture and Life Sciences, University of Maribor, Koroška 160, Maribor, Slovenia
e-mail: mitja.kaligaric@um.si

*Danijel IVAJNŠIČ*
Department of Biology, Faculty of Natural Sciences and Mathematics, University of Maribor, Koroška 160, Maribor, Slovenia
e-mail: dani.ivajnsic@um.si

## *ABSTRACT*

*We studied the potential distribution of silver fir (*Abies alba *Miller) in the Alpine and Dinaric phytogeographic regions of Slovenia and Croatia in the light of climate change. A decline of silver fir in southern Europe due to summer droughts and heat has already been observed, along with the spread of its range towards the north-east in continental Europe due to a warmer climate with milder winters. In this study, we modelled habitat suitability for the silver fir in regard to the most probable climate change scenarios. No major changes in habitat suitability were found in either region. Habitat suitability should slightly increase in the central and western parts of the Alpine region in more optimistic scenarios and on Pohorje and in the Dinaric region in more pessimistic scenarios. A more distinctive change of habitat suitability would probably be suppressed by weather extremes, such as summer drought and heat, a cold winter period, and extreme weather phenomena.*

**Key words:** global warming, ecological modelling, habitat suitability, RCP, silver fir, species range change

# DISTRIBUZIONE POTENZIALE DELL'ABETE BIANCO (*ABIES ALBA*) NELLE REGIONI FITOGEOGRAFICHE ALPINA SUD-ORIENTALE E DINARICA IN SLOVENIA E CROAZIA IN RELAZIONE AI CAMBIAMENTI CLIMATICI

## *SINTESI*

*Gli autori hanno studiato la distribuzione potenziale dell'abete bianco (*Abies alba *Miller) nelle regioni fitogeografiche alpina e dinarica della Slovenia e della Croazia in relazione ai cambiamenti climatici. Una diminuzione dell'abete bianco nell'Europa meridionale, dovuta alla siccità e al caldo estivi, era già stata osservata, insieme all'espansione del suo areale verso nord-est nell'Europa continentale. In questo studio gli autori hanno modellato l'idoneità dell'habitat per l'abete bianco in relazione agli scenari più probabili di cambiamento climatico. Non è risultato alcun cambiamento importante nell'idoneità dell'habitat in nessuna delle due regioni. Secondo scenari più ottimistici, l'adeguatezza dell'habitat dovrebbe aumentare leggermente nelle parti centrale e occidentale della regione alpina, mentre secondo scenari più pessimistici dovrebbe ingrandirsi sul Pohorje e nella regione dinarica. Un cambiamento più distintivo dell'idoneità dell'habitat verrebbe probabilmente soppresso da condizioni meteorologiche estreme, quali la siccità estiva e il caldo, un freddo periodo invernale e fenomeni meteorologici estremi.*

**Parole chiave:** riscaldamento globale, modellistica ecologica, idoneità dell'habitat, RCP, abete bianco, cambiamenti dell'areale della specie

## INTRODUCTION

Recently, the spatial distributions of many plant species, among them trees, including the silver fir (*Abies alba*), have been noted to be much different from their natural distributions owing to several anthropogenic factors and influences. With this research we aimed to reveal the potential distribution of silver fir without anthropogenic influences in the Alpine and Dinaric phytogeographic regions of Slovenia and Croatia. Furthermore, we also aimed to determine differences between the two areas. Primarily, we tested the present natural habitat suitability for the silver fir of those two study areas by considering environmental variables and ecological modelling techniques. Finally, we examined future potential spatial distributions of the silver fir based on the four most likely future climate scenarios grounded on four representative concentration pathways (RCP) of greenhouse gases.

The silver fir (*Abies alba*) grows up to 50 m high and 2.5 m thick, an evergreen tree with coniform to oviform crown, flat branches, flat needles with white lines and upright cones. It blossoms from April to June (Brus & Robič, 2002). Its main growth period is around 50 to 60 days from May to July (Aussenac, 2002). The silver fir is mainly a European tree species (Brus & Robič, 2002). Its natural habitat is located mostly in the mountain regions of eastern, western, southern and central Europe (Anić *et al.*, 2009). It grows in the Alps, Vosges and Jura, on the Balkan Peninsula and in the Carpathians. There are also some isolated ranges on the Apennine Peninsula, Corsica, in the Massif Central and Pyrenees (Brus & Robič, 2002). Its range spreads between 40° and 52° in latitude (between Poland and northern Greece) and between 5° and 27° in longitude (between the western Alps and the Carpathians) (Anić *et al.*, 2009). In the Alpine and Dinaric regions it prospers between 400 and 1200 meters above sea level (Brus & Robič, 2002) in humid habitats with more than 1000 mm annual rainfall, and in the Mediterranean with average annual temperatures between 7 and 13 °C (Aussenac, 2002). It prefers fresh, deep and nutrient rich soils and is not sensitive to geological bedrock: it grows on carbonate or non-carbonate substrates (Brus & Robič, 2002) despite water accessibility being lower on carbonate (Ficko *et al.*, 2011). The silver fir grows at late succession phases, mostly in a community with the common beech (*Fagus sylvatica*) and the spruce (*Picea abies*) (Brus & Robič, 2002). However, it is a rather weak competitor and as such prospers only in a narrow gradient of environmental conditions. In most of the favourable areas for the silver fir, the beech is more successful in less and spruce in more extreme conditions (Ellenberg, 1988). Nevertheless, the silver fir is more competitive in shady forests with slower growth during the spring compared to the beech (Diaci *et al.*, 2010). It does not tolerate extreme winter cold and summer drought and heath (Gazol *et al.*, 2015; Koprowski, 2013). In the Mediterranean, its growth is limited mostly by low precipitation and water accessibility in spring and summer, while in central Europe its growth is limited due to low temperatures in late winter and early spring (Gazol *et al.*, 2015). Forest managers gave preference to coniferous rather than to deciduous trees (Ellenberg, 1988; Ficko *et al.*, 2011). The silver fir is more common and widespread in the Dinaric region than in the Alpine (Slovenian Forest Service, 2010). Young specimens of fir are frequently consumed and damaged by deer (Brus & Robič, 2002). It is therefore no surprise that the silver fir population size is negatively correlated with deer population size (Diaci *et al.*, 2010).

The global increase of greenhouse gas concentration and mean temperature is currently well documented (Ogrin, 2004). The $CO_2$ concentration has risen from 280 to over 400 ppm since 1750 (Anić *et al.*, 2009). Without anthropogenic emissions, it only rose by 20 ppm between the years 8000 and 2000 B.C. (Anić *et al.*, 2009). In the study area, the increase of average annual temperature and decrease of annual rainfall was recorded during the 20th century (Ogrin, 2004; ARSO, 2016). Similar trends are expected in the 21st century. Average annual temperature increased by 1 to 1.5 °C over the 20th century (Ogrin, 2004; Gazol *et al.,* 2015) and it should increase additionally by 1.5 to 6 °C according to different scenarios during the 21st century (Ogrin, 2004). The variability in precipitation patterns are even higher (Ogrin, 2004). During the summer, the precipitation amount is expected to decrease by 20% followed then by a 30% increase during the winter (Kutner & Kobler, 2011). However, on the annual scale, a 10% decrease of precipitation is expected (Anić *et al.*, 2009). It should be emphasized that extreme weather events (heat and cold waves, droughts, fires, irregular precipitation, etc.) are more and more frequent and intensive (Kutner & Kobler, 2011; ARSO, 2016) and significantly affect the silver fir populations.

The silver fir population in the Mediterranean is expected to decrease due to more intensive summer droughts, heat waves and fires (Gazol *et al.*, 2015). The beech-fir forests in the Dinaric region are expected to be gradually replaced by thermophile forests (Kutner & Kobler, 2011). In central Europe, the silver fir range extension towards the northeast is expected because of less extreme cold conditions in late winter and early spring, as well as its present range stability despite the possible competition with thermophile tree species (Ruosch *et al.*, 2016).

We presumed that altitude, annual mean air temperature and precipitation variables represent the most important natural determinants of the silver fir´s spatial distribution. Climate change should affect silver fir populations and its distribution in the study area especially in the lowlands. On the other hand, it is not expected that silver fir will spread to altitudes higher than its upper limit, because winter cold is still too severe in the high mountains (ARSO, 2016; Ogrin, 2004). From that perspective, we focused on three hypotheses: 1. there are more potentially suitable silver fir habitats in the Dinaric than in the Alpine region; 2. its range will move towards higher altitudes and will be narrower along this gradient; 3. Potential silver fir range will decrease especially in the Dinaric region because it also occupies mountain peaks.

## MATERIALS AND METHODS

### Study area

The Alpine phytogeographic region of Slovenia and the Dinaric phytogeographic region of Slovenia and Croatia were chosen as the study area (Fig. 1). The Alpine region of Slovenia contains Alpine geographic region of Slovenia with Julian Alps, Kamnik-Savinja Alps and the Karawanks (they also contain several mountain plateaus – Pokljuka, Jelovica, Menina, Velika Planina and Dobrovlje), which are a part of the Southern Limestone Alps; and Pohorje and Kozjak as part of the Central Alps. The Dinaric region of Slovenia contains mostly the Dinaric geographic region of Slovenia with poljes and Dinaric plateaus (Senegačnik, 2012). The north-western border are the plateaus of Banjšice and Trnovski gozd. At the border with Croatia it stretches from Snežnik at the west across Kočevski Rog to the mountain edge of Bela Krajina at the east. In Croatia we marked off the Dinaric region from the Slovenian border with Gorski Kotar at the north across the Velebit and its continental hinterland with poljes to the southern edge of Velebit at the south.

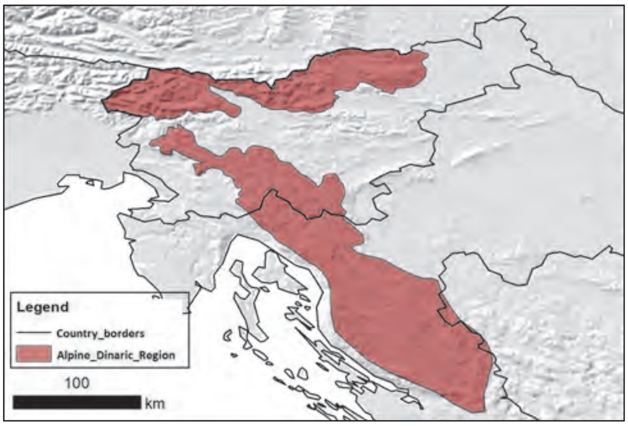

***Fig. 1: The chosen study area.***
***Sl. 1: Izbrano območje raziskave.***

***Tab. 1: Bioclimatic variables from the Worldclim database.***
***Tab. 1: Bioklimatske spremenljivke podatkovne baze Worldclim.***

| Symbol | Description |
|---|---|
| **BIO1** | Annual mean temperature |
| **BIO2** | Mean Diurnal Range (mean of monthly (max temp – min temp)) |
| **BIO3** | Isothermality (BIO2/BIO7) (*100) |
| **BIO4** | Temperature Seasonality (standard deviation *100) |
| **BIO5** | Max Temperature of Warmest Month |
| **BIO6** | Min Temperature of Coldest Month |
| **BIO7** | Temperature Annual Range (BIO5-BIO6) |
| **BIO8** | Mean Temperature of Wettest Quarter |
| **BIO9** | Mean Temperature of Driest Quarter |
| **BIO10** | Mean Temperature of Warmest Quarter |
| **BIO11** | Mean Temperature of Coldest Quarter |
| **BIO12** | Annual Precipitation |
| **BIO13** | Precipitation of Wettest Month |
| **BIO14** | Precipitation of Driest Month |
| **BIO15** | Precipitation Seasonality (Coefficient of Variation) |
| **BIO16** | Precipitation of Wettest Quarter |
| **BIO17** | Precipitation of Driest Quarter |
| **BIO18** | Precipitation of Warmest Quarter |
| **BIO19** | Precipitation of Coldest Quarter |

Alpine valleys stretch between 500 and 1000 m a.s.l., relief plateaus from 1000 to 1600 m a.s.l.; whereas the tree line extends up to 1900 m a.s.l., with the highest peaks reaching up to 1000 m above it. In the Dinaric region, poljes are distributed between 400 and 800 m a.s.l., plateaus between 800 and 1500 m a.s.l., and the highest peaks up to 1800 m a.s.l., thus stretching just above the tree line (Požar & Novak, 2005; Senegačnik, 2012). The average annual temperature of alpine plateaus is 2 to 6 °C, whereas on the Dinaric plateaus average temperatures are significantly higher and range from 4 to 7 °C. Annual rainfall on both considered regions reaches 1500 to 3000 mm (Zaninović *et al.*, 2008; ARSO, 2016).

### Collection of spatial data

Initially, silver fir spatial distribution data for the two phytogeographical regions in Slovenia and Croatia were gathered from the Slovenia Forest Service (Slovenia Forest Service, 2010; url: http://www.zgs.si/slo/gozdovi_slovenije/o_gozdovih_slovenije/karte/index.html) and the Flora Croatica database (Nikolić, 2015), which was established by the Faculty of Science in Zagreb (Url: https://hirc.botanic.hr/fcd/). Thereafter,

***Tab. 2: The considered future climate scenarios (RCP2.6, RCP4.5, RCP6.0, RCP8.5) according to CCSM global climate model.***

***Tab. 2: Upoštevani podnebni scenariji (RCP2.6, RCP4.5, RCP6.0, RCP8.5) po globalnem podnebnem modelu CCSM.***

| **Scenario** | **Solar radiation change (W/m²)** | **Increase of global annual temperature by year 2100 (°C) (variability)** |
|---|---|---|
| RCP2.6 | 2.6 | 1.0 (0.3 to 1.7) |
| RCP4.5 | 4.5 | 1.8 (1.1 to 2.6) |
| RCP6.0 | 6.0 | 2.2 (1.4 to 3.1) |
| RCP8.5 | 8.5 | 3.7 (2.6 to 4.8) |

bioclimatic (Worldclim 1.4; Hijmans *et al.*, 2005) and elevation data were considered as major contributions to potential future distribution of the silver fir under the selected climate change model (CCSM4) and four representative concentration pathways greenhouse gas scenarios (RCP2.6, RCP4.5, RCP6.0, RCP8.5 [Tab. 2]) until the end of the century (2070) (Tab. 1). The horizontal resolution of these geospatial datasets corresponds to 30 arc seconds (approximately 1 km² in mid latitudes).

The considered greenhouse gas (GHG) scenarios are named after possible changes of radiative forcing in the year 2100 relative to the preindustrial age (Meinshausen *et al.*, 2011). Scenario RCP2.6 anticipates a recent peak of emissions of GHG (between years 2010 and 2020); scenario RCP4.5 anticipates the GHG peak around 2040; scenario RCP6.0 around 2080; and finally, scenario RCP8.5 a continuous increase of GHG emissions until the end of the 21st century (Weyant *et al.*, 2009). Consequently, a global annual temperature increase is inevitable (Stocker et al., 2013) (Tab. 2).

## Spatial data processing and ecological modelling

The acquired spatial databases of silver fir distribution in the study area were unified (by leaning on the WGS84 coordinate system) and spatially filtered with ArcGIS software (ESRI, 2016). Selected environmental variables (bioclimatic and altitude) were extracted by using a background bias file (Barbet-Massin *et al.*, 2014). Additionally, all 19 bioclimatic variables were PCA transformed in order to avoid possible correlation of explanatory variables. The resulting first three components (BioPCA), explaining 87.5% of variability, together with altitude were considered using the habitat suitability modelling procedure.

In that light, the Mahalonobis Typicality species distribution modelling (SDM) approach within Idrisi Selva software (Clark Labs, 2015) was selected. This method is less sensitive to spatially auto-correlated occurrence data and is frequently being used to model plant distribution from the climate change perspective (Clark Labs, 2015). After completing the present scenario, the accuracy and reliability of the produced habitat suitability map was verified with ROC analysis and the resulting AUC value. The final processing of future environmental conditions, captured in future BioPCA components, gave us five habitat maps (present, and four future, RCP scenarios) for the silver fir in the study area. However, the continuous maps were simplified for easier interpretation into four suitability maps by applying the following thresholds: 1 = 0 – 25%, 2 = 25 – 50%, 3 = 50 – 75% and 4 = 75 – 100%. Finally, a comparative table summarizing the proportions of each suitability class within both phytogeographic regions was produced (Tab. 3)

## RESULTS

The ROC analysis results and the corresponding AUC value for the silver fir suitability in the study area by applying the Mahalonobis typicality model are shown

***Table 3: The proportion of potential habitat area for the silver fir in each phytogeographic region by considering four suitability thresholds and climate scenarios.***

***Tabela 3: Delež potencialnega habitata jelke po upoštevanih razredih ustreznosti in podnebnih napovedih na obravnavanih fitogeografskih območjih.***

| **Model** | **Alpine phytogeographic region** | | | | **Dinaric phytogeographic region** | | | | **Both regions together** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Habitat suitability (%)** | | | | **Habitat suitability (%)** | | | | **Habitat suitability (%)** | | | |
| | **0-25** | **25-50** | **50-75** | **75-100** | **0-25** | **25-50** | **50-75** | **75-100** | **0-25** | **25-50** | **50-75** | **75-100** |
| **Present** | 40 | 26 | 22 | 11 | 53 | 24 | 13 | 10 | 49 | 25 | 16 | 10 |
| **RCP2.6** | 40 | 23 | 24 | 13 | 54 | 25 | 15 | 7 | 50 | 24 | 18 | 9 |
| **RCP4.5** | 41 | 27 | 17 | 14 | 49 | 24 | 19 | 7 | 47 | 25 | 19 | 9 |
| **RCP6.0** | 39 | 26 | 21 | 14 | 40 | 27 | 26 | 8 | 39 | 27 | 24 | 9 |
| **RCP8.5** | 36 | 25 | 26 | 13 | 71 | 6 | 12 | 11 | 60 | 12 | 16 | 12 |

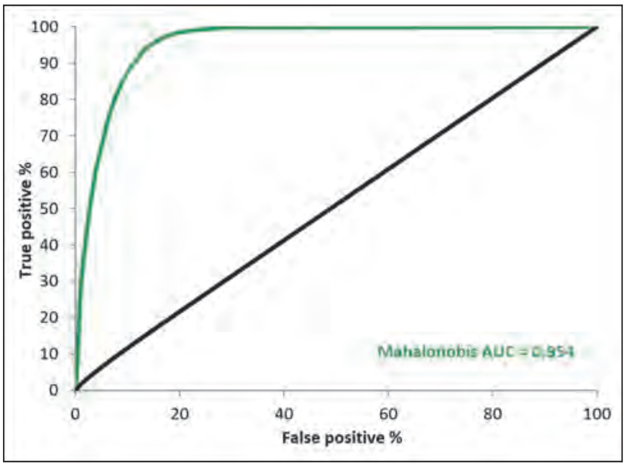

***Fig. 2: ROC analysis curve and the corresponding AUC value.***
***Sl. 2: Krivulja ROC analize in pripadajoča AUC vrednost.***

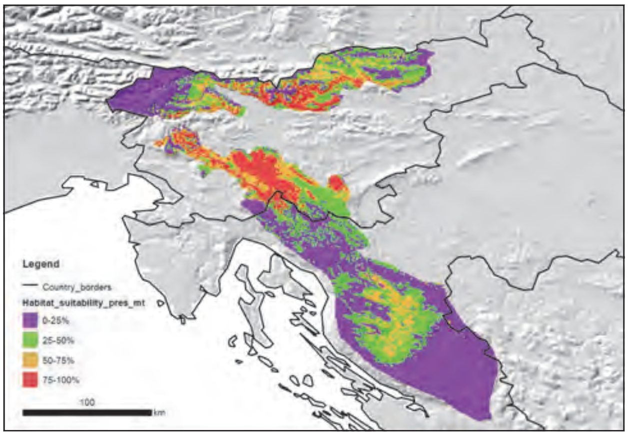

***Fig. 3: Recent habitat suitability for the silver fir*** *(Abies alba)* ***in the chosen Alpine and Dinaric phytogeographic regions.***
***Sl 3: Aktualna primernost jelke*** *(Abies alba)* ***v izbrani alpski in dinarski fitogeografski regiji.***

in Fig. 2. The curve for the model is steep and flattens quickly (Fig. 2), the AUC value is close to 1 (0.954) thus indicating a satisfactory level of agreement between occurrence data and predicted suitability.

Figure 3 shows the present silver fir´s (*Abies alba*) habitat suitability in the chosen Alpine and Dinaric phytogeographic regions, and figure 4 its future (year 2070) potential suitability according to considered GHG scenarios (RCP 2.6, 4.5, 6.0, 8.5) and the CCSM4 global climate model.

The present habitat suitability is the highest in Dinaric plateaus of Slovenia and Alpine plateaus and the middle mountain zone (Kamnik-Savinja Alps and the surrounding plateaus) (Fig. 3). Low suitability is detected in the western Julian Alps and in the Dinaric region of Croatia (Fig. 3). Quite distinctive contrasts are evident on both sides of the border (Fig. 3). The lowest suitability can be identified in the high mountains. The alpine valleys, the peak of Pohorje and several surrounding poljes exhibit low suitability as well (Fig. 3).

All future scenarios show common spatial features of the silver fir's potential habitat but differ in a few details (Fig. 4). They all show lower habitat suitability in the western Julian Alps and the southern part of the Dinaric region in Croatia (Fig. 4). The first scenario RCP2.6 is similar to the present one. The difference is noticeable in a somewhat lower habitat suitability on the Dinaric plateaus of Slovenia and higher on western Alpine plateaus of Slovenia (Pokljuka, Jelovica). The RCP4.5 scenario is the most similar to the present one. The only difference is a bit higher habitat suitability in the central Karawanks and a bit lower in the Trnovski gozd area. In comparison with RCP2.6 there is a bit higher silver fir habitat suitability on the Dinaric plateaus of Slovenia and a bit lower on Pokljuka and Jelovica (Fig. 4). The RCP6.0 scenario is similar to the present one and to RCP4.5. There is a bit lower suitability in the central part of the Dinaric region of Slovenia, whereas in Trnovski gozd it is similar to the present one. A noticeable difference of silver fir habitat suitability is in the eastern part of the Alpine region of Slovenia (eastern Karawanks, Pohorje) compared to other scenarios. There is a bit higher habitat suitability in the central part of the Dinaric region in Croatia as well (Fig. 4). The RCP8.5 scenario predicts the best conditions by the end of the century for the considered species mostly in Slovenia except in the western part of the Alpine region (Pokljuka, Jelovica, central Karawanks, western Kamnik-Savinja Alps) resulting in the highest habitat suitability. On the other hand, it is simultaneously the worst scenario for the Dinaric region of Croatia where there is very low habitat suitability almost throughout the whole region. This scenario assumes the highest contrast on both sides of the border (Fig. 4). Future scenarios also show a little tendency of potential habitat optimum shift from west to east in the Alpine region of Slovenia from less (RCP2.6) to the warmest scenario (RCP8.5) (Fig. 4).

The proportions of potential silver fir habitat area for each of the applied thresholds and considered regions separately and together are shown in Tab. 3. In the Alpine region, the proportions of the suitable area are similar considering all thresholds and RCP scenarios. However, in the 4th quartile of the potential habitat suitability a clear positive trend towards warmer climate conditions can be identified. In the Dinaric Region the largest potential habitat in the fourth class is predicted in the case of scenario RCP8.5. If climate scenarios RCP2.6, 4.5 or 6.0 are realized, the Dinaric region could be

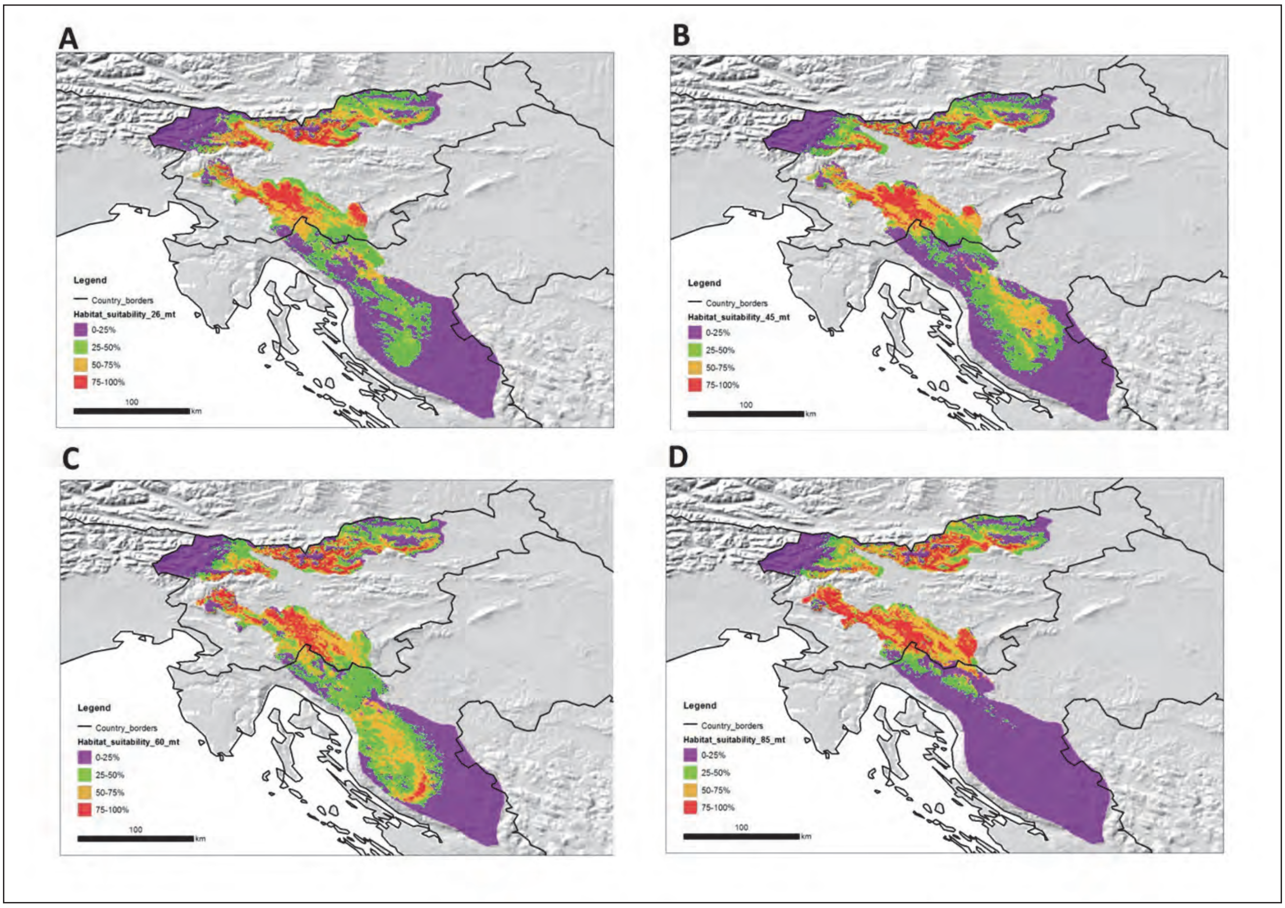

***Fig. 4: Potential habitat suitability for silver fir** (Abies alba) **for the year 2070 by four future climate scenarios (RCP2.6 [A], RCP4.5 [B], RCP6.0 [C], and RCP8.5 [D]) in the chosen Alpine and Dinaric phytogeographic regions.***
***Sl. 4: Primernost habitata jelke** (Abies alba) **leta 2070 po štirih prihodnjih klimatskih scenarijih (RCP2.6 [A], RCP4.5 [B], RCP6.0 [C], and RCP8.5 [D]) v izbrani alpski in dinarski fitogeografski regiji.***

occupied with less silver fir. Overall, high variability in silver fir potential habitat in the study area is assured in both - more optimistic and more pessimistic - thresholds considered.

## DISCUSSION

The influence of climate change on silver fir populations across Europe was already studied by Gazol *et al.* (2015). They outlined that in southwestern Europe, silver fir populations could decrease owing to increased aridity, but increase in the Continental temperate zone of central Europe due to climate warming. Ruosch *et al.* (2016) draw similar conclusions, predicting that silver fir range should decrease in southern Europe and spread northeast toward central Europe in future. They also predict that the present range should remain stable despite possible competition with thermophilous tree species. Kutner & Kobler (2011) tried to predict the change of forest vegetation in Slovenia by considering different climate scenarios with the use of ecological modelling. They calculated that the share of beech-fir forests will substantially decrease by the year 2100 and could be mostly replaced by thermophile forests. The coniferous forests with prevalent spruce and fir are expected to be replaced mostly by broadleaf forests. Koprowski (2013) tried to determine the response of silver fir growing outside its natural range concerning spring extreme weather phenomena in Poland. The higher March temperatures should stimulate silver fir growth especially in the western part of the study area, at the edge of continental plains with less spring frost and where colder winter periods are less pronounced. Anić *et al.* (2009) tried to reveal the influence of climate change on silver fir´s ecological niche in Croatia and proposed that the niche will gradually decrease in the 21st century because of temperature rise. Ficko *et al.* (2011) found that silver fir's range in Slovenia shifted towards cooler and more humid habitats over the last 40 years and slightly expanded.

Based on our results, none of the hypotheses can be completely proven. The first hypothesis about higher portion of suitable habitats in the Dinaric region can be at least partly proven, because the model, especially in Slovenia, shows more optimal potential habitats in the Dinaric compared to the Alpine region by applying the 4th quartile suitability threshold (75-100%) for the Mahalonobis probability distribution. Surprisingly, though the environment in Gorski Kotar is very similar to that on the Slovenian side of the border (Čavlović *et al.,* 2006; Kutnar & Kobler, 2011), the results show there much lower habitat suitability for the silver fir. However, the spatial pattern of the silver fir is heterogeneous; the tree grows in places like the Velebit and in its surroundings despite less favourable condition (Nikolić, 2015). The second hypothesis can neither be proven nor rejected because the model does not show any distinctive change in the Alpine region or the results are not distinctive enough to draw proper conclusions. In this case, a more accurate scale would be needed to adequately test this research question. The third hypothesis can be completely rejected, because the silver fir´s range will probably not decrease in the Dinaric region and could even increase, especially in the most pessimistic scenario, RCP8.5.

The results confirm the fact that silver fir is most common in the middle altitude (mountain) zone (Brus & Robič, 2002). Lower habitat suitability in the western and central Julian Alps might be the consequence of Mediterranean influence in the Soča valley (Ogrin, 2004). Thermophilic vegetation is also present there and could displace the silver fir (Kutner & Kobler, 2011), although all references do not confirm that (Ruosch *et al.*, 2016). However, climate conditions there are more variable; even though there are higher rates of precipitation and longer dry periods (ARSO, 2016). In the Dinaric region of Croatia the results show mostly low habitat suitability, but some scenarios (especially RCP6.0) still indicate better potential habitat suitability in the northern and central parts of the region. However, in the southern part all scenarios show mostly low potential habitat suitability. We could conclude that especially in Gorski Kotar, where the environment is currently similar to that on the Slovenian side of the border, potential habitat suitability is also similarly high. On the Velebit, especially its southern part and its continental hinterland, the habitats might actually be less suitable for silver fir today and still might be in the future. Maybe also the Mediterranean effect of summer droughts, heat and fires is and will be more distinctive there.

Future scenarios predict a lower share of optimal habitats in the Dinaric region in optimistic scenarios (RCP2.6 and RCP4.5) and higher in the most pessimistic (RCP8.5). In the Alpine region, all scenarios are similar; however, most of habitats of greater suitability are also shown by considering the RCP8.5 scenario. The Dinaric region could be placed in southern Europe and the Mediterranean, where the silver fir´s range should mostly decrease, especially because of hotter summers and more severe droughts (Aussenac, 2002; Kutnar & Kobler, 2011; Gazol *et al.*, 2015; Ruosch *et al.*, 2016). Such climate change consequences have already been spotted there (Anić *et al.,* 2009; Čavlović *et al.,* 2012; ARSO, 2016); but, surprisingly, our results do not confirm such a response of the silver fir. However, better potential habitat suitability for the silver fir in central Europe, where the Alpine region can be placed (Ruosch *et al.*, 2016), is confirmed but without significant change. That could be the consequence of a milder climate in the Alps, where more distinctive summer heat and droughts are not present yet (ARSO; 2016).

Finally, some restrictions and limitations regarding the research should be pointed out. We are aware that the bioclimatic variables of the *Worldclim* database are uncertain in some mountain areas, especially on geographically heterogeneous landscapes; this is why the results should be treated with some caution. Owing to more accurate data in Slovenia, the forecast of habitat suitability is probably much more representative. The exaggerated difference on both sides of the border, despite similar environmental conditions, is certainly not a representative result, but likely the consequence of unbiased spatial data or a highly variable spatial pattern of the considered species concerning the considered environmental predictors.

## CONCLUSIONS

The expected climate change could not have any distinctive influence on the silver fir distribution range. In western and central parts of the Alpine region, the optimistic future climatic scenarios predict somewhat more favourable conditions for the silver fir; in contrast, in the eastern part of its current range, in the Dinaric region, the pessimistic climate scenario (RCP 8.5) results in a more potentially suitable habitat area. Such results could be the consequence of higher mean air temperatures, but their favourable effect should probably be partly suppressed by more common and intensive weather extremes. Because of some research restrictions and limitations, the results can deviate from the actual expected state in the future.

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# POTENCIALNA RAZŠIRJENOST JELKE (*ABIES ALBA*) V JUGOVZHODNO-ALPSKEM IN DINARSKEM FITOGEOGRAFSKEM OBMOČJU SLOVENIJE IN HRVAŠKE V LUČI KLIMATSKIH SPREMEMB

*Aljaž KOŽUH*
Pševo 9, Pševo, 4000 Kranj
e-mail: aljazeko@gmail.com

*Mitja KALIGARIČ*
Department of Biology, Faculty of Natural Sciences and Mathematics and Faculty of Agriculture and Life Sciences, University of Maribor, Koroška 160, Maribor, Slovenia
e-mail: mitja.kaligaric@um.si

*Danijel IVAJNŠIČ*
Department of Biology, Faculty of Natural Sciences and Mathematics, University of Maribor, Koroška 160, Maribor, Slovenia
e-mail: dani.ivajnsic@um.si

## *POVZETEK*

*Zaradi vse bolj izrazitega vpliva klimatskih sprememb na vegetacijo smo s to raziskavo želeli ugotoviti njihov vpliv na potencialno razširjenost jelke (*Abies alba *Miller) v alpski in dinarski fitogeografski regiji na območju Slovenije in Hrvaške. Že danes je opazno krčenje areala jelke na južnem območju razširjenosti zaradi vse intenzivnejših poletnih suš in vročine v Sredozemlju ter širjenje areala proti severovzhodu zaradi toplejše klime in milejših zim kontinentalne Evrope. Preverjali smo primernost habitata za jelko s pomočjo ekološkega modeliranja za sedanje stanje in štiri najbolj verjetne prihodnje scenarije. Rezultati niso pokazali večjih sprememb v primernosti habitata v obeh regijah. Primernost habitata naj bi se nekoliko povečala, v osrednjem in zahodnem delu alpske regije ob bolj optimističnih scenarijih, na Pohorju in v Dinarski regiji pa ob bolj pesimističnih scenarijih. Izrazitejše izboljšanje primernosti habitata pa bodo najbrž vseeno zavrli vse intenzivnejši vremenski ekstremi, kot so poletna suša in vročina, zimski mraz in vremenske ujme.*

**Ključne besede:** ekološko modeliranje, jelka, globalno segrevanje, primernost habitata, RCP, sprememba areala

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# LE ORCHIDACEAE DI BALE-VALLE (ISTRIA, CROAZIA)

*Amelio PEZZETTA*
Via Monte Peralba 34 - 34149 Trieste
e-mail: fonterossi@libero.it

## *SINTESI*

*Bale-Valle è un Comune croato della costa sud-occidentale dell'Istria la cui superfice è di circa 82 Kmq. Nel presente lavoro, tenendo conto delle ricerche personali dell'autore, delle informazioni fornite da alcuni studiosi, dei dati ricavati da alcuni siti Internet e dei riferimenti bibliografici più recenti, è stato compilato un elenco floristico comprendente tutte le specie, le sottospecie e gli ibridi appartenenti alla famiglia delle Orchidaceae che sono presenti nel territorio comunale ed è stata fatta l'analisi corologica. Nel complesso sono segnalate 31 entità tra specie e sottospecie cui si aggiungono 10 ibridi. A sua volta l'analisi corologica evidenzia la prevalenza degli elementi mediterranei.*

**Parole chiave:** Orchidaceae, checklist comunale, Bale-Valle, Istria, Croazia

# THE ORCHIDS OF BALE-VALLE (ISTRA, CROATIA)

## *ABSTRACT*

*Bale-Valle is a Croatian municipality on the southwest coast of Istria, covering a surface of about 82 Kmq. This paper, taking into account the author's personal research, of the information provided by some researcher, data from some Internet sites and the most recent bibliographic references, lists all the members of the Orchidaceae family including hybrids, and provides a phytogeographical analysis. Overall, I found 31 species and subspecies, of orchids plus 10 hybrids. The phytogeographical analysis indicates the predominance of Mediterranean elements.*

**Key words:** Orchidaceae, municipal checklist, Bale-Valle, Istria, Croatia

## INTRODUZIONE

Bale-Valle (fino al 1945 Valle d'Istria), dal 1992 è un Comune censuario della Croazia (Cergna 2006) situato nell'Istria sud-occidentale che confina con Rovigno (Rovinj), Dignano (Vodnjan), Canfanaro (Kanfanar) e Sanvincenti (Svetvinčenat). La popolazione residente in base al Censimento del 2011 è di 1.129 abitanti mentre la superfice territoriale è di 82,06 Kmq. Oltre al centro comunale la popolazione vive sparsa in varie abitazioni e alcune frazioni: Černibek, Čubani, Golaš, Krmed, Pižanovac, Stancija Meneghetti e Sv. Bembo.

La densità di popolazione è molto bassa (meno di 14 abitanti per Kmq) e sino ad un recente passato, come nel resto della penisola istriana, gran parte del territorio vallese è stato utilizzato per pratiche agro-pastorali che hanno portato alla formazione di un paesaggio eterogeneo costituito da terreni aperti, in mosaico a formazioni boschive.

In tempi recenti il rapporto dell'uomo con il territorio è cambiato. Da un lato l'abbandono delle pratiche agro-pastorali tradizionali ha portato allo sviluppo di formazioni vegetali arbustive e a una ripresa spontanea del processo di riforestazione. Dall'altro lo sviluppo di forme di agricoltura intensiva ha ridotto i terreni aperti. Lungo la costa invece, la realizzazione di strutture turistico-ricreative ha contribuito a ridurre gli spazi naturali. A causa di ciò nell'ambito in esame il paesaggio è molto vario e si osserva un mosaico che associa insieme strutture turistiche, centri abitati, case sparse (molte delle quali chiamate localmente "*Stancija*"), infrastrutture stradali e di altro tipo, boschi, radure, terreni incolti e altri coltivati (generalmente oliveti e in minor misura seminativi, vigneti e altro).

### La geologia

Il Comune di Bale-Valle è attraversato dal 45° parallelo e quindi si trova a metà strada tra il polo nord e l'equatore. Il suo territorio occupa una linea di costa sassoso-ghiaiosa dalla lunghezza complessiva di circa 5 Km ed è caratterizzato da un altopiano calcareo leggermente ondulato in cui si elevano colline più o meno rotondeggianti che raggiungono l'altitudine massima di 244 m s.l.m. Esso è compreso nella cosiddetta "Istria rossa", una parte della penisola istriana costituita da diversi altipiani divisi tra loro da profondi solchi vallivi che è situata a sud di una linea spezzata che con diverse angolazioni collega Salvore (Savudrija) con Buie (Buje), Montona (Motovun), Pisino (Pazin) e il vallone di Fianona (Plomin). È cosi chiamata poiché in tale ambito prevalgono rocce calcaree ricoperte da terreni di colore rossastro con uno spessore compreso tra 2 e 7 metri che contengono silicati, ossidi di ferro, d'alluminio e altri materiali insolubili. La particolare colorazione è la conseguenza di un processo di rubefazione che avviene in superfice e porta alla formazione di vari tipi di ossidi e idrossidi ferrosi e ossidi di manganese (Merlak, 2014).

Il territorio vallese in particolare, è molto semplice essendo costituito da vari tipi di rocce calcaree databili al Cretaceo (Forti, 1988: 89; Alberi, 1997: 1568). In tale ambito non scorrono corsi d'acqua superficiali poiché a causa della natura permeabile del terreno, le precipitazioni s'infiltrano nel sottosuolo.

Un'interessante caratteristica del luogo è costituita dal ritrovamento avvenuto all'inizio degli anni '80 nel fondo del mare che bagna le coste comunali, di ossa fossilizzate di dinosauro risalenti all'Hauteriviano superiore-Barremiano inferiore, un periodo geologico compreso tra i 135 e i 129 milioni di anni fa (Boscarolli & Dalla Vecchia, 1999).

### Il clima

A Valle non esiste una stazione meteorologica che registri l'andamento delle precipitazioni e delle temperature e di conseguenza, i dati che si riportano sono valori medi registrati in più stazioni dell'Istria o quelli di località vicine quali Pola e Rovigno. In generale si può sostenere che tutto il territorio dell'Istria meridionale è caratterizzato dal clima mediterraneo con inverni miti, le stagioni estive generalmente lunghe e secche e le precipitazioni concentrate nel resto dell'anno.

Analizzando in dettaglio i valori delle temperature, Gorlato (1997) fa presente che la temperatura media annua lungo la fascia costiera istriana raggiunge i 14 °C. Le recenti osservazioni confermano tali dati e in particolare dimostrano che a Pola e Rovigno si registrano temperature medie annue di 14°C e 13,6°C (Zaninović *et al.*, 2008). In particolare a Pola: la media dei valori minimi si registra nel mese di gennaio e si aggira attorno a 6°C; la media dei valori massimi di temperatura si registra tra luglio e agosto e si aggira tra 25-26°C; la media delle precipitazioni nel periodo 1961-1990 è stata di 848 mm mentre nel periodo 2011-2013 di 722 mm (IDEOPLAN, 2015). Di solito il periodo più piovoso va da ottobre a novembre.

I venti dominanti sono: la bora, lo scirocco, il libeccio, il levante, il ponente e il maestrale. Altri venti con minore frequenza giungono da vari quadranti mentre alcuni locali tra cui le brezze, sono causati dalle escursioni termiche diurne e da fattori topografici di dettaglio.

### Il paesaggio vegetale

Le peculiarità geografiche del territorio, l'andamento climatico, le vicende storico-geologiche e la pressione antropica attuale e del passato si riflettono sul paesaggio vegetale e sulle sue particolarità floristiche e fitogeografiche. Le principali tipologie vegetali che si rinvengono nel territorio vallese sono le seguenti:

- ambiti di macchia mediterranea misti con sclerofille e caducifoglie;
- radure prative e prati-pascolo secondari inquadrabili in varie associazioni vegetali tra cui:

*Chryspogono-Euphorbietum nicaensis* Horvatić e *Danthonio -Scorzoneretum villosae* Horvatić;
- associazioni vegetali sinantropiche che attecchisco nei pressi dei campi coltivati, dei centri abitati, dei terreni incolti, le cave abbandonate, delle abitazioni sparse e dei bordi stradali;
- formazioni tipiche degli affioramenti rocciosi con *Saxifraga trydactilites L.*, varie specie di *Sedum*, etc.
- formazioni arbustive che lentamente stanno occupando pascoli e terreni abbandonati ed alla cui composizione, come osservato dallo scrivente, concorrono: *Carpinus orientalis* Mill., *Colutea arborescens* L., *Cornus mas* L., *Cornus sanguínea* L., *Coronilla emerus* L., *Erica arborea* L, *Ligustrum vulgare* L, *Juniperus oxycedrus* L., *Paliurus spina-christi* Mill., *Prunus spinosa* L., *Rosa canina* L, *Rosa sempervirens* L., *Ruscus aculeatus* L., *Smilax aspera* L., *Spartium junceum* L., vari tipi di *Cistus* L., *Rubus* L., etc.;
- formazioni di bosco submediterraneo con *Fraxinus ornus* L. *Ostrya carpinifolia* Scop., *Quercus pubescens* Willd. ed altre essenze arboree.

L'ambito litoraneo è caratterizzato dal bosco misto mediterraneo (*Orno-Quercetum-ilicis* Horvatić) che inizia a insediarsi a poche decine di metri dalla linea di battigia e si protrae sino ad alcuni Km dal mare. Questa particolare associazione vegetale è diffusa lungo le coste orientali adriatico-ioniche dalla Grecia sino al Golfo di Trieste ove raggiunge il limite settentrionale di distribuzione geografica (Poldini et al. 1980). Alle specie caratteristiche quali il leccio (*Quercus ilex* L.) e l'orniello (*Fraxinus ornus*) nel territorio in esame si accompagnano: *Anemone hortensis* L., *Arbutus unedo* L., *Asparagus acutifolius* L., *Clematis flammula* L., *Cyclamen repandum* Sibth & Sm., *Dorycnium hirsutum* (L.) Ser., *Lonicera etrusca* Santi, *Phillyrea latifolia* L., *Pistacia lentiscus* L., *Pistacia terebinthus* L., *Rosa sempervirens* L., *Rubia peregrina* L., *Viburnum tinus* L., etc. (Šugar, 1985).

Man mano si penetra verso l'interno, i parametri termici si abbassano, le infiltrazioni di essenze caducifoglie si accentuano e l'*Orno-Quercetum-ilicis* è sostituito dal bosco carsico sub-mediterraneo con la sua principale tipologia: *l'Ostryo-Quercetum pubescentis* (Ht.) Trinajstić dominato da *Fraxinus ornus*, *Ostrya carpinifolia* e *Quercus pubescens*. Inoltre, in diverse parti, a causa dell'azione antropica, l'*Orno-Quercetum-ilicis* è sostituito anche da: 1) prati-pascolo secondari e radure erbose più o meno vaste spesso ricche di orchidacee; 2) formazioni miste arboreo-arbustive e arbustive.

## MATERIALI E METODI

L'elenco floristico comprende le specie, le sottospecie e gli ibridi mentre non sono state prese in considerazione le varietà cromatiche e morfologiche. Esso è stato realizzato tenendo conto delle ricerche sul campo dell'autore, delle informazioni personali fornite da Remy Souche e Herbert Weyland e infine dei dati ricavati da:
- il sito internet della SFO- PCV (Societè Francaises d'Orchidophilie);
- le ricerche di Průša & Šmiták (2008) e Jelinec (2014) pubblicate sul sito internet denominato Orchidea klub Brno;
- la consultazione dei saggi dei seguenti autori: Biel (2001), Delforge (2006), Griebl (2009); Hertel & Hertel (2002), Hertel et al. (2016), Jakely (2016), Kranjčev (2005), Paulus (2000, 2014), Pericin (2001), Pezzetta (2016), Rottensteiner (2015, 2016) e Weyland (2010, 20113a)**.**

Le prime estemporanee e personali osservazioni nell'ambito di studio iniziarono circa venti anni fa e annualmente si sono protratte durante la stagione primaverile. Le stazioni in cui lo scrivente ha fatto dei ritrovamenti sono contrassegnate dai loro nomi con l'aggiunta del punto esclamativo. In tale sede sono state inserite in bibliografia gli studi più recenti che vanno dagli ultimi decenni del secolo scorso all'attualità. Accanto ad ogni taxon sono riportati: il tipo corologico, gli autori che l'hanno segnalato, le località di presenza e le eventuali osservazioni sul rango tassonomico.

Per la nomenclatura si è in genere seguita quella adottata nel recente volume del GIROS (2016) mentre per le specie non riportate in tale testo Delforge (2016) e/o nel caso di nuovi ritrovamenti i nomi assegnati alle singole piante dai loro autori. In diversi casi, alla nomenclatura sono state aggiunte varie precisazioni riportate nelle osservazioni e nelle considerazioni sui vari taxa dell'elenco floristico. Per l'assegnazione dei tipi corologici si è tenuto conto di quanto riportato in Pignatti (1982), Pezzetta (2011) e Delforge (2016).

## RISULTATI E DISCUSSIONE

### Elenco floristico

Nell'elenco sotto riportato al fine di non ripetere troppe volte gli stessi nomi, si è deciso di utilizzare le seguenti sigle costituite da lettere maiuscole che si riferiscono agli autori delle segnalazioni:

AX: PAULUS 2000; AY: BIEL 2001; BX: PERICIN 2001; BY: HERTEL & HERTEL 2002; CX: KRANJČEV 2005; CY: DELFORGE 2006; DX: PRŮŠA & ŠMITÁK 2008; DY: GRIEBL 2009; EX: WEYLAND 2010; EY: WEYLAND 2013a; FX: JELINEC 2014; FY: PAULUS 2014; GX: ROTTENSTEINER 2015; GY: HERTEL ET AL. 2016; HX: JAKELY 2016; HY: PEZZETTA 2016; IX: ROTTENSTEINER 2016; LX: SFO-PCV; LY: SOUCHE informazione personale;

MX: WEYLAND informazione personale.

1. *Anacamptis coriophora* (L.) R.M. Bateman, Pridgeon & M.W. Chase subsp. *fragrans* (Pollini) R.M. Bateman, Pridgeon & M.W. Chase – Euri-

mediterraneo. (AY, BY, CX, DY, LY). Stazione di rinvenimento: Valle!.

2. *Anacamptis morio* subsp. (*morio* L.) R.M. Bateman, Pridgeon & M.W. Chase - Europeo-Caucasico. (AY, BX, BY, CX, CY, DX, DY, FX, GX, LX, LY).Stazioni di rinvenimento: Golaš!, Krmed!, Stancija Golaš!, Stancija Negrin, Sv. Bembo, Valle!, Sono state ricondotte al taxon tutte le segnalazioni delle subsp. *caucasica* e *picta*.
3. *Anacamptis papilionacea* (L.) R.M. Bateman, Pridgeon & M.W. Chase - Eurimediterraneo. (AY, BY, CX, CY, DX, DY, FX, LY). Stazioni di rinvenimento: Golaš!, Stancija Golaš!, Sv. Bembo, Valle!.
4. *Anacamptis pyramidalis* (L.) Rich. subsp. *pyramidalis* – Eurimediterraneo. (AY, BY, CX, DY, EX, FX, FY, LX, LY). Stazioni di rinvenimento: Golaš, Stancija Golaš!, Sv. Bembo!, Valle!. Sono state ricondotte al taxon tutte le segnalazioni di *Anacamptis pyramidalis* subsp. *serotina* Presser.
5. *Cephalanthera longifolia* (L.) Fritsch – Eurasiatico. (MX). Stazione di rinvenimento: Valle.
6. *Gymnadenia conopsea* (L.) R. Br. in W.T. Aiton susbp. *conopsea* – Eurasiatico. (DX). Stazione di rinvenimento: Valle.
7. *Himantoglossum adriaticum* H. Baumann – Eurimediterraneo. (BY, GX, LY). Stazioni di rinvenimento:!, Sv. Bembo, Valle!.
8. *Limodorum abortivum* (L.) Sw. – Eurimediterraneo. (AY, CX, DX, DY). Stazioni di rinvenimento: Golaš, Valle,
9. *Neotinea maculata* (Desf.) Stearn - Mediterraneo-Atlantico. (BY). Stazione di rinvenimento: Valle.
10. *Neotinea tridentata* (Scop.) R.M. Bateman, Pridgeon & M.W. Chase – Eurimediterraneo. (BY, CX, DX, DY, FX, GX, LY). Stazioni di rinvenimento: Golaš!, Stancija Golaš!, Stancija Negrin, Valle.
11. *Neottia nidus-avis* (L.) Rich. – Eurasiatico. (AY). Stazioni di rinvenimento: Golaš, Valle.
12. *Ophrys apifera* Huds. – Eurimediterraneo. (AY, BY, CX, DX, DY, FX, LY). Stazioni di rinvenimento: Golaš, Stancija Golaš!, Valle!.
13. *Ophrys bertolonii* subsp. *bertolonii* Moretti –Appennino-Balcanico. (BY, HX, IX, LY). Stazioni di rinvenimento: Valle!.
14. *Ophrys holosericea* (Burm. f.) Greuter subsp. *holosericea*. – Eurimediterraneo. (BY, IX, LY). Stazioni di rinvenimento: Valle!.
15. *Ophrys holosericea* (Burm. f.) Greuter subsp. *tetraloniae* (W.P. Teschner) Kreutz - Appennino-Stazione di rinvenimento: Valle!. Il taxon nuovo per il Comune di Valle, ha nel Comune di Buzet e quindi in Istria, il suo locus classicus ove fu descritto da TESCHNER (1987).
16. *Ophrys holosericea* (Burm. f.) Greuter *subsp. untchjii (*M. Schulze) Kreutz – Subendemico. (CY, DY, EX, EY, FY, GX, IX, LX, LY). Stazioni di rinvenimento: Golaš, Stancija Negrin, Sv. Bembo, Valle!.
17. *Ophrys illyrica* S. Hertel & K. Hertel – Appennino-Balcanico. (BY, DY, EX, HY, LY). Stazioni di rinvenimento: Sv. Bembo, Valle!.
18. *Ophrys incubacea* Bianca subsp. *incubacea* – Stenomediterraneo. (AY, BY, CY, DX, DY, EX, FX, LX). Stazioni di rinvenimento: Golaš, Stancija Golaš, Sv. Bembo, Valle!.
19. *Ophrys istriensis* Hertel, Paulus & Weyland – Endemico. (EY, FY, GY, IX, LY). Stazioni di rinvenimento: Sv. Bembo, Stancija Golaš!, Valle!. Sono state ricondotte al taxon tutte le segnalazioni di *Ophrys* aff. *parvimaculata*.
20. *Ophrys sphegodes Mill.* subsp. *incantata* Devillers & Devillers-Tersch. – Endemico. (CY, LX). Stazioni di rinvenimento: Golaš, Valle!
21. *Ophrys sphegodes* subsp. *sphegodes* Mill. – Eurimediterraneo. (AY, CX). Stazioni di rinvenimento: Golaš, Valle!.
22. *Ophrys sphegodes* subsp. *tommasinii (*Vis.) Soó. – Appennino-Balcanico. (BY, CY, EX, LX). Stazioni di rinvenimento: Golaš, Stancija Golaš!, Valle!
23. *Ophrys sulcata*. Devillers-Tersch. & P. Devillers – Mediterraneo-Occidentale. (AX, BY). Stazioni di rinvenimento: Valle. Secondo Romolini (2002) la specie va assegnata a *O. funerea* Viv. Il taxon in Istria raggiunge il limite orientale di distribuzione geografica.
24. *Orchis pauciflora* Ten. – Stenomediterraneo. (BY, CX, EX, FY, GX). Stazione di rinvenimento: Valle!.
25. *Orchis provincialis* Balb. Ex Lam. – Stenomediterraneo. (BY, DY, EX, FY). Stazione di rinvenimento: Valle!.
26. *Orchis purpurea* Huds. – Eurasiatico. (BY, FX). Stazioni di rinvenimento: Krmed!, Valle!,.
27. *Orchis simia* Lam. – Eurimediterraneo. (BY, DX). Stazione di rinvenimento: Golaš!, Valle!.
28. *Platanthera chlorantha* (Custer) Rchb. – Eurosiberiano. (BY). Stazione di rinvenimento: Valle.
29. *Serapias lingua* L. – Stenomediterraneo. ( HX, LY). Stazione di rinvenimento: Valle!.
30. *Serapias vomeracea* (Burm.f.) Briq. subsp. *vomeracea* – Eurimediterraneo. (BY, EX). Stazione di rinvenimento: Valle.
31. *Spiranthes spiralis* (L.) Chevall. – Europeo-Caucasico. (BY). Stazione di rinvenimento: Valle.

### Ibridi

1. *Anacamptis ×gennarii* (Rchb. f.) Nazzaro & La Valva. (BY, CY, IX). Stazioni di rinvenimento: Golaš, Stancija Negrin, Sv. Bembo, Valle!.
2. *Ophrys bertolonii* × *O. illyrica* (HY, LY). Stazione di rinvenimento: Valle!.

3. *Ophrys bertolonii* × *O. istriensis* (IX come *Ophrys bertolonii* × *O.* cf. *parvimaculata*). Stazione di rinvenimento: Valle.
4. *Ophrys bertolonii* × O. *untchjii*. (HY). Stazione di rinvenimento: Valle!.
5. *Ophrys illyrica* × *O. tommasinii* (DY, EX). Stazione di rinvenimento: Valle.
6. *Ophrys illyrica* × *O. untchjii*. (DY, EX, LY). Stazione di rinvenimento: Valle.
7. *Ophrys incubacea* × *O. tommasinii* (DY). Stazione di rinvenimento: Valle.
8. *Ophrys incubacea* × O. *untchjii*. (HY).Stazione di rinvenimento: Valle.
9. *Ophrys* ×*lyrata* H. Fleischm. (*O. bertolonii* × *O. incubacea*) (BY, DY). Stazione di rinvenimento: Valle! Il taxon ha il suo locus classicus nell'isola di Lussino (Lošinj) in cui Fleischmann (1904) lo rinvenne e descrisse per la prima volta.
10. *Ophrys* ×*mansfeldiana* Soó (*O. incubacea* × *O. tommasinii)* (BY, DY). Stazione di rinvenimento: Valle.
11. *Orchis* ×*aurunca* W. Rossi & Minut. (*O. pauciflora* × O. *provincialis*) (DY, EX, FY). Stazione di rinvenimento: Valle!.

Nell'elenco floristico sono riportati 31 taxa infragenerici, corrispondenti a circa il 38,75 % del patrimonio orchidologico istriano che secondo Pezzetta (2013) ammonta a 80 taxa. Al loro insieme si aggiungono 11 ibridi e pertanto il numero complessivo dei taxa presenti è di 42. Tali numeri dimostrano l'importanza del patrimonio orchidologico dell'ambito di studio. Da ricerche sinora inedite dello scrivente, inoltre risulta che il Comune di Valle è tra i più ricchi di orchidacee della penisola istriana.

I seguenti taxa dell'elenco sono riportati nella lista rossa della flora croata (Vitasović Kosić *et al.*, 2009): *Anacamptis laxiflora*, *A. morio*, *A. papilionacea, A. pyramidalis*, *Neotinea tridentata*, *Ophrys apifera*, *O. bertolonii*, *O. bombyliflora*, *O. holosericea s.l.*, *O. insectifera*, *Orchis coriophora s.l.*, *O. mascula s.l.*, *O. militaris*, *O. provincialis*, *O. purpurea*, O. *simia*, *Platanthera chlorantha* e *Serapias vomeracea*.

Le specie e sottospecie comprese nell'elenco si ripartiscono in 11 generi e di questi il più rappresentato è il genere *Ophrys* con 11 taxa. Seguono: *Orchis* e *Anacamptis* con 4 taxa ciascuno, *Neotinea* e *Serapias* con 2 e poi tutti gli altri con un solo taxon. Alcuni taxa compresi nell'elenco, come si è potuto osservare, sono caratterizzati da alcune criticità. Ciò è la conseguenza del fatto che i ricercatori adottano criteri di classificazione e concetti di specie diversi cui seguono risultati di ricerche discordanti e non unanimemente condivisi (Paulus 2000, Hertel & Hertel 2002, Delforge 2006).

Nell'ambito in esame ha una certa importanza per la sua criticità *Ophrys sphegodes* subsp. *sphegodes* che in base alle ricerche dello scrivente nell'intera penisola istriana presenta caratteristiche morfologiche variabilissime, un fatto che ha contribuito ad assegnare i suoi popolamenti a taxa diversi il cui rango tassonomico è controverso. Tale osservazione è confermata anche da altri ricercatori tra cui Hertel & Hertel (2002, 2003) e Weyland (2013b). Secondo Devillers & Devillers-Terschuren (2004) e Delforge (2006) tutte le segnalazioni di *O. sphegodes* subsp. *sphegodes* fatte nelle zone mediterranee della Croazia devono essere attribuite ad altri taxa. Nel gruppo è discusso il rango tassonomico di *O. sphegodes* subsp. *incantata* descritta da Devillers & Devillers-Terschuren (2004) con locus classicus a Primosten (Dalmazia). Gli autori che l'hanno descritto e Delforge (2016) le attribuiscono lo status di specie, mentre per Hertel & Zirnsack (2006) il taxon deve considerarsi sinonimo di *Ophrys tommasinii.* Rispetto ad *O. tommasinii*, *O. incantata* è caratterizzata da una fioritura più precoce e a tal proposito Delforge (2016) fa presente che appartiene alla prima fase di fioriture delle specie del gruppo di *Ophrys sphegodes* a piccoli fiori. Ad avviso dello scrivente la fioritura precoce è un aspetto dell'isolamento riproduttivo che porta alla formazione del nuovo taxon ma i caratteri morfologici di *O. sphegodes* subsp. *incantata* non sono tali da poterla considerare una specie tipica; quindi è da ritenersi una sottospecie.

Un gruppo molto controverso è quello di *Ophrys holosericea* che nel territorio in esame è rappresentato da quattro entità: *O. holosericea* subsp. *tetraloniae*, *O. holosericea* subps. *holosericea*, *Ophrys holosericea* subsp. *untchji* e *Ophrys istriensis.* Biel (2001) fa presente che il gruppo di *O. holosericea* nella penisola istriana è molto vario, ha un periodo di fioritura che va dalla fine di marzo alla prima settimana di giugno ed è costituito da popolazioni che non sono facilmente classificabili. A suo avviso, questo fenomeno potrebbe essere la conseguenza della posizione geografica della regione che la porta a ricevere flussi genetici provenienti da sud-est (Balcani ed Egeo), occidente e settentrione (Europa centrale). Hertel & Hertel (2002, op. cit.) in base alle dimensioni del labello e altre caratteristiche individuano nell'Istria quattro varietà di *O. holosericea* di cui le prime tre indicano genericamente come Tipo1, Tipo 2 e Tipo 3 che segnalano anche nel territorio vallese mentre la quarta la identificano con *Ophrys tetraloniae*. Perazza & Lorenz (2013) nella classificazione degli individui del gruppo presenti nell'Italia Nord-Orientale attribuiscono alla specie nominale gli individui a fiori grandi, alla subsp. *untchjii* quelli a fiori medi con diverse colorature del perigonio e alla subsp. *tetraloniae* quelli con fiori piccoli e a fioritura più tardiva (giugno inoltrato). Paulus (2014) mette in dubbio il rango tassonomico di *Ophrys untchjii* affermando che potrebbe rappresentare una varietà locale di *O. serotina* caratterizzata da piante con un'alta percentuale di sepali di colore verde. Romolini & Souche (2012), a loro volta considerano sinonimi *O. serotina* e *O. tetraloniae*.

***Tab. 1: Corotipi delle Orchidaceae di Bale-Valle.***
***Tab. 1: Horotipi kukavičevk v občini Bale (Valle).***

| **Elementi geografici** | **Numero taxa** | **%** |
|---|---|---|
| **Endemico e Subendemico** | **3** | **9,68** |
| Endemico | 2 | |
| Subendemico | 1 | |
| **Mediterraneo** | **16** | **51,61** |
| Eurimediterraneo | 11 | |
| Stenomediterraneo | 4 | |
| Mediterraneo-Occidentale | 1 | |
| **Eurasiatico** | **7** | **22,58** |
| Eurasiatico s. s. | 4 | |
| Europeo-Caucasico | 2 | |
| Eurosiberiano | 1 | |
| **Europeo** | **4** | **12,9** |
| Appennino-Balcanico | 4 | |
| **Mediterraneo-Atlantico** | **1** | **3,23** |
| Mediterraneo-Atlantico | 1 | |
| **Totale** | **31** | **100** |

Nella Tabella 1 sono riportati i risultati dell'analisi corologica, con la ripartizione percentuale dei vari elementi geografici. I dati riportati dimostrano come l'elemento dominante sia il mediterraneo con 16 taxa corrispondente a oltre il 51 % delle entità presenti. Ad esso potrebbero aggiungersi le entità endemiche e appennino-balcaniche che sono strettamente legate a taxa affini mediterranei e che presentano un carattere di spiccata termofilia perché sono tipiche di ambienti caldi e soleggiati.

I corotipi con la maggior presenza di taxa sono l'Eurimediterraneo (11), l'Appennino-Balcanico (4), l'Eurasiatico *s. str.* (4) e lo Stenomediterraneo (4). In totale i corotipi rappresentati sono 10 e tale configurazione arealica, in accordo con Poldini (2009), si può ritenere il risultato dell'intreccio dei fattori ecologici e biogeografici che agiscono sulle varie specie. Nell'insieme i vari taxa appartengono a corotipi caratterizzati da entità tipiche di ambienti termofili mediterranei e submediterranei che ben si accordano con le caratteristiche ambientali locali. La presenza di un taxon a distribuzione eurosiberiana (*Platanthera chlorantha*), segnalata da Hertel & Hertel (2002, op. cit.), è indicativa del fatto che nel territorio vallese è presente qualche ambito molto riparato e fresco.

Nell'ambito in esame sono presenti due specie endemiche che sono esclusive della penisola istriana e/o dell'arcipelago cherso-lussignano (*Ophrys istriensis* e *O. zinsmeisteri*) e un endemismo istro-dalmata: *Ophrys sphegodes* subsp. *incantata*. Delforge (2016) considera *sphegodes* subsp. *incantata* presente anche in Abruzzo e alla luce di tale ipotesi (che lo scrivente non conferma), dovrebbe essere considerata un'entità appennino-balcanica. Il taxa subendemico *Ophrys untchjii,* invece, è condiviso con alcune regioni italiane.

Nell'ambito di studio sono segnalate anche quattro specie appennino-balcaniche (*Ophrys bertolonii* subsp. *bertolonii, Ophrys holosericea* subsp. *tetraloniae*, *Ophrys illyrica* e *Ophrys sphegodes* subsp. *tommasinii*) che potrebbero rappresentare attuali testimonianze di processi migratori avvenuti in ere geologiche passate tra le penisole italiana e balcanica. Un altro gruppo interessante è costituito dall'elemento mediterraneo-atlantico e mediterraneo-occidentale rappresentato in totale da due taxa che documenta possibili movimenti migratori avvenuti in direzione orientale.

## CONCLUSIONI

L'elevato numero di Orchidacee presenti è un indicatore della grande qualità e integrità ambientale del territorio vallese poiché tali piante attecchiscono su terreni oligotrofici e stabili non alterati da dissodamenti, concimazioni e largo uso di diserbanti e insetticidi. Tali pratiche agrarie modificando le caratteristiche fisico-chimiche dell'aria, dell'acqua e del suolo, possono essere la causa dell'estinzione dei funghi micorrizici e degli insetti pronubi da cui dipende la vita delle piante appartenenti alla famiglia in esame (Scopece *et al.,* 2007, Newman, 2009, Swarts & Dixon, 2009, Ingeborg, 2010, Slaviero *et al*., 2016). Tuttavia le trasformazioni in atto quali lo sviluppo di un'agricoltura intensiva e delle infrastrutture stradali, turistiche e commerciali, tendono a ridurre gli spazi in cui possono attecchire. Anche l'abbandono di certe forme tradizionali di attività agro-pastorali porta alla trasformazione del territorio cui segue la scomparsa di orchidacee tipiche di prati-pascolo e la maggiore diffusione di quelle di ambiti boschivi e cespugliosi.

# KUKAVIČEVKE OKOLICE BAL (VALLE, ISTRA, HRVAŠKA)

*Amelio PEZZETTA*
Via Monte Peralba 34 - 34149 Trieste
e-mail: fonterossi@libero.it

## *POVZETEK*

*Hrvaška občina Bale (Valle) se nahaja na jugozahodni istrski obali in obsega površino približno 82 km$^2$. V pričujočem delu avtor predstavlja popis vrst, podvrst in križancev kukavičevk na območju občine, ki izhajajo iz lastnih izsledkov, podatkov, ki so mu jih priskrbeli drugi raziskovalci, nekaterih spletnih virov in recentnih objav. Poleg tega je avtor opravil še horološko analizo. Skupno je popisal 31 vrst in podvrst ter 10 križancev. Horološka analiza je pokazala prevladovanje sredozemskih elementov.*

**Ključne besede:** Orchidaceae, popis vrst v občini, Bale-Valle, Istra, Hrvaška

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# FAVNA

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# RANGE EXPANSION OF ALIEN NUDIBRANCH *MELIBE VIRIDIS* (KELAART, 1858) IN THE NORTHERN ADRIATIC SEA

*Lovrenc LIPEJ & Borut MAVRIČ*
Marine Biology Station, National Institute of Biology, Fornace 41, Piran, Slovenia
E-mail: lovrenc.lipej@nib.si

## *ABSTRACT*

*The alien nudibranch* Melibe viridis *(Kelaart, 1858) was recorded in the waters of Slovenia (Gulf of Trieste, northern Adriatic Sea). A specimen was photographed and filmed on rocky hard bottom of the Natural Monument Cape Madona in October 2016. This is the first record of this large sized nudibranch for Slovenia and for the Gulf of Trieste, as well, and a new one among otherwise rare records on this species in the Adriatic Sea.*

**Key words:** Mollusca, Gastropoda, Nudibranchia, Slovenia, non indigenous species, Adriatic Sea

# ESPANSIONE DELL'AREALE DEL NUDIBRANCO ALIENO *MELIBE VIRIDIS* (KELAART, 1858) NELL'ADRIATICO SETTENTRIONALE

## *SINTESI*

*Il nudibranco alieno* Melibe viridis *(Kelaart, 1858) è stato trovato nel mare della Slovenia (Golfo di Trieste, Adriatico settentrionale). Un esemplare è stato fotografato e filmato sul fondale roccioso all'interno del Monumento naturale Punta Madonna, nell'ottobre del 2016. Si tratta del primo ritrovamento di questo nudibranco di grandi dimensioni per la Slovenia e per il Golfo di Trieste, e di una delle poche segnalazioni di questa specie per il mare Adriatico.*

**Parole chiave:** Mollusca, Gastropoda, Nudibranchia, Slovenia, specie non-indigena, mare Adriatico

## INTRODUCTION

*Melibe viridis* (Kelaart, 1858) (Tethyididae: Nudibranchia) is a large sized tropical nudibranch with a typical head hood and inflated cerata. It is widespread across the west Indo-Pacific region. The first Mediterranean record of this species (as *Melibe fimbriata*) was reported from the island of Cephalonia in the Ionian Sea in 1970 (Moosleitner, 1986), but it has also been recorded from the coastal waters off peninsular Greece (Tzouval & Pettas, 2005), Aegean waters off the island Milos (Kotsoubas & Cinelli, 1997), both the Ionian (Thompson & Crampton, 1984; Cariglio *et al*., 2004; Mastrototaro *et al*., 2004) and Tyrrhenian coasts of Calabria (Crocetta *et al*., 2009), the waters off Sardegna (Doneddu & Trainito, 2008), the waters off Cyprus (Sanchez Villarejo, 2007; Tsiakkiros & Zenetos, 2011), the Strait of Messina (Mojetta, 1998), north-eastern Sicily (Scuderi & Russo, 2003), Maltese islands (Borg *et al.,* 2016), southern Turkey (Van Bragt, 2001; Yokes & Rudman, 2004), off Israeli coast (Mienis, 2010) and the island of Djerba in the Gulf of Gabes (Cattaneo-Vietti *et al.,* 1990) (maps and references in Despalatović *et al.,* 2002; Zenetos *et al.,* 2004).

Despalatović *et al*. (2002) published the first records of *M. viridis* for the Adriatic Sea in waters off the island of Hvar (Croatia) and Jančić (2004) reported it close to the city of Herceg Novi (Montenegro). Recently, Mandić *et al*. (2016) reported on spawning of this species in Boka Kotorska Bay (Montenegro) in October 2014.

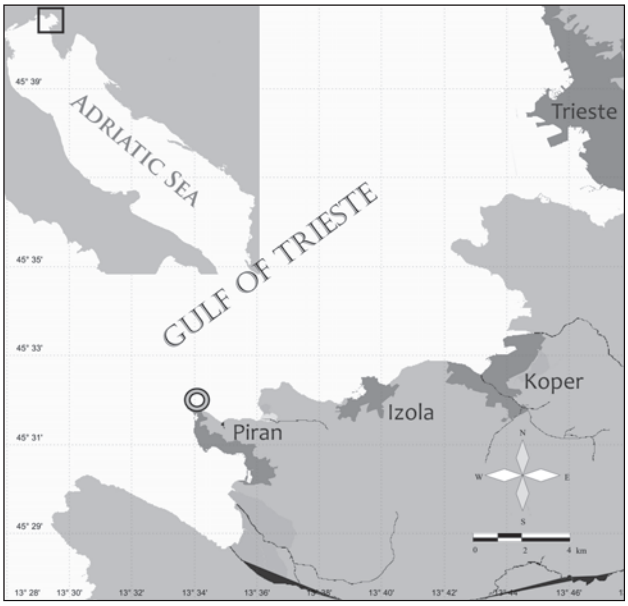

***Fig. 1: Map of the northern Adriatic Sea with the studied area. The sampling locality of** Melibe viridis **is presented with a circle.***

***Sl. 1: Zemljevid Jadranskega morja z obravnavanim območjem. Lokaliteta, kjer je bil najden primerek vrste,** Melibe viridis **je označena s krogcem.***

In this note we report on the first record of *M. viridis* in the Slovenian part of the Adriatic Sea, which also represent the first finding for the Gulf of Trieste.

## MATERIAL AND METHODS

On the 25th of October 2016 diver Kristijan Murn photographed and filmed a 10 cm long specimen of *Melibe viridis* with Go-Pro camera at the location of Natural Monument of Punta Madona in Piran (Slovenia) (Fig. 1) The specimen was sighted at 7 m depth, creeping on rocks at the sea bottom (Fig. 2) in front of the Norik diving club Piran. Photographs and film shots of the studied specimen are stored as a part of species record collection of Marine Biology Station (National Institute of Biology) in Piran.

## RESULTS AND DISCUSSION

The observed seaslug was easily determined due to the typical shape, large and dilated buccal hood and other diagnostic features (Thompson & Crampton, 1984; Gosliner & Smith, 2003). This is the first record of this nudibranch in the Slovenian part of Adriatic and at the same time in the Gulf of Trieste and the northernmost record in the Adriatic Sea, as well. The specimen was photographed in rocky environment while crawling over the substrate and scaning the sediment between rocks with its oral veil. The habitat was not typical one for this species, since in most reports *M. viridis* was found in seagrass meadows of *Cymodocea nodosa*. However, it is also true that the rocky habitat host a high abundance of many small sized decapods and other crustaceans.

The occurrence of *M. viridis* in the waters off Piran occurred in October, where temperatures are still rather high (above 20°C). At the end of summer the Gulf of Trieste is facing the intrusion of southern water masses into the area (Adriatic ingression) which could affect the occurrence of thermophilous faunistic elements (Dulčić *et al.,* 2004). The possibility that *M. viridis* could be related with the ingression of southern Adriatic water masses was previously already pointed by Mandić *et al*. (2016) for Montenegrin waters. Tsiakkiros & Zenetos (2011) considered the ballast waters as a probable vector of *M. viridis* introduction into the Mediterranean Sea, since this species was not reported neither from the Levantine waters nor from the Red Sea.

The new Adriatic record on this species proved again the importance of an effective cooperation between SCUBA diving organisations and scientific institutes.

In Slovenian coastal sea at least nine alien mollusks were recorded (Tab. 1) prior the finding of *M. viridis* (De Min & Vio, 1997, 1998; Lipej *et al*., 2008., Mavrič *et al*., 2010; Crocetta, 2011; Dailanis *et al*., 2016; Lipej *et al*., 2017). Six of them are bivalves, while other are gastropods with three of them being seaslugs (*Bursatella*

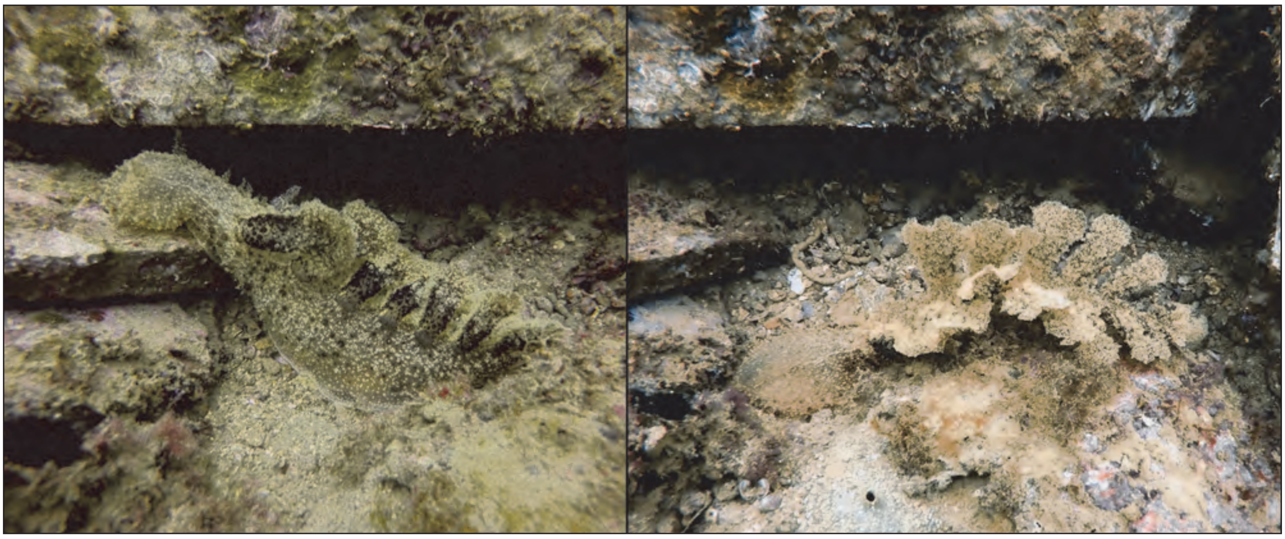

***Fig. 2: A specimen of* Melibe viridis *photographed on hard bottom in the Nature Monument Punta Madona, Piran in October 2016 (Photo: K. Murn).***
***Sl. 2: Primerek vrste* Melibe viridis*, fotografiran na skalnatem dnu znotraj naravnega spomenika Rt Madona v Piranu oktobra 2016 (Foto: K. Murn).***

***Tab. 1: Alien molusks up to date recorded in the Slovenian part of the Adriatic Sea with the locality, year of first record and literature reference.***
***Tab. 1: Tujerodni mehkužci v slovenskem delu Jadranskega morja s podatki o lokaliteti, prvem zapisu in literaturnih virih.***

| **Alien mollusk** | **Pathway/ Probable vector** | **Locus** | **year** | **source** |
|---|---|---|---|---|
| *Arcuatula senhousia* (Benson, 1842) | mariculture | Gulf of Koper | 2005 | Mavrič *et al.* (2010) |
| *Magallana gigas* (Thunberg, 1793) | mariculture | Sečovlje Bay | 1971 | De Min & Vio (1998) |
| *Ruditapes philippinarum* (Adams & Reeve, 1850) | mariculture | Sečovlje salina | 1993 | Lipej (1994) |
| *Anadara kagoshimensis* (Tokunaga, 1906) | Shipping/ Ballast waters | Gulf of Trieste (Slo) sandy environments | 1996 | De Min & Vio (1997) |
| *Anadara transversa* (Say, 1822) | Shipping/ Ballast waters | Piran, Debeli rtič | 2011 | Crocetta (2011) |
| *Brachidontes pharaonis* (P. Fischer, 1870) | Shipping/ Ballast waters or fouling | Cape Strunjan | 2012 | Lipej *et al.* (2017) |
| *Bursatella leachi* Blainville, 1817 | Suez Canal/ spreading | Marine Biology Station, Piran | 2001 | Lipej *et al.* (2008) |
| *Rapana venosa* (Valenciennes, 1846) | Shipping/Ballast waters | Many infralittoral sites | 1983? | De Min & Vio (1997) |
| *Polycera hedgpethi* Er. Marcus, 1964 | Shipping/fouling and/ or mariculture | Škocjan Inlet | 2015 | Dailanis *et al.*, (2016) |
| *Melibe viridis* (Kelaart, 1858) | Shipping/ Ballast waters? | Cape Madona, Piran | 2016 | This paper |

*leachi* de Blainville, 1817, *Polycera hedgpethi* Marcus, 1964, *M. viridis*). The introduction of the bulk of them is related to shipping or to mariculture (Zenetos *et al.,* 2004). Some of them such as *B. leachi*, *Rapana venosa* (Valenciennes, 1846), *Arcuatula senhousia* (Benson, 1842), *Magallana gigas* (Turnberg, 1893), and *Ruditapes philippinarum* Adams & Reeve, 1850 could already be considered as established in the area. The fate of *M. viridis* in the northernmost Adriatic area remains questionable, since these waters are facing low winter temperatures (<10 °C), which have an important impact on the survival of newcomers and probably prevent the establishment of viable populations. For now the occurrence of this seaslug species in the Gulf of Trieste should be considered purely as a casual one.

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# *ŠIRJENJE AREALA TUJERODNEGA GOLOŠKRGARJA MELIBE VIRIDIS* (KELAART, 1858) V SEVERNI JADRAN

*Lovrenc LIPEJ & Borut MAVRIČ*
Morska Biološka Postaja, Nacionalni Inštitut za biologijo, Fornače 41, Piran, Slovenija
E-mail: lovrenc.lipej@nib.si

## *POVZETEK*

*Tujerodnega morskega polža gološkrgarja vrste* Melibe viridis *(Kelaart, 1858) so v oktobru 2016 opazili v slovenskih vodah (Tržaški zaliv, severni Jadran). Potapljač je primerek fotografiral in posnel s filmsko kamero na skalnatem dnu znotraj akvatorija Naravnega spomenika Rt Madona. To je prvi zapis o pojavljanju te velike vrste gološkrgarja v Sloveniji in eden izmed maloštevilnih opažanj te vrste v Jadranskem morju.*

**Ključne besede:** Mollusca, Gastropoda, Nudibranchia, Slovenija, tujerodna vrsta, Jadransko morje

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# ANTITUMORAL ACTIVITY IN INKS OF *SEPIA OFFICINALIS* AND *OCTOPUS VULGARIS* (CEPHALOPODA) FROM THE NORTHERN TUNISIAN COAST (CENTRAL MEDITERRANEAN SEA)

*Emna SOUFI-KECHAOU & Ichrak SARIYA*
Department of Animal Sciences, Halieutics and Food Technology, National Institute of Agronomy of Tunis, 43 avenue Charles Nicolle,
1082-Tunis-Mahrajène,Tunisia
e-mail: esoufi@gmail.com

*Amine BEZAA, Neziha MARRAKCHI & Mohammed EL AYEB*
Laboratory of Venoms and Toxins, Pasteur Institute of Tunis. 13, place Pasteur,
B.P. 74. 1002Tunis-Belvédère, Tunisia

## *ABSTRACT*

*In the present study, authors investigate the antitumor effects of peptides from the ink of two cephalopod species: the common cuttlefish,* Sepia officinalis *(Linnaeus, 1758), and the common octopus,* Octopus vulgaris *(Cuvier, 1797), from specimens sampled in the northern Tunisian coasts (central Mediterranean). The results indicate that the crude ink show anti-adhesion properties of the IGR39 cells depending on the Extra Cellular Matrixes (ECM) tested. The partially purified fractions with a molecular weight inferior to 10 kDa for* Sepia officinalis *($F_{inf10}$) and the superior to 10 kDa for* Octopus vulgaris *($F_{sup10}$) revealed anti-invasion, anti-migration and anti-adhesive activities on the U87 glioma cell lines, with a dose-dependent response. No antiproliferative activity was found for both of the partially purified fractions and the MTT assay showed toxicity effect only for high ink fraction concentrations.*

**Key words:** Cephalopoda, *Sepia* ink, *Octopus* ink, antitumor, enzymatic hydrolysis, oligopeptide, Tunisia, central Mediterranean

# ATTIVITÀ ANTITUMORALE DI INCHIOSTRI DI *SEPIA OFFICINALIS* E *OCTOPUS VULGARIS* (CEFALOPODA) PROVENIENTI DALLA COSTA SETTENTRIONALE DELLA TUNISIA (MEDITERRANEO CENTRALE)

## *SINTESI*

*Nel presente studio gli autori analizzano gli effetti antitumorali dei peptidi ricavati dall'inchiostro di due specie di cefalopodi: la seppia comune,* Sepia officinalis *(Linnaeus, 1758), e il polpo comune,* Octopus vulgaris *(Cuvier, 1797), provenienti da campioni prelevati lungo la costa settentrionale della Tunisia (Mediterraneo centrale). I risultati indicano che l'inchiostro grezzo mostra proprietà di anti-adesione delle cellule IGR39, in dipendenza delle matrici extra cellulari (ECM) analizzate. Le frazioni parzialmente purificate, con un peso molecolare inferiore a 10 kDa per* Sepia officinalis *(Finf10), e superiore a 10 kDa per* Octopus vulgaris *(Fsup10), hanno evidenziato attività anti-invasione, anti-migrazione e anti-adesività sulle linee cellulari degli gliomi U87, con una risposta dose-dipendente. Nessuna attività antiproliferativa è stata trovata per entrambe le frazioni parzialmente purificate, e il dosaggio MTT ha dimostrato l'effetto tossicità solo per elevate concentrazioni di frazioni di inchiostro.*

**Parole chiave:** Cefalopoda, inchiostro di seppia, inchiostro di polpo, antitumorale, idrolisi enzimatica, oligopeptide, Tunisia, Mediterraneo centrale

## INTRODUCTION

In recent years, researchers have focused on identifying novel natural products as anticancer drugs. Anticancer peptides have characteristics of multi-function, high sensitivity and stability (Leng *et al.*, 2005; Simmons *et al.*, 2005).

Molluscan species, such as sea hares show a wide range of uses in pharmacology as they produce bioactive metabolites used in the treatment of cancerous tumors (Chakraborty & Ghosh, 2010). Peptides as antitumor drugs can improve immune response, inhibit the tumor angiogenesis and metastasis of tumor cells, directly eradicate tumor cells and induce the apoptosis of tumor cells and stop the cell cycle (Shen *et al.*, 2000; Aneiros & Garateix, 2004; Zheng *et al.*, 2007). The most studied, *Dolastatins* is a family of cytotoxic peptides isolated from the mollusk *Dollabella auricularia*, where the linear pentapeptide *Dolastatin*-10 and the depsipeptide *Dolastatin* 15 have had the most promising antiproliferative activity reported (Pettit *et al.*, 1995; Garteiz *et al.*, 1998; Pettit *et al.*, 1998). The pentapeptide Dolastatin-10 is characterized by four of the residues being structurally unique but with many side effects. Also, the Keenamide A is a cytotoxic cyclic hexapeptide isolated from the mollusk *Pleurobranchus forskalii*, elicits antitumor activity via unknown mechanisms. This compound exhibited significant activity against the P388, A549, MEL-20 and HT-29 tumor cell lines (Wesson *et al.*, 1996). Strong anticancer peptides were also found from *Meretix meretrix* with IC$_{50}$ of 10 µg·mL$^{-1}$ (Liu & Qiu, 2004).

Cephalopoda ink had been used in the treatment of hemostasis for centuries in Chinese traditional medicine (Zhong *et al.*, 2009). As early as 1982, it was reported that *Sepia* ink could regulate gastric juice secretion and had antiulceration activity (Andersen & Roepstorff, 1982). Researchers in Japan found that the peptidoglycan extracted from *Sepia* ink had higher antitumor activity than the other fractions (Takaya *et al.*, 1996, 1997). Other research works reported antitumor activity of cephalopoda ink (Naraoka *et al.*, 2000; Palumbo *et al.*, 2000). For example, it has been reported that *Sepia* ink has antitumor activity against Meth-A fibrosarcoma in BALB/c mice and its fraction containing peptidoglycan showed higher antitumor activity than the other fractions (Tetsushi *et al.*, 2000; Mayer *et al.*, 2010). Nowadays, none of the currently available anticancer drugs acts solely on carcinoma cells. Anticancer drugs are usually extremely toxic and kill both malignant and normal cells. However, despite its wide spectrum of clinical uses, they are known to cause several adverse effects. These limits on the use of chemotherapeutic agents thus constrain their use in effective therapy.

Protein hydrolysates formed by the enzymatic digestion of aquatic and marine by-products are an important source of bioactive peptides. Purified peptides from these sources show cytotoxic effect on several human cancer cell lines such as HeLa, AGS, and DLD-1 (Wang *et al.*, 2010). These characteristics imply that the use of peptides from marine sources has a great potential for the prevention and treatment of cancer, and that they might also be useful as molecular models in anticancer drug research.

In this paper, the inks from *Sepia officinalis* and *Octopus vulgaris* had been used for *in vitro* antitumor activities. These two marine species have been chosen because of their widespread geographical distribution in Tunisia and also because of the popularity of this seafood. The cephalopoda ink, which is the natural substance released for defence purpuses against predators is composed mainly of melanin and proteoglycans (Shen *et al.*, 2007; Mayer *et al.*, 2013). It is produced by the ink gland, a by-product of marine-product processing, generated after gutting procedures. The objective of this research work was the characterization and the evaluation of anticancer potential from the ink of *S. officinalis* and *O. vulgaris* through the antiproliferative effect, inhibition on invasion, migration of tumor cells, as well as cytotoxicity. A characterization of the nature the peptide has also been carried out after an enzymatic hydrolysis process.

## MATERIAL AND METHODS

### Biological material

Like all the cephalopoda species, *S. officinalis* and *O. vulgaris* (Fig. 1) are positioned in a high level in the marine food web and are carnivorous since egg hatchling *S. officinalis* is a demersal and neritic species present in the infra and circalittoral zones, on sandy or muddy-sandy bottoms and phanerogam meadows, from the coast up to 150 meters. The cuttlefish specimens are present in the coastal waters from April till October. In the winter, they migrate to deeper zones searching for abundant food and more adequate temperatures.

*O. vulgaris* is a benthic, neritic species occurring from the coastline to the outer edge of the continental shelf (in depths from 0 to 200 m), where it is found in a variety of habitats, such as rocks, coral reefs, and grass beds. Throughout its distribution range, this species is known to undertake limited seasonal migrations, usually overwintering in deeper waters and occurring in shallower waters during summer. In the western Mediterranean, large mature or maturing individuals migrate inshore in early spring, followed later on by smaller, immature individuals. These two groups begin their retreat into deeper waters by August/September and November/December respectively.

Specimens of *S. officinalis* and *O. vulgaris* were captured off Bizerte coasts (Fig. 1), (North of Tunisia, Mediterranean Sea) by deep-sea trawling for the Cuttlefish and by traditional coast-fishing for *Octopus*, during April 2010. The specimens were then transported to the

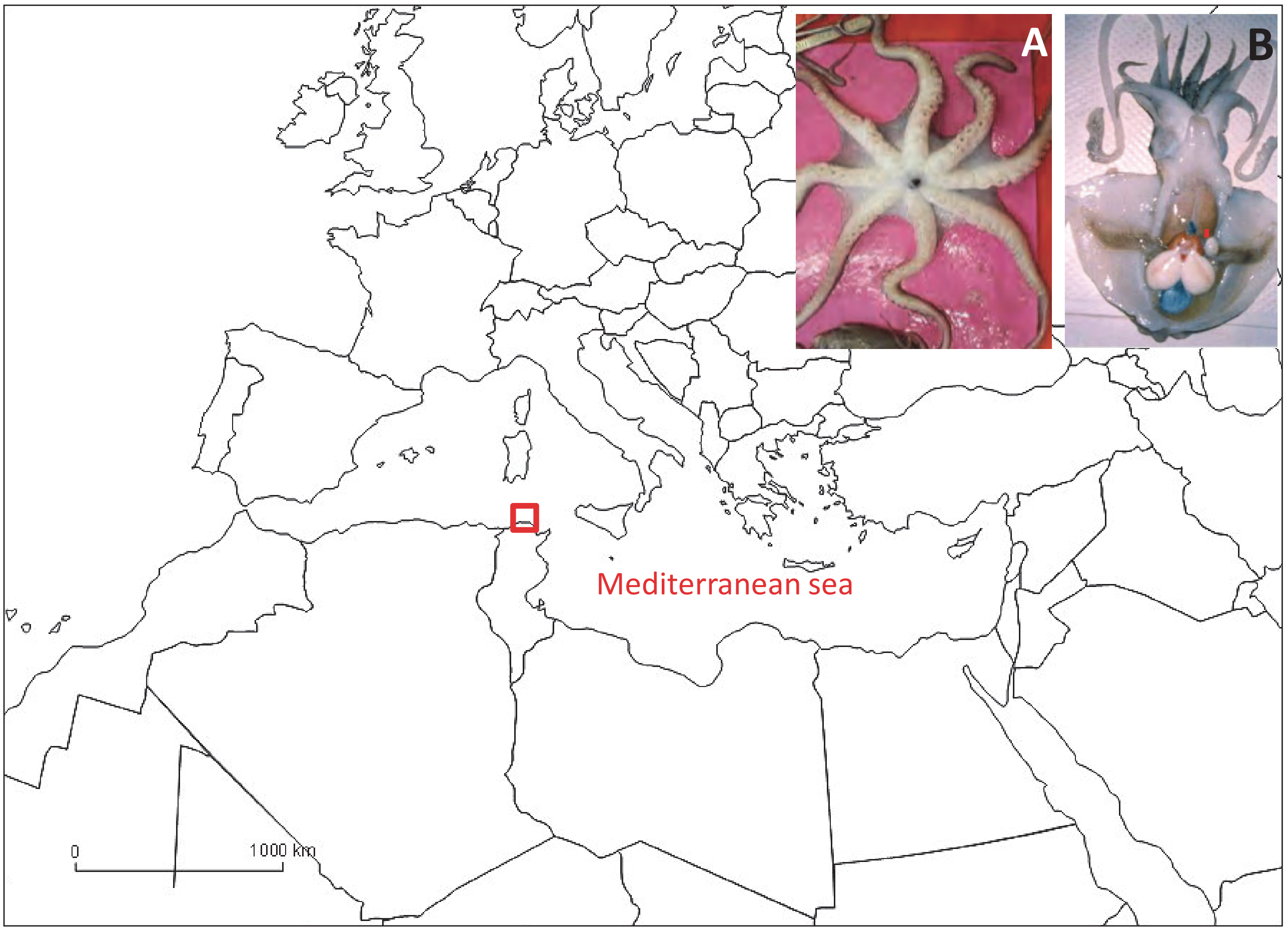

***Fig. 1: Location of the capture sites (FAO fisheries department 2005) of the biological material. A.*** *Sepia officinalis* ***and B.*** *Octopus vulgaris****.***
***Sl. 1: Lokalitete, kjer je bil nabran biološki material (FAO fisheries department 2005). A.*** *Sepia officinalis* ***in B.*** *Octopus vulgaris****.***

laboratory packed in ice and the ink gland was extracted. In order to avoid biochemical variation due to the physiological state of the animals, inks were homogenized by triturator and stored at -20°C before use.

### Ink extraction and preparation

At a first time, the crude *cuttlefish* or *Octopus* inks was dissolved in PBS buffer (500 mL). After centrifugation at 1000 *g* during 10 min., the supernatants were collected for the antitumoral tests on tumor cell lines deriving from human melanoma IGR39. The sediment is stored at -80°C for further analyses. In a second time, the crude *cuttlefish* or *Octopus* inks were homogenized with acetone at -30°C (4V), according to the method of Takaya *et al*. (1994). The supernatant was collected after centrifugation at 1000 *g* during 15 min. and lyophilised. The sediment was stored at -80°C for further analyses. The lyophilised extracts were dissolved in a 0.1 M Tris-HCl solution (PH = 6.8) (40 v) for 72 hours at 4°C and then centrifuged at 11.000 *g* during 30 min (Mikro 200 R, Hettich Zentrifuger), in Amicon centrifuging cells (YM10) in order to separate the ink extracts according to their molecular weight. Two fractions were obtained for each cephalopoda species: the $F_{\mathbf{sup\ 10}}$ molecular compounds with a molecular weight higher then 10kDa and another fraction denoted $F_{\mathbf{inf\ 10}}$ with the molecular weight inferior to 10 kDa. For Cuttlefish, as well as Octopus, these two fractions were lyophilised under vacuum (LABCONCO, 2.5 $_{(\mathrm{Plus})}$ Freezone). Finally, the freeze-dried fractions, $F_{\mathbf{inf\ 10}}$ and the $F_{\mathbf{sup\ 10}}$ of *Sepia* and *Octopus* were dissolved in PBS (v/v) according to the method of Naraoka *et al.* (2000). Aliquots were stored at -80°C.

### Enzymatic hydrolysis

The Pepsin (EC 3.4.23.1) was provided by DSM. The enzymatic hydrolysis conditions were: a temperature of

45°C, pH2, an E/S ratio of 0.1% and an hydrolysis time of 10 hours. The reaction was stopped by heating the solution to 85°C to inactivate the enzyme. The resulting hydrolysate was centrifuged at 20,000 *g* for 20 min.

### Isolation and purification of anticancer peptide

*Sepia* ink hydrolysates were fractionated into a high and low molecular weight fractions and by ultrafiltration at 4°C by PM-10 membrane (MWCO = 3000 Da) and kept for use in gel filtration. Prior to use, the membrane was washed with 10 mL of distilled water. The ultrafiltrate was filtered again through a Millipore membrane filter (0.45 µm) and applied to a (2.8 cm × 90 cm) column saturated in Sephadex G-25 resin. The Sephadex G-25 column was eluted with distilled water and fractions were collected every 4 minutes with a fraction collector. The absorbance was measured at 280 nm. The hydrolysate was fractionated into five fractions by gel filtration chromatography. Each fraction was tested for anticancer activities.

The fraction showing the highest anticancer activity was further purified using reverse-phase HPLC on a Primesphere 10 $C_{18}$ column (10 mm × 250 mm) with a linear gradient of acetonitrile (0-50% for 20min) containing 0.1% trifluoroacetic acid (TFA) at a flow rate of 1 mL·min$^{-1}$. The absorbance of the eluent was monitored at 280 nm. Active peak representing anticancer activity was pooled and freeze-dried immediately for a future analysis of the bioactive fraction.

### The yield of *Sepia* ink oligopeptides

The fraction provided by the G-25 gel chromatography was concentrated in vacuum under 25°C and concentrated liquid was dried under 70°C by vacuum freeze-drying. Then the powder of *Sepia* ink oligopeptides was collected and weighed.

### Cell line and cell culture

Human glioma cell lines U87 and melanoma cell lines IGR 39 were used for anticancer activity tests with non purified ink fractions. The human prostate cancer cell lines PC-3 were used later for the purified and isolated active peptide. The cell lines were grown at 37°C in a 5% $CO_2$, 95% air humidified atmosphere, in MEM medium for U87 and DMEM-Ham's F12 medium for IGR39. The medium was supplemented with 10% FCS + 5% heat inactivated horse serum to which streptomycin (100 µg/mL) and penicillin (100 U/mL) had been added. The cells grown in flasks were washed with PBS, trypsinized (3 mL of trypsine-EDTA, 500 µg/ml), centrifuged at 800 rpm for 5 min and dissolved in fresh culture medium at 104 cells/90 µL. Subsequently, a 90 µL volume of suspension cells was added to each well of a 96-well microplate and incubated at 37°C for 24 hours.

### Cell migration and invasion assay in vitro

The assays were achieved in Boyden Chambers (Becton Dickinson). A 24-well transwell (Corning, NY, USA) was used to evaluate the motility and invasive ability of U87 and IGR29 cells in vitro. The upper surface of polycarbonate filters with 8 µm pores (0,3 cm$^2$) was coated with 5 mg of Matrigel, fibrinogen (Sigma-Aldrich) at 50 µg/mL of PBS and incubated during 2 hours at 37°C . The lower chambers were filled with the medium (MEM/BSA 0,1%) (Sigma-Aldrich) (500 µl in the lower well, and 200 µl in the upper one). The glioma cells U87 were pre-incubated with different doses of the molecular fractions $F_{inf\ 10}$ and the $F_{sup\ 10}$ of *Sepia* and *Octopus* or 1% BSA (negative control) for 24 hours at 37 °C in a $CO_2$ incubator and then washed with PBS, detached with the versene (Gibco) and resuspended in serum-free MEM. A suspension of cells (2 x10$^5$ cells/200 mL) was placed in the upper chambers. After 5 h of incubation at 37°C under optimal conditions, the supernatant was completely removed and the upper and lower faces of the membranes are washed with PBS. Cells on the upper surface of the filter (that did not migrate) were completely removed by wiping them with a cotton swab. Cells that invaded the Matrigel and were fixed during 10 min with glutaraldehyde 1% and then stained with crystal violet at 0.5 % for 30 min. The cellular migration was quantified by counting the number of cells that migrated using a microscope (Leica) with 5 mm$^2$ fields at a magnification of x 400, or by measuring the absorbance at 560 nm after solubilization of the colorant in SDS 1%.

### Antiproliferative activity assay

This assay aims to evaluate the effect of the molecular fractions, $F_{inf\ 10}$ and the $F_{sup\ 10}$ of *Sepia* and *Octopus* on the multiplication of tumor cells. U87 cells are incubated in the wells of a microplate (5 x 10$^3$ cells/well) in 50 µl MEM/10% FCS (Fœtal Calf Serum/ 5% horse serum. After one hour incubation, the medium is renewed in presence of the fractions to test. Each day, 3 wells are washed with PBS and the cells are fixed with glutaraldehyde 1% then fixed with PBS. At the end of the week, the cells are stained with crystal violet 0.1% and quantified by measuring the absorbance at 560 nm.

### Cytotoxicity assay

The MTT assay (Mosmann, 1983) allows the evaluation of the effect of the molecular fractions $F_{inf\ 10}$ and the $F_{sup\ 10}$ on the cell viability of cancer cells U87. The peptidic ink fractions from Cuttlefish and Octopus, at a concentration of 10 mg/mL was prepared in PBS 0.1 M (pH 7.4), and diluted 10-fold in cell culture medium containing the cells. The microplate was then incubated at 37 °C for 24, 48 and 72 h, changing the culture medium every 24 h and adding the ink fractions at a final concentration of 1 mg/mL. At the end of every incubation period, 15 µL of 5 mg/mL tetrazolium salt (MTT) solution was added to each well, and the plate was incubated for 3 h. To stop succinate-tetrazolium reductase activity and solubilize formazan crystals, 200 µL of dimethyl sulfoxide (DMSO) was then added to each well and kept at 37 °C for 1 h. Absorbance was read on a plate reader at 570 nm.

### Cellular adhesion assay

The aim of this assay was to analyze the ability of ink fractions to inhibit the adhesion of tumor cells IGR39 on the proteins of the extracellular matrix (ECM). Four proteins were assyed: fibrinogen, fibronectin, collagen type I and polylysin. The substrates of adhesion were prepared by coating the wells of a microplate (Nunc) by 50 µL of a proteic solution : fibrinogen at 50 µg/mL, la polylysine (PL) at 20 µg/mL, the collagen type I (Coll I) at 10µg/mL and fibronectine (Fn) at 5µg/mL and incubating it during 2 hours at 37°C. The wells are then saturated with 50 µL PBS/BSA 0.5 % during 1 hour at 37°C. During saturation, the cells were detached by PBS/EDTA and washed twice with adhesion buffer (MEM, NaHCO3 1,2 g/l, HEPES 15 mM pH 7.3 and BSA 0,2%). For the assay of the ink fractions, the cells were pre-incubated during 30 min at room temperature with stirring and then deposited in the wells where 50 µL cellular suspension ($10^6$ cells/mL adhesion buffer) were added and incubated during 1 hour at 37°C. After incubation, the non adherent cells are eliminated by washing with an adhesion buffer. The adherent cells are fixed with glutaraldehyde 1% during 10 min at room temperature and are washed twice with distilled water and stained during 30 min by 100 µL of a crystal violet solution at 0.5%. Cellular adhesion was quantified by measuring the absorbance at 560 using a microplate reader (Σ960 Metertech). All the data were analyzed by the software of SPSS.

## RESULTS AND DISCUSSION

### Effect of the crude ink on the adhesion of IGR23 to the proteins of the ECM

Cephalopoda ink had been studied for its antimicrobial and antiviral activities, but also for its toxicity for some cell lines (Derby *et al.*, 2007). To this context, we investigated the antitumor potential of *S. officinalis* and *O. vulgaris* inks. At a first time, the crude inks were diluted in PBS and then centrifuged. We evaluated the anti-adhesive effect of the supernatants on IGR39 cell lines deriving from human melanoma, on 3 different ECM: fibrinogen, fibronectin and collagen type I. The results showed that cuttlefish ink - at a concentration of 5,28 µg/ml - significantly ($p < 0.05$) inhibits the adhesion to fibrinogen, with an inhibition percentage of 60%. This inhibition was 25% on fibronectin. There was no inhibition of the adhesion of the IGR 39 cells on the collagen type I (Fig. 2). Concerning Octopus ink, we noticed that the adhesion of IGR 39 cells on fibronectin is significantly ($p < 0.05$) decreased by 40% (concentration of 8,75 µg/mL), but there was no inhibition for fibrinogen and collagen type I.

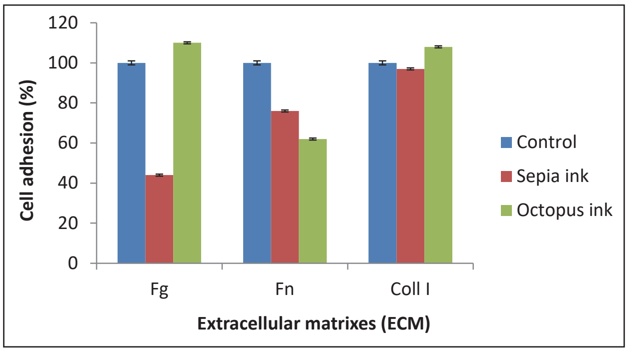

***Fig. 2: Effect of crude*** *Sepia* ***and*** *Octopus* ***ink on the adhesion of the melanoma IGR 39 cells to fibrinogen, fibronectin and collagen type I.***
***Sl. 2:*** Učinek surovega črnila sipe in hobotnice na lepljenje celic ***melanoma IGR 39 na fibrinogen, fibronectin in kolagen tipa I.***

### Effect of ink fractions on the adhesion of tumor cells U87 to the proteins of the ECM

After the fractionation of the inks in Amicon cells, two fractions were obtained for each cephalopoda specie, the $F_{sup\ 10}$ (MW > 10 kDa) and the $F_{inf\ 10}$ (MW < 10 kDa). With a concentration of 30 µg/mL, the cuttlefish $F_{sup\ 10}$ poorly inhibits the adhesion of U87 cells on fibrinogen (Fig. 3.A), whereas the $F_{inf\ 10}$ fraction inhibits cell adhesion on fibrinogen in a dose-dependent manner, with an $IC_{50}$ of 25µg/ml (Fig. 3.B). In the same way, *Octopus* $F_{sup\ 10}$ inhibits cell adhesion with an $IC_{50}$ of 75 µg/mL (Fig. 4.A). However, the fraction $F_{inf\ 10}$ assayed at dose 100µg/mL did not show a significant antitumor effect at the level of 5%. (Fig. 4.B). The adhesion assays of the U87 cells on Polylysine-L showed an inhibition that did not exceed 20 % with cuttlefish $F_{inf\ 10}$ and *Octopus* $F_{sup\ 10}$. At that point we cannot conclude yet that these inhibitions are integrin-dependent (Fig. 5.A and B).

### Effect of ink fractions on the migration of U87 cells

The cellular migration plays a very important role in the metastatic dissemination and requires cellular adhesion to the proteins of the extracellular matrixes (ECM). Because the extracts of the active fractions, $F_{inf\ 10}$ of *Sepia* ink and $F_{sup\ 10}$ of *Octopus* ink exhibited an inhibiting potential of the adhesion of U-87 tumoral cells, we have essayed the effect of these extracts on their migration.

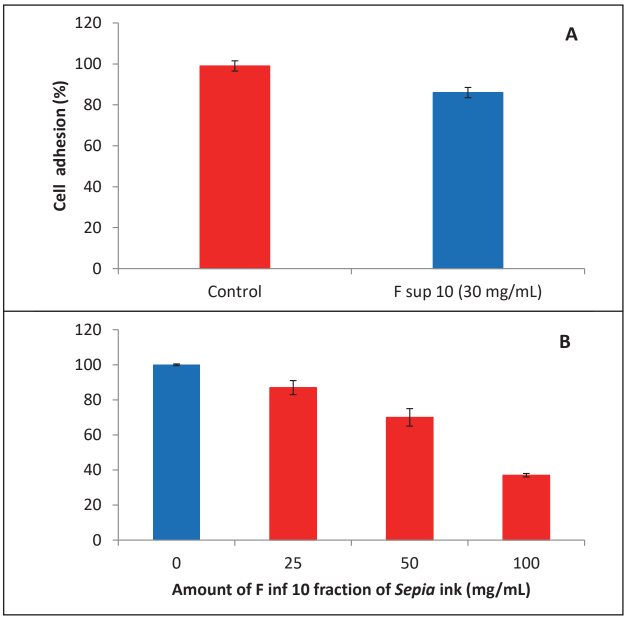

***Fig. 3: Effect of the* Sepia *ink fractions on the adhesion of the glioma U87 cells on fibrinogen. A: $F_{sup\ 10}$ (30µg/ml), B: Amount of $F_{inf\ 10}$ fraction.***
***Sl. 3: Učinek frakcije surovega črnila sipe na lepljenje celic glioma U87 na fibrinogen. A: $F_{sup\ 10}$ (30µg/ml), B: Količina $F_{inf\ 10}$ frakcije.***

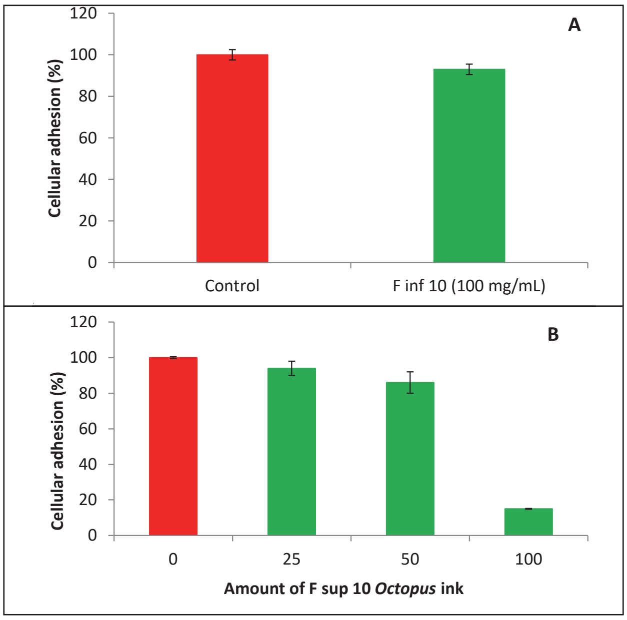

***Fig. 4: Effect of the* Octopus *ink fractions on the adhesion of the glioma U87 cells on fibrinogen. A: $F_{inf\ 10}$ (100µg/mL); B: Amount of $F_{sup\ 10}$ fraction.***
***Sl. 4: Učinek frakcije surovega črnila hobotnice na lepljenje celic glioma U87 na fibrinogen. A: $F_{sup\ 10}$ (30µg/ml), B: Količina $F_{sup\ 10}$ frakcije.***

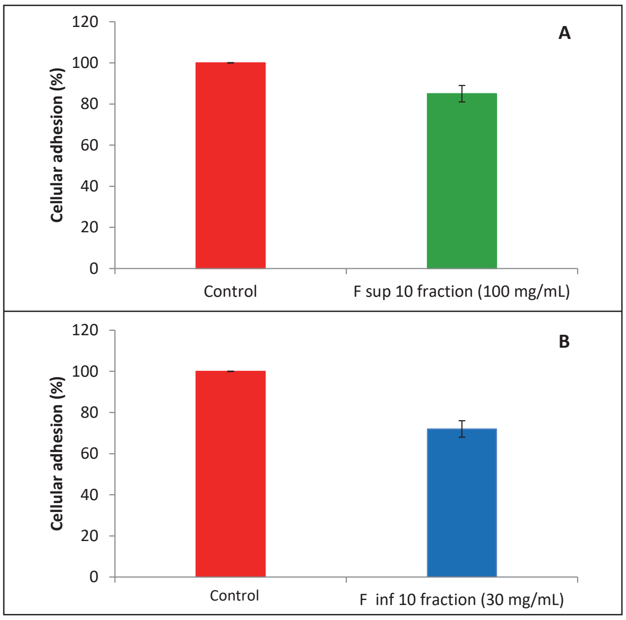

***Fig. 5: Dose-response effect of ink fractions on the adhesion of U87 cells to Polylysin-L. A:* Sepia *$F_{sup\ 10}$, B:* Octopus *$F_{inf\ 10}$***
***Sl. 5: Učinek frakcij črnila na lepljenje U87 celic na polilizin-L v odvisnosti od doziranja. A:* Sepia *$F_{sup\ 10}$, B:* Octopus *$F_{inf\ 10}$.***

The U87 cells treated with increasing concentrations of cuttlefish $F_{inf10}$ or *Octopus* $F_{sup\ 10}$ stopped the migration of the cells and their adhesion to fibrinogen, with a dose-dependence ($IC_{50}$ =15µg/ml) for *Sepia* $F_{inf\ 10}$ and ($IC_{50}$ = 40µg/ml) for *Octopus* $F_{sup\ 10}$ (Fig. 6 and Fig. 7).

### Antiproliferative effect of ink fractions on the U87 cells

**Because the ink fractions** *Sepia* $F_{inf\ 10}$ and *Octopus* $F_{sup\ 10}$ showed interesting antitumor activities (inhibits adhesion and migration of U87), their antiproliferative potential was assayed. Our results showed that the ink fractions did not show any significant inhibition of cell proliferation during 4 days (Fig. 8 A and B).

### Isolation and purification of the *Sepia* ink peptide

Because the peptidic fraction inferior to 10 kDa of *Sepia* ink ($F_{inf\ 10}$) is the one who was the most active, and because biologically active peptides have low molecular weight in general, we decided to concentrate our study on *Sepia* ink anticancer peptides. After ultrafiltration using a 3000 Da MWCO membrane the permeates of Pepsin hydrolysates were loaded on a gel filtration column (Sephadex G-25). Five anticancer peptide fractions with the highest activity were collected. The purity was

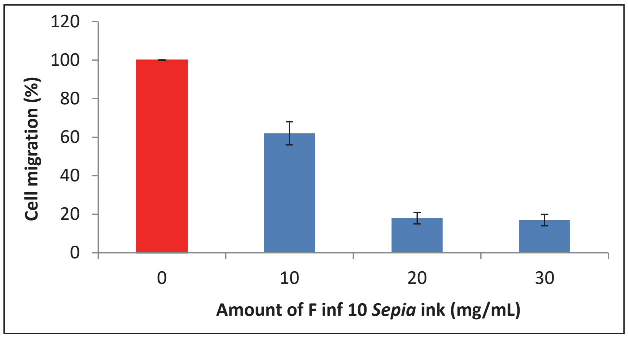

***Fig. 6: Effect of the* Sepia *$F_{inf\ 10}$ ink fraction on the U87 cell migration and morphological changes. (I): Microscopic observation. A-D: Cells were incubated with $F_{inf\ 10}$* Sepia *ink fractions (0, 10, 20, 30 µg/mL) during 5 hours at 37°C. (II): U87 cellular migration (%) according to the concentration of* Sepia *ink fraction $F_{inf10}$ (0, 10, 20, 30 µg/mL).***

***Sl. 6: Učinek frakcije črnila sipe ($F_{inf\ 10}$) na migracijo celic U87 in morfološke spremembe. (I): Mikroskopska opazovanja. A-D: Celice inkubirane s frakcijami $F_{inf\ 10}$ črnila sipe (0, 10, 20, 30 µg/mL) v peturnem obdobju pri 37°C. (II): U87 celična migracija (%) glede na koncentracijo frakcij črnila sipe $F_{inf10}$ (0, 10, 20, 30 µg/mL).***

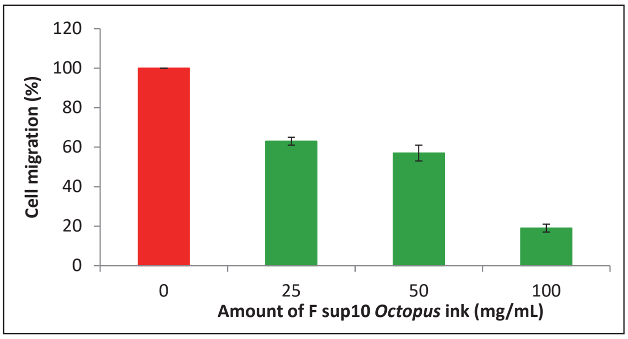

***Fig. 7: Effect of the* Octopus *$F_{sup\ 10}$ ink fraction on the U87 cell migration and morphological changes. (I): Microscopic observation. A-D: Cells were incubated with $F_{sup\ 10}$* Octopus *ink fractions (0, 25, 50, 100 µg/mL) during 5 hours at 37°C. (II): U87 cellular migration (%) according to the concentration of* Octopus *ink fraction $F_{sup\ 10}$ (0, 25, 50, 100 µg/mL).***

***Sl. 7: Učinek frakcije črnila hobotnice ($F_{sup\ 10}$) na migracijo celic U87 in morfološke spremembe. (I): Mikroskopska opazovanja. A-D: Celice inkubirane s frakcijami $F_{sup\ 10}$ črnila sipe (0, 10, 20, 30 µg/mL) v peturnem obdobju pri 37°C. (II): U87 celična migracija (%) glede na koncentracijo frakcij črnila hobotnice $F_{sup\ 10}$ (0, 25, 50, 100 µg/mL).***

then detected by HPLC. The sample was divided into five peaks and the peak 2 had the highest anticancer activity. After a further reverse-phase HPLC, the second peak had a molecular weight of 340.6. After freeze drying, 1.74 g *Sepia* ink oligopeptide was obtained from 100 g *Sepia* ink. So the yield was 1.74 %.

### Effect of *Sepia* ink oligopeptides on cell viability

The PC-3 cells (human prostate cancer cell lines) were treated with 2-10 µg·mL$^{-1}$ of *Sepia* ink oligopeptides for 24-72 h. PC-3 cells displayed dose-dependent decreases in viability, detectable as early as 24 h. At 24 h, the threshold concentration which caused a decrease in PC-3 cell viability was 1.89 µg·mL$^{-1}$ (89 % of control, $P > 0.05$). The *Sepia* ink oligopeptide concentration that produced the maximal effect was 10 µg/mL (26% of control, $P < 0.05$), and the half inhibitory concentration (IC ) was 7.45 µg·mL$^{-1}$.

$_{50}$At 48 h, the threshold concentration was 2,2 µg·mL$^{-1}$ (33 % of control, $P < 0.05$), the maximal effect was 10 µg·mL$^{-1}$ (0.24% of control, $P < 0.05$), and the $IC_{50}$ was 1.23 µg·mL$^{-1}$. At 72 h, the threshold concentration was again 2 µg·mL$^{-1}$ (43 % of control, $P < 0.05$). The maximal effect was 10 µg·mL$^{-1}$ (0% of control, $P < 0.05$), and the $IC_{50}$ was 1.64 mg·mL$^{-1}$. The threshold concentration at 24, 48 and 72 h were the same (2 µg·mL$^{-1}$), even though the level of the significance increased from day 1 to days 2 and 3. The $IC_{50}$ decreased from 7.45 µg·mL$^{-1}$ at 24 h to 1.23 µg·mL$^{-1}$ at 48 h. These results suggest that *Sepia* ink oligopeptides had a dose-dependent deleterious effect on PC-3 cell viability.

Biologically active antitumor compounds have been isolated from different marine sources. Recently research has been focused on peptides from marine animal sources, since they have been found as secondary metabolites from sponges, ascidians, tunicates, and mollusks. The structural characteristics of these peptides include various unusual amino acid residues which may be responsible for their bioactivity. However, many side effects had been observed in clinical trials and the complexity and low yield of chemical synthesis, together with low water solubility, have been significant obstacles to broader clinical evaluation, triggering the development of analog compounds (De Arruda *et al.*, 1995; Pitot *et al.*, 1999; Tamura *et al.*, 2007).

Even if the bioactive peptides from marine mollusks had been well documented, there had been a few publications on anticancer peptides from cephalopoda, specifically the species *O. vulgaris* and *S. officinalis* ink wastes.

Interestingly in our study, the crude *Sepia* and *Octopus* inks assayed on tumor cells IGR39, showed a selective inhibition according to the cellular matrix used. In order to refine this investigation, we adopted an acetone fractioning of the ink and interested particularly to the supernatant. The two fractions obtained

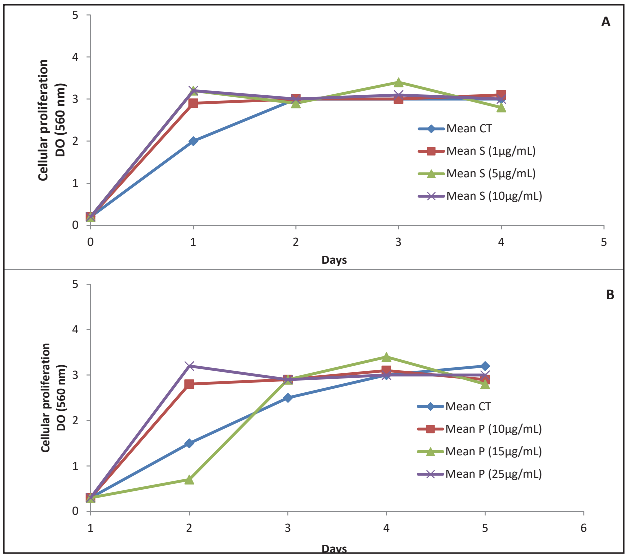

***Fig. 8: Anti-proliferative effect of the active fractions on U87 cells. (A):*** *Sepia* $F_{inf\ 10}$, ***(B):*** *Octopus* $F_{sup\ 10}$.
***Sl. 8: Anti-proliferativni učinek aktivnih frakcij na U87 celice. A):*** *Sepia* $F_{inf\ 10}$, ***(B):*** *Octopus* $F_{sup\ 10}$.

$F_{inf\ 10}$ (MW< 10kDa) and $F_{sup\ 10}$ (MW> 10kDa) were then assayed *in vitro* on glioma cell lines U87. The in anti-adhesive activities of the ink fractions belonging to the two cephalopoda species are not comparable. *Sepia* $F_{inf\ 10}$ and *Octopus* $F_{sup\ 10}$ inhibit the adhesion of U87 cells on fibrinogen, according to their concentrations (dose-dependent) with an $IC_{50}$ = 25µg/mL for cuttlefish ink fraction and 75µg/mL for *Octopus*. However, both of the ink extracts slightly inhibit the non-specific adhesion of U87 cells on Polylysin-L. This result suggests that the fractions would own an inhibition mechanism through one or more membrane receptors. It is also important to mention that the anti-adhesive effect requires a high concentration of the *Octopus* $F_{sup\ 10}$ (~50µg/mL), unlike *Sepia* $F_{inf\ 10}$ fraction (~10 µg/mL). We can thus emit the hypothesis that these ink fractions may contain antagonistic activities. According to the literature, the antitumor effect is correlated to a synergy between different chemical ink compounds. This action is related to the tyrosinase activity and peptidoglycans (Naraoka *et al.*, 2000). We also showed that with concentrations of 10 µg/mL of *Sepia* ink fraction, the cell migration is reduced and is completely stopped with a concentration of 30 µg/ml. However, concerning the *Octopus* ink fraction, we observe an inhibition of cell migration starting from a concentration of 25 µg/mL. This inhibition is complete at 100 µg/mL. Somehow, there was no inhibition of cell proliferation. Our results are in concordance with the research work on squid (*Ommastrephes bartrami*) ink where the authors did not detect evident antiproliferive activity on tumor cells Hep G2, but induces a suppression of cell invasion and cell migration, according to the concentrations of ink fractions (Chen *et al.*, 2010). The cytotoxicity assays of the $F_{inf\ 10}$ and $F_{sup\ 10}$ of Sepia and *Octopus* during 5 hours showed that these fractions are toxic only at very high concentrations. It had previously been reported that the tyrosinase (MW=94 kDa) is responsible of the toxic effect of cephalopoda ink (Prota et *al.,* 1981, Palumbo et *al.*, 1985, 1994, Takaya et *al.*, 1994, Naraoka et *al.*, 2000, 2003). At this point, we can only hypothesize that the cytotoxicity of the *Octopus* $F_{sup10}$ is also due to the enzymatic effect of tyrosinase, but this is to be confirmed.

The *Sepia* ink oligopeptides extracted using the protease Pepsin also inhibited the growth of PC-3 cells. In U-87 cells, *Sepia* ink oligopeptides caused a linear decrease of cell viability in a dose-dependent manner. However, the mechanism of the anticancer activity is unclear. Therefore, further studies are needed to identify the mechanism of the potent antitumor activity.

Finally we can deduce that the fractions $F_{inf\ 10}$ et $F_{sup\ 10}$, respectively from *S. officinalis* and *O. vulgaris*, do not have antiproliferative but are responsible of antiadhesive and anti-migration activity. However, we still have to investigate whether these antitumor activities are due to one or more chemical components and to determine their chemical nature and molecular mechanisms that are implied. The results of our study also demonstrated the effect of *Sepia* ink oligopeptides on growth inhibition and could be a potentially useful adjunct in the treatment of cancer. Hence, since the cephalopod species *S. officinalis* and *O. vulgaris* are easily accessible Tunisian marine resources, their ink protein wastes are attractive as a protein source for the future industrial production of functional peptides.

# PROTITUMORSKA AKTIVNOST ČRNILA PRI SIPI *SEPIA OFFICINALIS* IN HOBOTNICI *OCTOPUS VULGARIS* (CEPHALOPODA) IZ SEVERNE TUNIZIJSKE OBALE (OSREDNJE SREDOZEMSKO MORJE)

## *POVZETEK*

*Avtorji poročajo o protitumorskih* učinkih peptidov iz črnila dveh glavonožcev in sicer sipe, Sepia officinalis *(Linnaeus, 1758), in hobotnice,* Octopus vulgaris *(Cuvier, 1797), dobljenih na primerkih, ujetih ob severnotunizijskih obalah (osrednje Sredozemsko morje). Rezultati prikazujejo, da učinkovine iz surovega črnila kažejo protiadhezijsko aktivnost na celice IGR39 v odvisnosti od testiranih izvenceličnih matriksov. Delno prečiščena frakcija z molekulsko maso, manjšo od 10 kDa pri vrsti* Sepia officinalis *($F_{inf10}$) in višjo od 10 kDa pri vrsti* Octopus vulgaris *($F_{sup10}$) sta pokazali koncentracijsko odvisno protiinvazivno, protimigracijsko in protiadhezivno aktivnost na celičnih linijah glioma U87. Delno prečiščene frakcije niso pokazale nobenih protiproliferativnih aktivnosti, MTT protokol pa je pokazal toksični učinek le v primeru visoke koncentracije frakcije črnila.*

**Ključne besede:** Cephalopoda, črnilo sipe**,** črnilo hobotnice, protitumorska aktivnost, encimatska hidroliza, oligopeptidi, Tunizija, osrednje Sredozemsko morje

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# SREDOZEMSKI MORSKI PSI

# *SQUALI MEDITERRANEI*

# *MEDITERRANEAN SHARKS*

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# REMARKS ON INCIDENTAL CAPTURE OF DEEP-SEA SHARKS IN MARMARA SHELF WATERS

*Hakan KABASAKAL*
Ichthyological Research Society, Tantavi mahallesi, Menteşoğlu caddesi, İdil Apt., No: 30, D: 4, Ümraniye, TR-34764, İstanbul, Turkey
e-mail: kabasakal.hakan@gmail.com

## *ABSTRACT*

*Between 23 November 2015 and 10 May 2017, 11 deep-sea sharks representing 3 families and 3 species were incidentally captured by commercial fishermen in the shelf waters of the Sea of Marmara. These species were: the bluntnose sixgill shark,* Hexanchus griseus *(Bonnaterre, 1788), the bramble shark,* Echinorhinus brucus *(Bonnaterre, 1788), and the angular rough shark,* Oxynotus centrina *(Linnaeus, 1758). All of the examined deep-sea sharks were captured at depths from 45 to <200 m. Gear-abrasions and injuries induced by gaffing or rough handling were observed in the majority of the examined specimens (n = 10; 90.9%). The present study points out that, in the Sea of Marmara,* H. griseus, E. brucus *and* O. centrina *are exposed to pressure by different fishing gears not only deployed in slope waters, but in shelf waters, too. Conservation of Marmara deep-sea sharks is a critical issue that requires an integrative approach to the implementation of protective measurements, covering both deep- and shelf water fishery.*

**Key words:** deep-sea, sharks, continental shelf, bycatch, Marmara, conservation

# OSSERVAZIONI SU CATTURE ACCIDENTALE DI SQUALI DI ACQUE PROFONDE NELLA PIATTAFORMA CONTINENTALE DEL MAR DI MARMARA

## *SINTESI*

*Tra il 23 novembre 2015 e il 10 maggio 2017, 11 squali di acque profonde, che rappresentano 3 famiglie e 3 specie, sono stati accidentalmente catturati nelle acque della piattaforma continentale del Mar di Marmara. Le specie in questione sono: lo squalo capopiatto,* Hexanchus griseus *(Bonnaterre, 1788), il ronco,* Echinorhinus brucus *(Bonnaterre, 1788) e il pesce porco,* Oxynotus centrina *(Linnaeus, 1758). Tutti gli squali esaminati sono stati catturati a profondità tra i 45 e i <200 m. Nella maggior parte dei campioni esaminati (n = 10, 90,9%) sono state osservate abrasioni da attrezzature e lesioni indotte da uncini o manipolazione rude. Il presente studio rileva che nel Mar di Marmara* H. griseus, E. brucus *e* O. centrina *sono esposti a pressioni dovute all'uso di diversi attrezzi da pesca anche nelle acque della piattaforma continentale. La tutela degli squali delle acque profonde di Marmara è di cruciale importanza e richiede un approccio integrativo all'attuazione delle misure di protezione, considerando sia la pesca in acque profonde che in acque della piattaforma continentale.*

**Parole chiave:** acque profonde, squali, piattaforma continentale, cattura accessoria, Marmara, conservazione

## INTRODUCTION

The deep sea has always been considered inaccessible, isolated, difficult and expensive to reach and explore. The deep-sea floor is a vast habitat, covering more than 65% of the Earth's surface (Thistle, 2003), and cartilaginous fishes – sharks, rays and their relatives – are remarkable members of the ichthyofauna of this remote environment. Kyne & Simpendorfer (2007) define deep-sea chondrichthyans as those sharks, rays and holocephalans whose distribution is predominantly at or restricted to depths below 200 m, or those that spend the majority of their lifecycle below this depth. For the moment, approximately half of the known chondrichthyans, 575 of the 1207 species (47.6%), live in deep oceans, below 200 m (Cotton & Dean Grubbs, 2015).

Our knowledge of Mediterranean deep-sea sharks has remarkably and progressively increased over the past few decades (see *e.g.*, Hemida & Capapé, 2002; De Maddalena & Zuffa, 2003; Sion *et al.*, 2004; Capapé *et al.*, 2003, 2008; Kousteni & Megalofonou, 2012; Kabasakal & Bilecenoğlu, 2014; Kabasakal, 2015). Furthermore, research efforts on the deep-sea sharks of the Sea of Marmara, a subregion of the Mediterranean ecosystem, also demonstrated a promising increase during almost the same period, and the current status of Marmara deep-sea sharks has been reviewed recently in a deep-sea inventory study (Gönülal & Topaloğlu, 2016) and shark specific studies (Kabasakal & Karhan, 2015).

Every new data on deep-sea sharks of the Sea of Marmara can also be considered as a contribution to the knowledge about the deep-sea chondrichthyans of the Mediterranean Sea. In the present article, the author reports on recent cases of incidental capture of deep-sea sharks in Marmara shelf waters and discusses the risk factors decreasing the survivability of these vulnerable species.

## MATERIAL AND METHODS

### Study area

The Sea of Marmara is a 280 km long and 80 km wide intracontinental sea on the waterway between the Mediterranean and the Black Seas (Fig. 1) (Çağatay *et al.*, 2016). Its maximum depth is 1370 m, and the sea consists of three deep basins with depths exceeding 1100 m (Çağatay *et al.*, 2016). The northern part of the Sea of Marmara is characterized by a narrow shelf area, the southern sublittoral, on the other hand, is covered by a remarkably wider continental shelf (Fig. 1). The map of the Sea of Marmara depicted in Fig. 1 is based on bathymetric surveys carried out by Claude *et al.* (2001).

### Study material

The deep-sea sharks examined in the present study were incidentally captured by commercial purse-seiners and stationary netters (mainly gill-netters) (Tab. 1). Since the present study was set to be a fishery-dependent survey of Marmara deep-sea sharks, fixed-station sampling was not applicable and the observations were irregularly spread over a 2-year survey period. The author collected data on the examined species whenever and wherever a sampling occasion presented itself. The following data were recorded for the examined specimens: total length (TL), weight (W), sex, fishing date, fishing depth, fishing locality, fishing gear, and the presence of anthropogenic or fishing-gear induced injuries. The fishing localities related to the examined specimens are plotted on map (Fig. 1). The TL represents a horizontal line reaching from the tip of the snout to the tip of the upper lobe of the caudal fin, where the caudal fin is depressed to the body axis (Serena, 2005). The documentation of the species

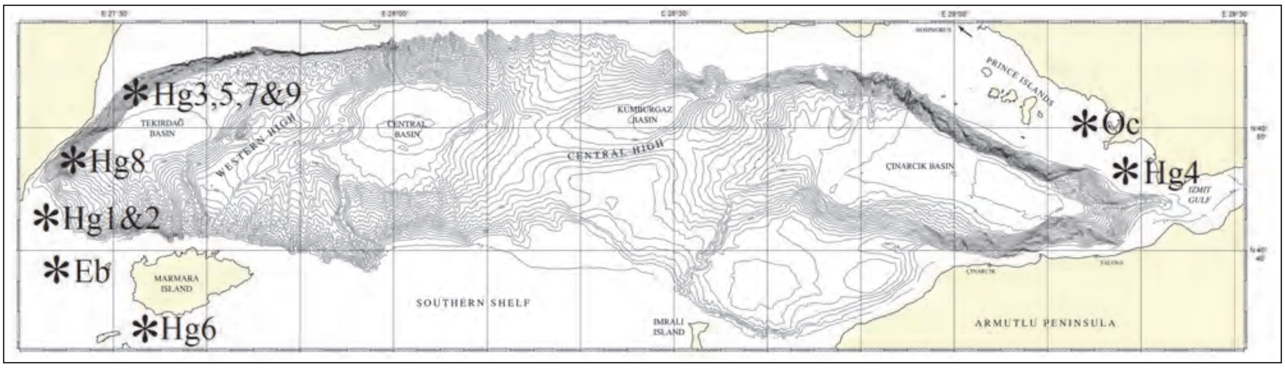

**Fig. 1: Map showing the fishing localities (*) of the examined deep-sea sharks in Marmara shelf waters during the 2015–2017 period (the numbers correspond to the numbers of specimens in Tab. 1). Hg,** *Hexanchus griseus* **; Eb,** *Echinorhinus brucus***; and Oc,** *Oxynotus centrina***.**

**Sl. 1: Zemljevid ribolovnega območja z lokalitetami (*), kjer so bili ujeti globokomorski psi na kontinentalnem pragu v Marmarskem morju med letoma 2015 in 2017 (številke se nanašajo na primerke v Tab. 1). Hg,** *Hexanchus griseus***; Eb,** *Echinorhinus brucus***; in Oc,** *Oxynotus centrina***.**

***Tab. 1: An overview of the recent capture (2015–2017) of deep-sea sharks in Marmara shelf waters. Abbreviations as follows: NW, northwestern; NE, northeastern; SW, southwestern; SM, Sea of Marmara.***
***Tab. 1: Pregled novejših ulovov (2015-2017) globokomorskih morskih psov na kontinentalnem pragu Marmarskega morja. Okrajšave: NW, severozahodni; NE, severovzhodni; SW, jugozahodni; SM, Marmarsko morje.***

<table>
<tr><td colspan="8"><b>HEXANCHIDAE</b><br><i>Hexanchus griseus</i> (Bonnaterre, 1788)</td></tr>
<tr><th>No</th><th>Date</th><th>Fishing depth (m)</th><th>Fishing locality</th><th>Fishing gear</th><th>TL (cm)</th><th>W (kg)</th><th>Sex</th></tr>
<tr><td>1</td><td>23.11.2015</td><td><100</td><td>off Şarköy, SW SM</td><td>Stationary net</td><td>200</td><td>150</td><td>♂</td></tr>
<tr><td>2</td><td>2.12.2015</td><td><100</td><td>off Şarköy, SW SM</td><td>Stationary net</td><td>200</td><td>130</td><td>♀</td></tr>
<tr><td>3</td><td>13.03.2016</td><td>150</td><td>off Tekirdağ, NW SM</td><td>Purse seine</td><td>500</td><td>?</td><td>♀</td></tr>
<tr><td>4</td><td>10.11.2016</td><td>80</td><td>off Darıca, NE SM</td><td>Stationary net</td><td>350</td><td>200</td><td>♀</td></tr>
<tr><td>5</td><td>13.12.2016</td><td>150</td><td>off Tekirdağ, NW SM</td><td>Stationary net</td><td>500</td><td>500</td><td>♀</td></tr>
<tr><td>6</td><td>24.12.2016</td><td><100</td><td>off Avşa island, SW SM</td><td>Stationary net</td><td>525</td><td>?</td><td>?</td></tr>
<tr><td>7</td><td>20.02.2017</td><td><200</td><td>off Tekirdağ, NW SM</td><td>Stationary net</td><td>200</td><td>200</td><td>♀</td></tr>
<tr><td>8</td><td>19.03.2017</td><td><100</td><td>off Mürefte, SW SM</td><td>?</td><td>300</td><td>200</td><td>♂</td></tr>
<tr><td>9</td><td>10.05.2017</td><td><100</td><td>off Tekirdağ, NW SM</td><td>Stationary net</td><td>300</td><td>150</td><td>?</td></tr>
<tr><td colspan="8"><b>ECHINORHINIDAE</b><br><i>Echinorhinus brucus</i> (Bonnaterre, 1788) (Fig. 2)</td></tr>
<tr><th>No</th><th>Date</th><th>Fishing depth (m)</th><th>Fishing locality</th><th>Fishing gear</th><th>TL (cm)</th><th>W (kg)</th><th>Sex</th></tr>
<tr><td>1</td><td>24.01.2017</td><td>45</td><td>off Şarköy, SW SM</td><td>Gill net</td><td>160</td><td>100</td><td>♀</td></tr>
<tr><td colspan="8"><b>OXYNOTIDAE</b><br><i>Oxynotus centrina</i> (Linnaeus, 1758)</td></tr>
<tr><th>No</th><th>Date</th><th>Fishing depth (m)</th><th>Fishing locality</th><th>Fishing gear</th><th>TL (cm)</th><th>W (kg)</th><th>Sex</th></tr>
<tr><td>1</td><td>28.02.2017</td><td>50</td><td>off Pendik, NE SM</td><td>Stationary net</td><td>45</td><td>1,2</td><td>♂</td></tr>
</table>

followed the best practice protocol for ichthyological first records proposed by Bello *et al*. (2014), particularly with regard to photographic identification of deep-sea sharks. For the purpose of photographic identification of previously recorded deep-sea shark species, a clear image, shot laterally and depicting details allowing conclusive identification (Bello *et al*., 2014), was required, otherwise the record was not included in the study. Raw data and photographs of the examined deep-sea sharks are preserved in the personal archives of the author.

## RESULTS AND DISCUSSION

Between 23 November 2015 and 10 May 2017, 11 deep-sea sharks representing 3 families and 3 species were incidentally captured by commercial fishermen in the shelf waters of the Sea of Marmara (Tab. 1; Fig. 1). These species were: the bluntnose sixgill shark, *Hexanchus griseus* (Bonnaterre, 1788), the bramble shark, *Echinorhinus brucus* (Bonnaterre, 1788), and the angular rough shark, *Oxynotus centrina* (Linnaeus, 1758). The majority of the specimens captured belonged to the species *H. griseus* (n=9; 81.8%), followed by *E. brucus* (n=1; 9.09%) and *O. centrina* (n=1; 9.09%) (Tab. 1). Most of them were captured in northern Marmara shelf waters (n=7; 63.6%), only 4 (36.3%) in the southern shelf waters (Tab. 1; Fig. 1). Ten of the examined specimens (90.9%) were captured by stationary-netters (gill nets, trammel nets), 1 examined *H. griseus* by a purse-seiner (Tab. 1). All of the examined deep-sea sharks were captured at depths <200 m, the recorded fishing depths ranging from 45 to <200 m. The TL of the examined *H. griseus* varied between 200 and 525 cm (Tab. 1). Injuries induced by fishing gear interactions

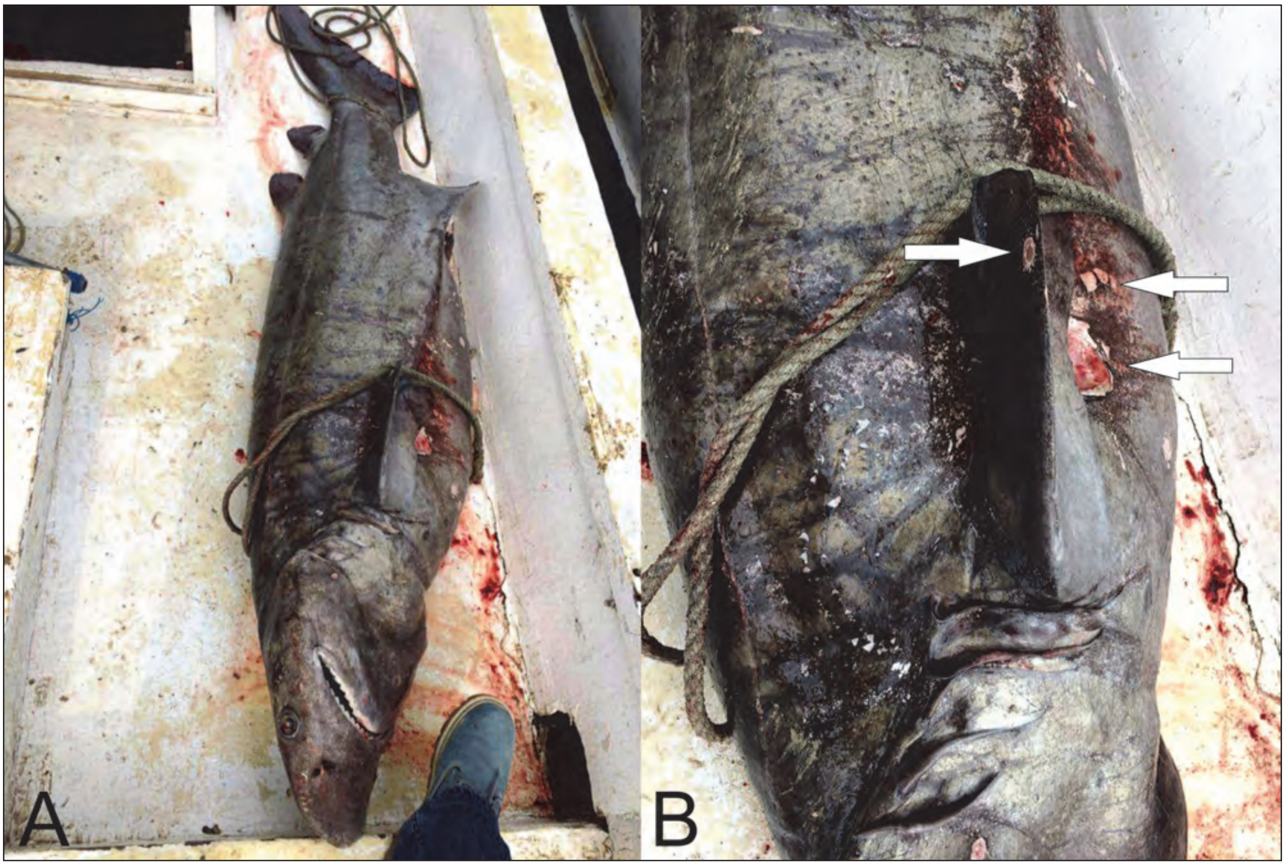

***Fig. 2: (A) The bramble shark,* Echinorhinus brucus*, captured in the south-western Sea of Marmara. (B) Arrows indicate fishing gear-induced injuries, such as deep lacerations on the left pectoral fin and ventral surface of the body. (Photo: Ichthyological Research Society Archives).***

***Sl. 2: Sl. 2: (A) Bodičasti morski pes,* Echinorhinus brucus*, ujet v jugozahodnem Marmarskem morju. (B) Puščice označujejo poškodbe nastale zaradi ribolovnega orodja kot so globoke rane na levi prsni plavuti in na trebušnem delu telesa. (Foto: Ichthyological Research Society Archives).***

(abrasions), gaffing or rough handling were observed in most of the examined specimens (n=10; 90.9%) (Fig. 2).

The pioneering writings on deep-sea sharks in the Sea of Marmara date back to the early 20th century. Based on the sharks landed at the İstanbul fish market, Ninni (1923) and Deveciyan (1926) reported on capture of *H. griseus* and *E. brucus*, providing brief biological notes on these species. Later, Erazi (1942), Kocataş *et al.* (1993), Meriç (1995) and Kabasakal (2003) confirmed the occurrence of *H. griseus* and *O. centrina* in Marmara waters; whereas the occurrence of *E. brucus* in the Sea of Marmara was only reconfirmed about a century after the first record (Kabasakal *et al.*, 2005). Contemporary occurrence of *H. griseus*, *E. brucus* and *O. centrina* in the Sea of Marmara was reported in recent shark-specific reviews (Kabasakal & Karhan, 2015).

Compared to the 9 specimens of *H. griseus* incidentally captured during the 2-year study period, only 1 specimen of *E. brucus* and 1 of *O. centrina* were captured during the same period, corroborating the suggested rarity of these two deep-sea sharks in the Sea of Marmara (Kabasakal & Karhan, 2015). Based on the Sixgill Shark Database of Turkey, 60% of the incidentally captured specimens of *H. griseus* (n=90) were recorded in the Sea of Marmara between 1967 and 2013 (Kabasakal, 2013). Following the species reoccurrence in Marmara waters in October 2002 (Kabasakal *et al*., 2005), 5 new specimens of *E. brucus* were captured in the Sea of Marmara, the last occurrence dated to 19 May 2010 (Kabasakal & Bilecenoğlu, 2014). In a recent review of *O. centrina* occurrences in eastern Mediterranean, Kabasakal (2015) stated that as of October 2012, 21.5% (n=19) of catches of angular rough shark were recorded in the Sea of Marmara. In a recent survey on the large sharks of Turkish waters, Kabasakal *et al*. (2017) concluded that 43.2% of the incidentally captured large sharks between 1990 and 2015 were *H. griseus* specimens (n=169), with the contribution of *E.*

*brucus* to total specimens captured (n=392) accounting for less than 2% (n=7).

The deep-sea sharks examined in the present survey, *H. griseus*, *E. brucus* and *O. centrina,* occur at a depth of 100–1000 m, 200–900 m, and 60–660 m, respectively (Serena, 2005). However, the published maximum depths for each of the mentioned species can be different, as reported by Sion *et al*. (2004) and Kabasakal *et al*. (2005). According to Sion *et al*. (2004), *H. griseus* was recorded at a depth of 1300 m in the eastern Ionian Sea, and *O. centrina* was recorded at a depth of 800 m in the western Ionian Sea. Moreover, imaging surveys carried out by remotely operated vehicles in the northern Sea of Marmara revealed the presence of *E. brucus* at a depth of 1214 m in the Tekirdağ deep basin (Kabasakal *et al*., 2005).

Deep areas in the Mediterranean Sea and adjacent waters, where trawling did not occur in the past, provided secure shelter for deep-sea sharks. However, with the advancement of deep fishing gear, commercial fishing operations in these former shelters are gradually increasing and contributing importantly to the vulnerability and the depth shift of deep-sea sharks. During the DESEAS survey carried out in the Mediterranean Sea (depth range 600 to 2800 m; Sion *et al*., 2004) and imaging surveys carried out in Marmara deep basins (>1100 m depth; Kabasakal *et al*., 2005), the mentioned depth shifts were recorded for *H. griseus*, *E. brucus* and *O. centrina*, and are summarised in the above paragraph. However, in the present study, the majority of the examined specimens (n=8; 72.7%; Tab. 1) were recorded at depths shallower than their published minimum depth limits. Thus, these findings suggest that *H. griseus*, *E. brucus* and *O. centrina* can be incidentally captured by commercial fishermen operating in Marmara shelf waters.

Previous research has also shown that *H. griseus*, *E. brucus* and *O. centrina* can occur in waters shallower than their published minimum depth limits. According to Kabasakal (2013), *H. griseus* can occur in coastal waters between depths of 10 and 50 m. A specimen of *E. brucus* was recorded in shelf waters at a depth of 100 m, and another one at 150 m (Kabasakal & Bilecenoğlu, 2014). Occurrence of *O. centrina* in shallow waters ($\leq$30 m depth) off Prince Islands and in the pre-Bosphoric area was reported by Kabasakal (2009, 2015). An examination of the localities of shallow water with records of deep-sea shark occurrence, as indicated in the present study, as well as in previous reports, reveals that they are situated near slope areas in the vicinity of Marmara deep basins (Fig. 1). In the light of these findings, the following question arises: What makes the shelf waters a death zone for deep-sea sharks?

Broadly speaking, Marmara shelf waters represent an important fishing ground and an area where 17% of the Turkish fishing fleet operates (Demirel & Gül, 2016). Furthermore, the Sea of Marmara is an important area for demersal fishery, and besides the legally permitted demersal fishing gears (*e.g*., stationary nets, bottom long-lines, hand-lines), illegal bottom-trawls and beam-trawls are also used on the Marmara sea floor (Demirel & Gül, 2016). Illegal bottom-trawling and beam-trawling are two leading sources of bycatch in the Sea of Marmara. According to Demirel & Gül (2016), 55% of total catch by illegal trawlers is discarded and chondrichthyan fish are one of the major groups observed in the discards. In most instances ($\geq$90%) the incidental capture of *E. brucus* and *O. centrina* in the Sea of Marmara was linked to demersal fisheries (Kabasakal & Bilecenoğlu, 2014; Kabasakal, 2015).

In addition to illegal bottom- and beam-trawling, stationary-netting and purse seining are also among the leading causes of mortality of Marmara deep-sea sharks. In a recent review of fishing pressure on Marmara chondrichthyans, Yığın *et al*. (2016) reported that incidental capture of chondrichthyans is mostly recorded in purse-seining and stationary-netting. In the present study, 83% of the examined deep-sea sharks were incidentally captured by demersal fishing gears (Tab. 1). Only 1 bluntnose sixgill shark was captured by purse-seining (Spec. No. 3; Tab. 1). However, previous studies showed that the leading cause of incidental capture of *H. griseus* in the Sea of Marmara was purse-seiners catching small pelagics (*e.g*., sardine, anchovy) and other pelagics (*e.g*., bonito, greater amberjack) (Kabasakal *et al*., 2017). Since *H. griseus* is a demersal shark species, it might be expected for incidental catches of this shark to occur more frequently in demersal fishery. However, the bluntnose sixgill shark is known to rise to surface waters at night in pursuit of its prey (diel migration pattern), which includes schools of small and other pelagics with high commercial value in purse-seine fishery (Andrews *et al*., 2009; Kabasakal, 2013). Its diel migration for feeding purposes accounts for the frequent occurrence of incidental capture of *H. griseus* in purse-seine fishery conducted in northern Marmara shelf waters. According to Serena (2005), in the Mediterranean Sea, *H. griseus* is caught as bycatch by bottom trawls and longlines in epibathyal and bathyal grounds. In one of his previous surveys, Kabasakal (2013) reported on the catch data of *H. griseus* in the Sea of Marmara and stated that the bluntnose sixgill shark is mostly caught on the shelf and upper slope regions of Marmara waters. During the 46-year research period, only one bluntnose sixgill shark was caught at a 1000 m depth by means of a drop-line (Kabasakal, 2013). In the Sea of Marmara, commercial fishermen do not deploy their fishing gear over bathyal grounds and their fishing activities are concentrated on shelf and upper slope waters (Hakan Kabasakal, *pers. obs*.), which could explain the rarity of bathyal records of *H. griseus* in the fishing business conducted in the Sea of Marmara.

Based on this data, it is clear that the examined deep-sea sharks – *H. griseus*, *E. brucus* and *O. centrina* – came in contact with multiple gear types (*e.g*., bottom- and beam-trawls, gill- and trammel-nets, bottom long-lines and purse-seines) used in Marmara shelf water fishery, and that these species are under severe multi-gear fishing pressure. According to Stevens *et al*. (2000), chondrichthyans are a common, though unspecified bycatch in many fisheries, particularly those using demersal trawls, long-lines or gill nets. However, there is yet another risk factor jeopardising the survivability of deep-sea sharks hauled on the decks of fishing boats: injuries induced by fishing gear or human handling.

Physical injuries induced by fishing gear interactions (abrasions), gaffing or rough handling may have lethal consequences and contribute to post-release mortality of sharks (Skomal & Chase, 2002; Skomal, 2007). Kabasakal (2010) reported that the post-release survivability of *H. griseus* specimens incidentally captured in Turkish waters decreased because of these injuries, and emphasized that gear-induced injuries can contribute to the cryptic mortality of this species. In the present study, gear abrasions or injuries induced by gaffing or rough handling were observed in most of the examined deep-sea sharks (n=10; 90.9%; Fig. 2). No such injuries were observed on the body surface of the single specimen of *O. centrina*, which was released immediately after the hauling. Since gear-induced injuries can cause serious damage and pose a future health risk, it is questionable whether the hauled and released specimens of *H. griseus* and *E. brucus* can survive with these serious injuries. Observations of a released bluntnose sixgill shark captured by a commercial bottom-trawler in the Bay of Saros (NE Aegean Sea) revealed that, upon release, it exhibited aberrant behaviour with successive "rise and sink" movements, lost its equilibrium and eventually died (Kabasakal, 2010).

Based on depth ranges recorded in several surveys, Gönülal & Topaloğlu (2016) grouped the deep-sea sharks of the Sea of Marmara into 1st group (200–500 m), 2nd group (500–1000 m) and 3rd group (>1000 m) species. Accordingly, *Centrophorus granulosus*, *C. uyato*, *Dalatias licha*, *Mustelus asterias*, *O. centrina*, *Scyliorhinus canicula*, *Squalus acanthias* and *S. blainvillei* are included in the 1st group; *E. brucus* in the 2nd group; and *Galeus melastomus* in the 3rd group. However, the list by Gönülal & Topaloğlu (2016) omits *H. griseus*, the most common deep-sea shark occurring in the Sea of Marmara (Kabasakal & Karhan, 2015). Kabasakal (2016) reviewed the status of chondrichthyan fishes in the Sea of Marmara and concluded that, due to their absence from fishery records in the last 20 years or more, the presence of the *Centrophorus* species and of *D. licha* in Marmara waters should be considered questionable and required confirmation.

In conclusion, the present study confirms the contemporary presence of *H. griseus*, *E. brucus* and *O. centrina* in the Sea of Marmara, and points out that Marmara deep-sea sharks are exposed to fishing pressure by different fishing gears not only deployed in slope waters but in shelf waters, too. Since only 1 specimen of *E. brucus* and 1 of *O. centrina* were captured during the 2-year survey, the study also confirms the suggested rarity (Kabasakal & Karhan, 2015) of these two species in the Sea of Marmara. In the light of available data it is possible to assume that the combined effects of rarity and fishing pressure makes *E. brucus* and *O. centrina* more vulnerable; further study is necessary to clarify whether these two shark species should be classified as "in critical danger of extinction." Due to injuries by fishing gear, Marmara deep-sea sharks may be at risk for post-release disability or mortality. Slow growth, longevity, long life span, delayed maturity and low fecundity are common patterns of *k*-selected deep-sea ichthyofauna (Shotton, 2005), and deep-sea sharks cannot be excluded from this definition (Camhi *et al*., 1998; Stevens *et al*., 2000). Therefore, deep-sea sharks are highly vulnerable to targeted or untargeted fishery, and in case of overexploitation, the recovery of their populations could take several decades or more. Conservation of Marmara deep-sea sharks is a critical issue that requires an integrative approach to the implementation of protective measurements, covering both deep- and shelf water fishery.

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# OPAŽANJA O NAKLJUČNEM ULOVU GLOBOKOMORSKIH MORSKIH PSOV NA CELINSKEM PRAGU V MARMARSKEM MORJU

*Hakan KABASAKAL*
Ichthyological Research Society, Tantavi mahallesi, Menteşoğlu caddesi, İdil Apt., No: 30, D: 4, Ümraniye, TR-34764, İstanbul, Turkey
e-mail: kabasakal.hakan@gmail.com

## *POVZETEK*

*Med 23 novembrom 2015 in 10 majem 2017 so komercialni ribiči naključno ulovili 11 globokomorskih morskih psov treh vrst na kontinentalnem pragu Marmarskega morja. Morski psi so pripadali sledečim vrstam:* šesteroškrgarju, Hexanchus griseus *(Bonnaterre, 1788), bodičastemu morskemu psu,* Echinorhinus brucus *(Bonnaterre, 1788) in morskemu prašiču,* Oxynotus centrina *(Linnaeus, 1758). Preiskani morski psi so bili ujeti na globinah med 45 m in skoraj 200 m. Pri večini primerkov so bile vidne poškodbe in rane, nastale s kavlji in zaradi grobega rokovanja. Pričujoče delo potrjuje, da se v Marmarskem morju vrste* H. griseus, E. brucus *in* O. centrina *soočajo z ribolovnimi pritiski z različnimi ribolovnimi orodji ne samo v globinah, večjih od 200 m, ampak tudi na kontinentalnem pragu. Da bi zavarovali globokomorske pse v Marmarskem morju je ključen celovit pristop pri uveljavitvi varovalnih ukrepov, tako na nivoju ribolova v kontinentalnem pragu kot tudi v globljih vodah.*

**Ključne besede:** globokomorski morski psi, kontinentalni prag, prilov, Marmarsko morje, varovanje

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# FIRST RECORDS OF VELVET BELLY LANTERN SHARK *ETMOPTERUS SPINAX* (CHONDRICHTHYES: ETMOPTERIDAE) FROM THE SYRIAN COAST (EASTERN MEDITERRANEAN)

*Christian CAPAPÉ*
Laboratoire d'Ichtyologie, case 104, Université Montpellier 2, Sciences et Techniques du Languedoc 34095 Montpellier cedex 5, France
E-mail: capape@univ-montp2.fr

*Malek ALI*
Marine Sciences Laboratory, Basic Sciences Department, Faculty of Agriculture, Tishreen University, Lattakia, Syria

## *ABSTRACT*

*This paper reports the first records of velvet belly lantern shark* Etmopterus spinax *(Linnaeus, 1758) from the Syrian coast. They are related to three juvenile females measuring 298.6 mm, 306.3 mm and 317.1 mm in total length, respectively, and weighing 102.3 g, 110.4 g and 106.6 g, respectively. The specimens are described and commented with respect to other records from the local area and the Mediterranean Sea.*

**Key words:** Etmopteridae, *Etmopterus spinax*, deep sea shark, Eastern Mediterranean

# PRIMI RITROVAMENTI DI MORETTO *ETMOPTERUS SPINAX* (CHONDRICHTHYES: ETMOPTERIDAE) LUNGO LA COSTA SIRIANA (MEDITERRANEO ORIENTALE)

## *SINTESI*

*L'articolo riporta i primi ritrovamenti del sagrì nero o moretto* Etmopterus spinax *(Linnaeus, 1758) lungo la costa siriana. Si tratta di tre giovani femmine, rispettivamente di 298,6 mm, 306,3 mm e 317,1 mm di lunghezza totale, e 102,3 g, 110,4 g e 106,6 g di peso. Gli autori forniscono una descrizione degli individui e ne discutono la presenza su scala locale e più ampia del Mediterraneo.*

**Parole chiave:** Etmopteridae, *Etmopterus spinax*, squalo abissale, Mediterraneo orientale

## INTRODUCTION

Velvet belly lantern shark *Etmopterus spinax* (Linnaeus, 1758) is a small-sized common shark, well-known in the eastern Atlantic from Iceland and Norway to Portugal (Quéro *et al.*, 2003). To the south of the Strait of Gibraltar, the species is reported off Morocco (Lloris & Rucabado, 1998), Mauritania (Maurin & Bonnet, 1970), Senegal (Cadenat & Blache, 1981), Guinea Bissau (Sanches, 1991), the Azores (Santos *et al.*, 1997), Madeira (Sanches, 1986) and the Cape Verde Islands (Menezes *et al.*, 2004), as well as in southern Africa (Compagno, 1984).

*E. spinax* is known to be more commonly caught in the western Mediterranean Basin (Capapé, 1989; Capapé *et al.*, 2000); mainly off the Tunisian and Sicilian coasts (Capapé *et al.*, 2001; Porcu, 2014). Lipej & Dulcic (2010) noted its occurrence in the Adriatic Sea; eastwards, the species is reported in the Aegean Sea (Papaconstantinou, 2014), in Turkish waters (Bilecenoglu *et al.,* 2014), off the Egyptian coast (Farrag, 2016), and in the Levant Basin (Golani, 2005). Conversely, the species is not recorded off the Lebanese coast (Mouneimne, 1979). However, investigations conducted nearby, on the Syrian coast, have allowed the collection of three specimens, the records of which form the subject of the present paper and are here described and commented on with respect to their distribution in this new capture area, as well as in the Mediterranean Sea.

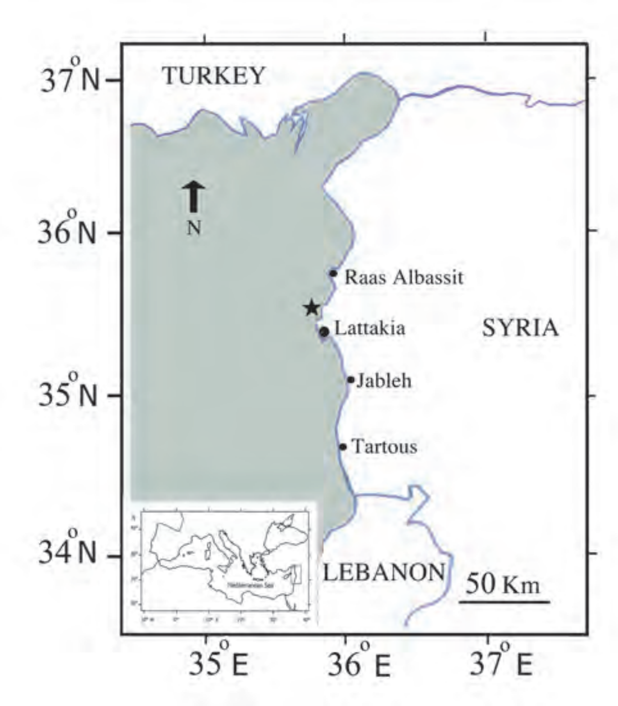

***Fig. 1: Map of the Mediterranean showing Syria and map of the Syrian coast indicating the capture site of*** *Etmopterus spinax* ***(black star).***
***Sl. 1: Zemljevid Sredozemskega morja in sirske obale z označbo lokalitete, kjer so bili ujeti primerki žametnega trneža*** *Etmopterus spinax* ***(črna zvezdica).***

## MATERIAL AND METHODS

Information on the capture of *E. spinax* was provided by local fishermen aware of the fishing grounds. The researchers engage the help of the local communities, referring to it as local ecological knowledge (*sensu* Anadòn *et al.*, 2009), in order to spread and heighten the awareness in fisheries research. The description of the specimens in the present paper follows the protocol recommended by Bello *et al.* (2014) for first records.

On 19 August 2017, three specimens of *E. spinax* (Linnaeus, 1758) were caught in Syrian marine waters during a trawl trip from the city of Lattakia southwards as far as the city of Jableh (between 35° 31´ N, 35° 39´ E and 35° 16´ N, 35° 49´ E), at a depth of about 375 m, on sandy and muddy bottoms (Fig. 1). All measurements were carried out using digital caliper and recorded to the nearest 0.1 millimetre, the weights to the nearest 0.1 gram. The three specimens were preserved in 10% buffered formalin and deposited in the Ichthyological Collection of the Laboratory of Marine Sciences, Faculty of Agriculture, Tishreen University, Syria, under catalogue numbers M.S.L. 2316, 2317 and 2318, respectively (Fig. 2).

## RESULTS AND DISCUSSION

All specimens were identified as *E. spinax* through a combination of the following characteristics: body robust with a fairly long tail, snout moderately long, broad and flattened, both dorsal fins bearing stout, grooved spines at the front, with the second fin much longer than the first and curved; mouth with thin, smooth lips; upper teeth small with a narrow central cusp and two pairs of lateral cusplets; lower teeth larger with a strongly slanted, blade-like cusp at the top and interlocking bases (Fig. 3); five pairs of tiny gill slits, comparable in size to spiracles; first dorsal fin originating behind short and rounded pectoral fins; second dorsal fin larger than the first and originating behind the pelvic fins; anal fin absent; tail slender, leading to a long caudal fin with a small lower lobe and a

***Fig. 2: An*** *Etmopterus spinax* ***captured off the Syrian coast (specimen Ref. 2316); scale bar 50 mm.***
***Sl. 2: Primerek žametnega trneža*** *Etmopterus spinax* ***(kataloška številka Ref. 2316); merilo 50 mm.***

***Fig. 3: Ventral surface of the head of** Etmopterus spinax* ***(specimen Ref. 2316), showing teeth on the upper and lower jaws, and a dermal denticle; scale bar 10 mm.***
***Sl. 3: Spodnja polovica glave primerka vrste** Etmopterus spinax* ***(kataloška številka Ref. 2316). Vidijo se zobje v zgornji in spodnji čeljusti ter kožni dentikli; merilo 10 mm.***

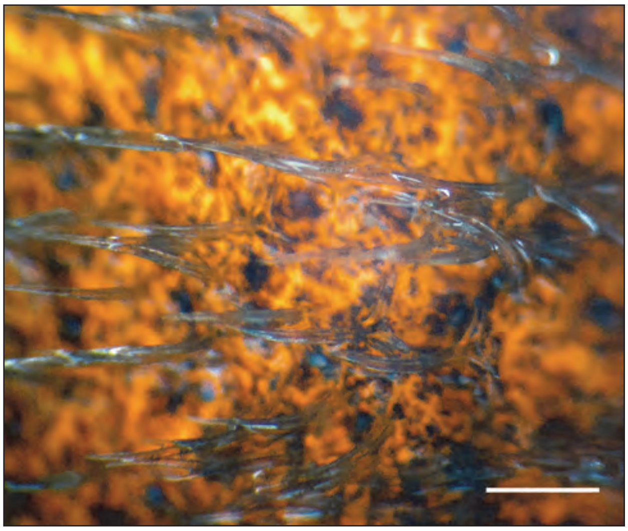

***Fig. 4: Dermal denticles removed from** Etmopterus spinax* ***(specimen Ref. 2317); scale bar 0.2 mm.***
***Sl. 4: Kožni dentikli vrste** Etmopterus spinax* ***(kataloška številka Ref. 2317); merilo 0,2 mm.***

low upper lobe with a prominent ventral notch near the tip; dermal denticles thin with hooked tips, occurring in an irregular pattern and well separated from one another (Fig. 4); coloration brown above, abruptly transitioning to black below; black markings on flanks above and behind the pelvic fins, and along the caudal fin.

The morphology, measurements, counts and colour are in total agreement with previous descriptions of *E. spinax* by Tortonese (1956), Cadenat & Blache (1981), Compagno (1984) and Mc Eachran & Branstetter (1984). The three captures of *E. spinax* that constitute the first records of the species from the Syrian coast have increased the number of elasmobranch reported to date in the region to a total of 43. Following Mc Eachran & Branstetter (1984), a second species belonging to the genus *Etmopteru*s Rafinesque, 1810 is believed to occur in the Mediterranean Sea, namely the smooth lantern shark *E. pusillus* (Lowe, 1839), which can be easily distinguished from *E spinax* by crater-shaped dermal denticles without the medial spine and flanks without the conspicuous black markings.

Previous studies on the reproductive biology of *E. spinax* showed it to be a viviparous aplacental species with a gestation period not exceeding one year. Sexual maturity is reached at similar sizes as in specimens from the areas off the British Isles (Hickling, 1963) and the Tunisian coast (Capapé, *et al*., 2001), in each area the males mature at a smaller size than the females, at 350 mm and 380 mm, respectively, with 460 mm as the maximum size recorded for both sexes. However, Compagno (1984) observed that the maximum size for this species was 600 mm. The three specimens presented in this study were females, measuring 298.6 mm, 306.3 mm and 317.1 mm in TL, respectively, and weighing 102.3 g, 110.4 g and 106.6 g, respectively. The dissection of the abdominal cavities of all three specimens revealed whitish and undeveloped ovaries, thread-like oviducts and inconspicuous oviducal glands – patterns characteristics of juvenile females (Hickling, 1963; Capapé *et al*., 2001). This could mean they did not reach the size at first sexual maturity, which in females from different Mediterranean marine regions ranges between 340 and 380 mm (Porcu *et al*., 2014). Conversely, in specimens from the Atlantic, southern Portugal (Coelho & Erzini, 2008), the first sexual maturity is reached at a smaller size. Therefore, further records are needed before determining the size at first sexual maturity for *E. spinax* from the Syrian coast. The stomachs of the three Syrian specimens were empty; however, Capapé *et al*. (2003) and Fanelli *et al*. (2009) noted that small specimens from Tunisian waters and western Mediterranean fed on crustaceans and cephalopods, while larger specimens fed on small teleost species, as corroborated by ontogenetic changes caused by such a diet.

*E. spinax* is reported in both Mediterranean Basins in waters ranging between 150-200 m and 400 m, and probably deeper (Quignard & Capapé, 1971), as it has been recorded at depths as low as 2,200 m in the Ionian Sea (Sion *et al*, 2004). Due to economic and technical reasons, deep-sea waters are rather poorly exploited by commercial vessels; also, this species is not of interest to fishermen and is generally discarded at sea when caught. Such patterns are observed throughout the Mediterranean Sea, including the Syrian coast. This could explain why *E. spinax* had not been captured here before and why the records of these three specimens constitute only the first report of the species from this area.

***Tab. 1: Morphometric measurements (in mm) with percentages of total length (%TL) recorded for the three specimens of* Etmopterus spinax *captured off the Syrian coast.***
***Tab. 1: Morfometrične meritve (v mm) in njihov delež glede na celotno dolžino telesa (%TL), opravljene na treh primerkih vrste* Etmopterus spinax, *ujetih ob obali Sirije.***

| **References** | **2316 M.S.L.** | | **2317 M.S.L.** | | **2318 M.S.L.** | |
|---|---|---|---|---|---|---|
| Morphometric measurements | mm | TL% | mm | TL% | mm | TL% |
| Total length | 317.1 | 100.0 | 298.6 | 100.0 | 306.3 | 100.0 |
| standard length | 251.2 | 79.2 | 236.1 | 79.1 | 242.1 | 79.0 |
| Head length | 71.5 | 22.5 | 66.8 | 22.4 | 68.3 | 22.3 |
| Prespiracular length | 38.9 | 12.3 | 36.9 | 12.4 | 37.9 | 12.4 |
| Spiracle length | 5.2 | 1.6 | 4.7 | 1.6 | 5.1 | 1.7 |
| Preorbital length | 25.9 | 8.2 | 24.6 | 8.2 | 25.3 | 8.3 |
| Eye length | 19.2 | 6.1 | 18.1 | 6.1 | 18.5 | 6.0 |
| Prenarial length | 6.4 | 2.0 | 6.1 | 2.0 | 6.3 | 2.1 |
| Preoral length | 31.7 | 10.0 | 29.9 | 10.0 | 30.2 | 9.9 |
| Nostril width | 9.4 | 3.0 | 9.1 | 3.0 | 8.6 | 2.8 |
| Mouth width | 28.9 | 9.1 | 27.4 | 9.2 | 28.4 | 9.3 |
| Pre-first dorsal-fin length | 104.3 | 32.9 | 98.3 | 32.9 | 101.6 | 33.2 |
| First dorsal-fin length | 27.7 | 8.7 | 26.2 | 8.8 | 37.6 | 12.3 |
| First dorsal-fin base | 11.2 | 3.5 | 10.7 | 3.6 | 10.8 | 3.5 |
| First dorsal-fin height | 12.6 | 4.0 | 11.9 | 4.0 | 12.1 | 4.0 |
| First dorsal fin spine length | 16.1 | 5.1 | 15.1 | 5.1 | 15.2 | 5.0 |
| Pre-second dorsal-fin length | 193.6 | 61.1 | 182.6 | 61.2 | 189.3 | 61.8 |
| Second dorsal-fin length | 36.4 | 11.5 | 34.1 | 11.4 | 36.2 | 11.8 |
| Second dorsal-fin base | 22.4 | 7.1 | 21.3 | 7.1 | 22.4 | 7.3 |
| Second dorsal-fin height | 20.2 | 6.4 | 19.3 | 6.5 | 20.1 | 6.6 |
| Second dorsal fin spine length | 19.4 | 6.1 | 18.2 | 6.1 | 19.1 | 6.2 |
| Prepectoral-fin length | 77.1 | 24.3 | 72.8 | 24.4 | 74.6 | 24.4 |
| Pectoral-fin base | 19.2 | 6.1 | 18.3 | 6.1 | 18.3 | 6.0 |
| Pectoral-fin length | 27.6 | 8.7 | 26.3 | 8.8 | 26.3 | 8.6 |
| Prepelvic-fin length | 166.2 | 52.4 | 156.8 | 52.5 | 161.8 | 52.8 |
| Pelvic-fin length | 37.1 | 11.7 | 35.1 | 11.8 | 24.9 | 8.1 |
| Pelvic-fin base | 25.5 | 8.0 | 23.9 | 8.0 | 23.8 | 7.8 |
| Precaudal-fin length | 251.2 | 79.2 | 236.8 | 79.3 | 243.8 | 79.6 |
| Dorsal caudal-fin margin | 66.1 | 20.8 | 62.2 | 20.8 | 34.5 | 11.3 |
| Preventral caudal-fin margin | 29.7 | 9.4 | 28.3 | 9.5 | 29.2 | 9.5 |
| Upper postventral caudal-fin margin | 47.3 | 14.9 | 44.3 | 14.8 | 46.4 | 15.1 |
| Tooth rows on upper jaw | 25 | | 25 | | 25 | |
| Tooth rows on lower jaw | 25 | | 25 | | 25 | |
| Total weight (g) | 106.6 | | 102.3 | | 110.4 | |

# PRVI ZAPIS O POJAVLJANJU ŽAMETNEGA TRNEŽA *ETMOPTERUS SPINAX* (CHONDRICHTHYES: ETMOPTERIDAE) IZ SIRSKIH VODA (VZHODNI MEDITERAN)

*Christian CAPAPÉ*
Laboratoire d'Ichtyologie, case 104, Université Montpellier 2, Sciences et Techniques du Languedoc 34095 Montpellier cedex 5, France
E-mail: capape@univ-montp2.fr

*Malek ALI*
Marine Sciences Laboratory, Basic Sciences Department, Faculty of Agriculture, Tishreen University, Lattakia, Syria

## *POVZETEK*

*Avtorja poročata o prvih podatkih o pojavljanju žametnega trneža* Etmopterus spinax *(Linnaeus, 1758) iz sirskih voda. Nanaša se na ulov treh samic, ki so merile 298,6 mm, 306,3 mm in 317,1 mm v dolžino in tehtale 102,3 g, 110,4 g in 106,6 g. Avtorja te primerke opisujeta in razpravljata o pojavljanju vrste v lokalnem in širšem sredozemskem merilu.*

**Ključne besede:** Etmopteridae, *Etmopterus spinax*, globokomorski morski pes, vzhodno Sredozemsko morje

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# ON THE JAWS OF A SHORTFIN MAKO SHARK, *ISURUS OXYRINCHUS*, CAUGHT OFF THE İZMİR PENINSULA (CENTRAL AEGEAN SEA, TURKEY)

*Hakan KABASAKAL*
Ichthyological Research Society, Tantavi mahallesi, Menteşoğlu caddesi, İdil Apt., No: 30, D: 4, Ümraniye, TR-34764, İstanbul, Turkey
e-mail: kabasakal.hakan@gmail.com

## *ABSTRACT*

*An old record of shortfin mako shark,* Isurus oxyrinchus*, is discussed based on the set of upper and lower jaws dissected from a specimen caught off the İzmir Peninsula, central Aegean Sea, Turkey, which is now on display at the Kuşadası fish market. The specimen was captured as bycatch by a purse-seiner in the early 1990s. Based on the lower jaw circumference to total length ratio for* I. oxyrinchus*, the total length of this specimen was estimated at 1.8 m.*

**Key words:** shortfin mako shark, *Isurus*, Kuşadası, jaws, historical record

# MASCELLE DI SQUALO MAKO, *ISURUS OXYRINCHUS*, CATTURATO AL LARGO DELLA PENISOLA DI İZMİR (MAR EGEO CENTRALE, TURCHIA)

## *SINTESI*

*Nell'articolo viene discusso un vecchio ritrovamento di squalo mako,* Isurus oxyrinchus*, sulla base della dissezione delle mascelle superiore e inferiore di un esemplare catturato al largo della penisola di İzmir (Turchia), nel mar Egeo centrale, ora esposte al mercato dei pesci di Kuşadası. L'esemplare è stato prelevato come cattura accessoria da una rete da circuizione nei primi anni 90 del secolo scorso. In base al rapporto fra la circonferenza della mascella inferiore e lunghezza totale per la specie in questione, la lunghezza totale di questo esemplare è stata stimata a 1,8 m.*

**Parole chiave:** squalo mako, *Isurus*, Kuşadası, mascelle, ritrovamento storico

## INTRODUCTION

Shark experts often rely on teeth and jaws as valuable aids in identifying the species and size of a given shark specimen. Since shark jaws and teeth are considered attractive decoration for coastal facilities, such as restaurants, fishmongers and others, these trophies can, if reliable fishing data are available, provide valuable data sources of historical records of local sharks.

The occurrence of *I. oxyrinchus* in Turkish waters was reviewed by Kabasakal (2015), and a most recent northern Aegean Sea record was reported by Tunçer & Kabasakal (2016). In the present note, the author reports on the mentioned jaw of *I. oxyrinchus*.

## MATERIAL AND METHODS

In early September 2017, during a field trip, the author of the present article had the opportunity to examine a set of dried upper and lower jaws of a shortfin mako shark, *Isurus oxyrinchus* Rafinesque, 1810, which is on display in Kuşadası, Turkey. The shark, the dried jaw of which is shown in Figure 1, was caught by a purse-seiner off the İzmir Peninsula (Fig. 2) in the early 1990s. The dental features of the examined jaw were compared with the dental characters of *I. oxyrinchus* as described by Compagno (2002).

## RESULTS AND DISCUSSION

Anterior teeth of the examined jaw enlarged, with single dagger-shaped cusps and not forming a continuous cutting edge. Intermediate teeth very small, less than half the height of the adjacent anterior teeth (Fig. 1). Cusps of upper and lower anterior teeth flexed, tips reversed (Fig. 1). The dental features of the examined jaw coincided with the dental characters of *I. oxyrinchus* as described by Compagno (2002). The examined jaw of the shortfin mako shark is displayed as decoration in the Kuşadası fish market.

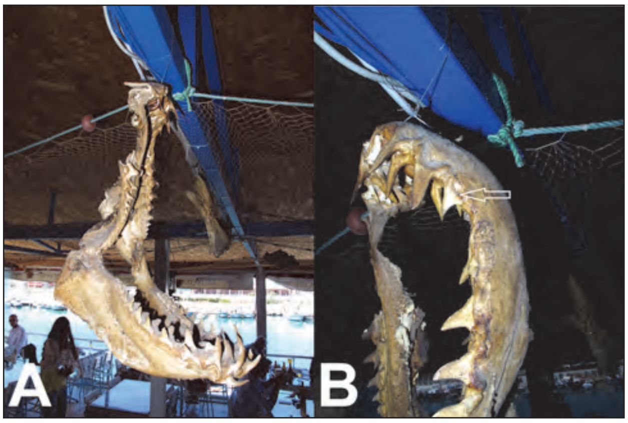

***Fig. 1: (A) Examined dried jaw of the** Isurus oxyrinchus* ***caught off İzmir peninsula in the early 1990s. (B) Anterior and intermediate teeth of the examined jaw; the arrow indicates the 3rd anterior tooth.***

***Sl. 1: (A) Preiskana posušena čeljust atlantskega maka, ujetega ob izmirskem polotoku v zgodnjih devetdesetih. (B) Sprednji in vmesni zobje iz preiskane čeljusti; puščica označuje tretji sprednji zob.***

***Fig. 2: Map depicting the approximate site of capture (★) of the examined specimen in the central Aegean Sea.***

***Sl. 2: Zemljevid obravnavanega območja z označeno približno lokaliteto (★), kjer je bil primerek ujet v osrednjem Egejskem morju.***

The circumference of the lower jaw of the examined specimen was 377.4 mm, and based on this measurement, the total length of the shortfin mako shark was estimated at 1.809 m. This measurement and the resultant total length of the examined shortfin mako shark coincided with the lower jaw circumference range (203–505 mm) and estimated total length range (1,360–3,200 mm) stated by Lowry *et al*. (2009). The height of the 1st anterior tooth on the lower jaw was 32 mm. The height of the 1st anterior tooth measured by Celona *et al*. (2004) on an estimated 390 cm long female shortfin mako, caught off Scaletta Zanclea, Sicily, on 26 July 2003, was 45 mm.

The dental formula for the upper and lower jaws of the examined shortfin mako was 12-12 / 13-13, respectively. The tooth count in *I. oxyrinchus* is remarkably variable. Based on the lower-jaw tooth count information for shortfin mako sharks from the Pacific, the Atlantic-Mediterranean and the Indian Oceans, Garrick (1967) reported the dental formulae as 11-16, 11-15 and 10-13, respectively. The dental count of the upper and lower jaws of a male shortfin mako shark (123.6 cm TL) caught in the Bay of Saroz was 14-14 / 14-14 (Kabasakal & Kabasakal, 2013). The dental formula of the Scaletta Zanclea specimen was 12-12 / 12-12 (Celona *et al*., 2004).

In a recent review of the occurrence of *I. oxyrinchus* off the Turkish coast, Kabasakal (2015) reported on the capture of 17 shortfin mako sharks in the period between 1950 and 2013. Following this review, a juvenile male (74.7 cm TL) was caught in the coastal waters of the Bay of Edremit on 8 April 2016 (Tunçer & Kabasakal, 2016). According to Kabasakal *et al*. (2017), 5.3% (n=21) of large elasmobranchs captured by the Turkish fishing fleet between 1990 and 2015 was comprised of *I. oxyrinchus*. Both historical and contemporary occurrences of shortfin mako shark in Turkish seas are corroborated by several studies (Kabasakal & De Maddalena, 2011; Ergüden *et al*., 2013; Kabasakal & Kabasakal, 2013; Kabasakal, 2015; Tunçer & Kabasakal, 2016). Most of the data on the occurrence of *I. oxyrinchus* in the waters of Turkey were obtained by fishery-dependent studies. Such studies are valuable data sources for clarifying the contemporary occurrence of shortfin mako shark and other sharks in Turkish waters. While available data on the occurrence of *I. oxyrinchus* suggest that the shortfin mako shark is a rarely occurring large shark in Turkish Aegean and Mediterranean waters (Kabasakal, 2015; Tunçer & Kabasakal, 2016), the examined jaw provides further evidence supporting its historical occurrence in the mentioned marine region.

The forensic analysis method used to determine the size of a shark based on the circumference of the jaws (Lowry *et al*., 2009) has proved to be a valuable tool in shark research. Following this method, it could be possible to estimate the sizes of previously recorded local sharks caught off the Turkish coasts of the Aegean and Mediterranean Seas – at least the species examined by Lowry *et al*. (2009) – based on their preserved jaws. The size estimations of historical records can provide valuable data in determining whether nowadays the local sharks are decreasing in size or not in comparison with historical specimens. Although the historical and contemporary occurrence of shortfin mako shark in Turkish Aegean and Mediterranean waters has been confirmed, the available knowledge does not allow us to make a reliable prediction of the seasonality of *I. oxyrinchus* in the mentioned region. Since several large specimens (TL ≥ 250 cm) have been caught in the mentioned region, and *I. oxyrinchus* is considered dangerous and responsible for unprovoked attacks on swimmers and boats (Bonfil & Abdallah, 2004), the seasonal occurrence of this species in Turkish waters should be monitored, as emphasised by Kabasakal (2015). Today, the aforementioned coastline is intensively used for aquaculture, fishery and recreational activities. Therefore, the seasonal co-existence of man and shortfin mako shark could evolve into a major problem, possibly triggering a headhunt and thus jeopardizing the survival of *I. oxyrinchus* as well as other local large predatory sharks.

## ACKNOWLEDGMENTS

I am grateful to my wife Özgür, and my son Derin, for their endless love, patience and support. I am also grateful to staff of Kuşadası Fish Market, allowing me to examine the present jaws of shortfin mako shark, and provide valuable information on the catch of the specimen, as well as two anonymous referees for their valuable comments, improving the content of the article.

# ČELJUSTI PRIMERKA ATLANTSKEGA MAKA, *ISURUS OXYRINCHUS*, UJETEGA OB IZMIRSKEM POLOTOKU (OSREDNJE EGEJSKO MORJE, TURČIJA)

*Hakan KABASAKAL*
Ichthyological Research Society, Tantavi mahallesi, Menteşoğlu caddesi, İdil Apt., No: 30, D: 4, Ümraniye, TR-34764, İstanbul, Turkey
e-mail: kabasakal.hakan@gmail.com

## *POVZETEK*

*Avtor poroča o starejšem zapisu, ki se nanaša na ulov primerka atlantskega maka,* Isurus oxyrinchus, *ob izmirskem polotoku v osrednjem Egejskem morju. Ohranjene so njegove čeljusti, ki jih razkazujejo na ribji tržnici Kuşadası. Primerek se je ujel kot prilov v povlečni mreži iz zgodnjih devetdesetih let. Na podlagi odnosa med obodom spodnje čeljusti in celotno dolžino telesa je bila ocenjena velikost primerka 1,8 m dolžine.*

**Ključne besede:** atlantski mako, *Isurus*, Kuşadası, čeljusti, zgodovinski zapis

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# IHTIOLOGIJA

# *ITTIOLOGIA*

# *ICHTHYOLOGY*

short scientific article
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# HAS A VIABLE POPULATION OF COMMON LIONFISH, *PTEROIS MILES* (SCORPAENIDAE), ESTABLISHED OFF THE SYRIAN COAST (EASTERN MEDITERRANEAN)?

*Malek ALI*
Marine Sciences Laboratory, Basic Science Department, Faculty of Agriculture, Tishreen University, Lattakia, Syria

*Christian REYNAUD*
Laboratoire Interdisciplinaire de Recherche sur la Didactique, l'Education et la Formation, E.A. 3749, Composante «Didactique et Socialisation», Faculté d'Éducation, Université de Montpellier, 2, Place Marcel Godechot, B.P. 4152, 34095 Montpellier cedex 5, France

*Christian CAPAPÉ*
Laboratoire d'Ichtyologie, case 104, Université Montpellier 2, Sciences et Techniques du Languedoc, 34095 Montpellier cedex 5, France
E-mail: capape@univ-montp2.fr

## *ABSTRACT*

*A new record of a mature common lionfish* Pterois miles *(Bennet, 1828) from the Syrian coast and captures reported by fishermen in the context of local ecological knowledge suggest that a viable population of the species has successfully established in the area. Monitoring activities need to be implemented to contain the invasion of a species considered, for many reasons, a huge threat not only for this area, but for the entire Mediterranean marine environment.*

**Key words:** Scorpaenidae, *Pterois miles*, alien species, Eastern Mediterranean Sea

# È POSSIBILE CHE UNA POPOLAZIONE VITALE DI PESCE SCORPIONE, *PTEROIS MILES* (SCORPAENIDAE), SI SIA STABILITA NELLE ACQUE AL LARGO DELLA COSTA SIRIANA (MEDITERRANEO ORIENTALE)?

## *SINTESI*

*Un nuovo ritrovamento di un esemplare maturo di pesce scorpione* Pterois miles *(Bennet, 1828) lungo la costa siriana e le catture riportate dai pescatori suggeriscono, nel contesto delle conoscenze ecologiche locali, che una popolazione vitale della specie si sia stabilita con successo nella zona. Secondo gli autori, nuove attività di monitoraggio dovrebbero venir implementate al fine di contenere l'invasione di una specie considerata, per svariate ragioni, un'enorme minaccia non solo per quest'area, ma per l'intero ambiente marino mediterraneo.*

**Parole chiave:** Scorpaenidae, *Pterois miles*, specie aliena, mare Mediterraneo orientale

## INTRODUCTION

The common lionfish *Pterois miles* (Bennet, 1828) is well-known in the eastern Mediterranean, where it entered the Red Sea through the Suez Canal. The first record was in the Levant Basin, off Herzliya (Golani & Sonin, 1992), since then, other records have been reported off the northern coast of Lebanon (Bariche *et al.*, 2013), in Turkish waters (Turan *et al.*, 2014), off the coast of Cyprus (Jimenez *et al.*, 2016), and throughout the Levant Basin (Jimenez *et al.*, 2017). The species has migrated westwards and is presently known in the Aegean Sea (Crocetta *et al.*, 2015), while recently also reaching the Tunisian coast and southern Italy (Azzurro *et al.*, 2017).

The first records of *P. miles* from the Syrian coast were presented by Ali *et al.* (2016), who noted that these findings did not represent sufficient data to state that a substantial population had successfully established in the area. Due to the fact that *P. miles* has drastic effects on native fish species, including the destruction of ecosystems and negative economic impacts on fisheries and tourism, investigations have been regularly conducted in the concerned area and the most recent results are presented in this paper.

## MATERIAL AND METHODS

Our methodology was mainly based on the information provided by fishermen, referred to as Local Ecological Knowledge (LEK), which has been applied in the collection of data about natural systems and the monitoring of recent biodiversity changes in the marine environment (Anadón *et al.*, 2009; Azzurro *et al.*, 2011). The approach from Azzurro & Bariche (2017) was adopted to investigate the status and distribution of alien species, such as the *P. miles* lionfish, in the Mediterranean.

On 29 May 2017, a specimen of *P. miles* was captured together with 2 specimens of striped eel catfish *Plotosus lineatus* (Thunberg, 1787) 12 km north of Lattakia City, 2 km offshore (35° 46´ E, 35° 39´N) (Fig. 1), on rocky bottom, at a depth of 25 m, using a bottom cage net made of metal wire. Prior to the dissection, the fresh specimen was measured to the nearest 0.1 millimetre using a digital caliper, and weighed to the nearest 0.1 gram. After the dissection, the ovaries and the digestive tract were reinserted into the abdominal cavity, and subsequently, the entire specimen was preserved in 10% buffered formalin and deposited in the Ichthyological Collection of the Marine Science Laboratory, Agriculture Faculty of the University of Tishreen, under the catalogue number 2290 M.S.L (Fig. 2 A).

## RESULTS AND DISCUSSION

The captured specimen was 226.1 mm in total length, its total body weight was 194.2 g (Tab. 1). It was identified as a *Pterois miles* based on a combination of morphological characters, morphometric measurements, meristic counts and colour, which were in total agreement with previous descriptions of the species by Golani & Sonin (1992), Carpenter & Niem (1999), Golani *et al.* (2002), Turan *et al.* (2014) and Ali *et al.* (2016). The stomach content was removed and four small, undetermined fish weighing 0.6 g were found. The dissected specimen exhibited large and developed ovaries, allowing the conclusion it was mature and able to reproduce, thus corroborating the presence of a viable population in this or the neighbouring areas, most likely Turkish waters, where new records confirm that the species is successfully established there (Özbek *et al.*, 2017).

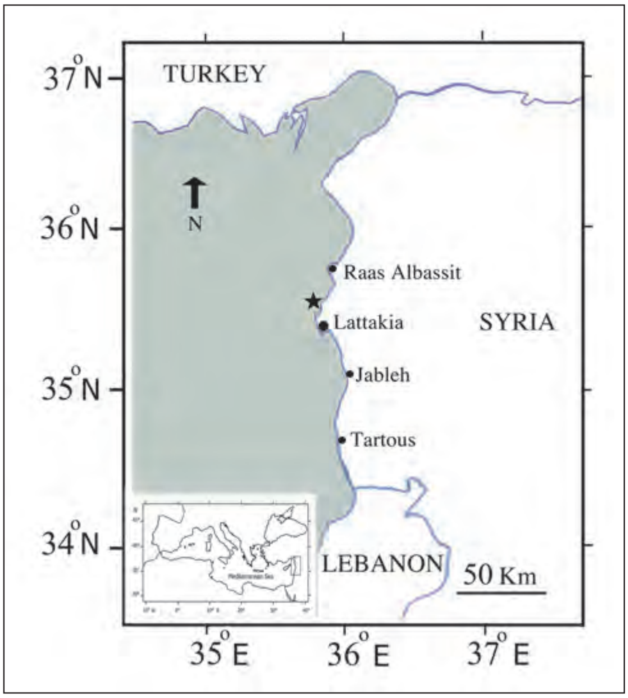

***Fig. 1: Map of the Mediterranean showing Syria, and map of the coast of Syria indicating the capture site of*** *Pterois miles* **(black star).**

***Sl. 1: Zemljevid Sirije in širšega Sredozemskega morja z označeno lokaliteto, kjer je bila ujeta plamenka (črna zvezdica).***

A single mature specimen does not constitute sufficient evidence of a definitive establishment, however, experienced local fishermen aware of the fishing grounds report that the species has lately been a common bycatch in their nets. They are worried about a progressive invasion of this species, about increased competitive pressure for food exerted on native species with a high commercial interest or endangered species, about the destruction of local ecosystems, but also about human envenomation during handling (Frazer *et al.*, 2012). According to Jimenez *et al.* (2016), some divers

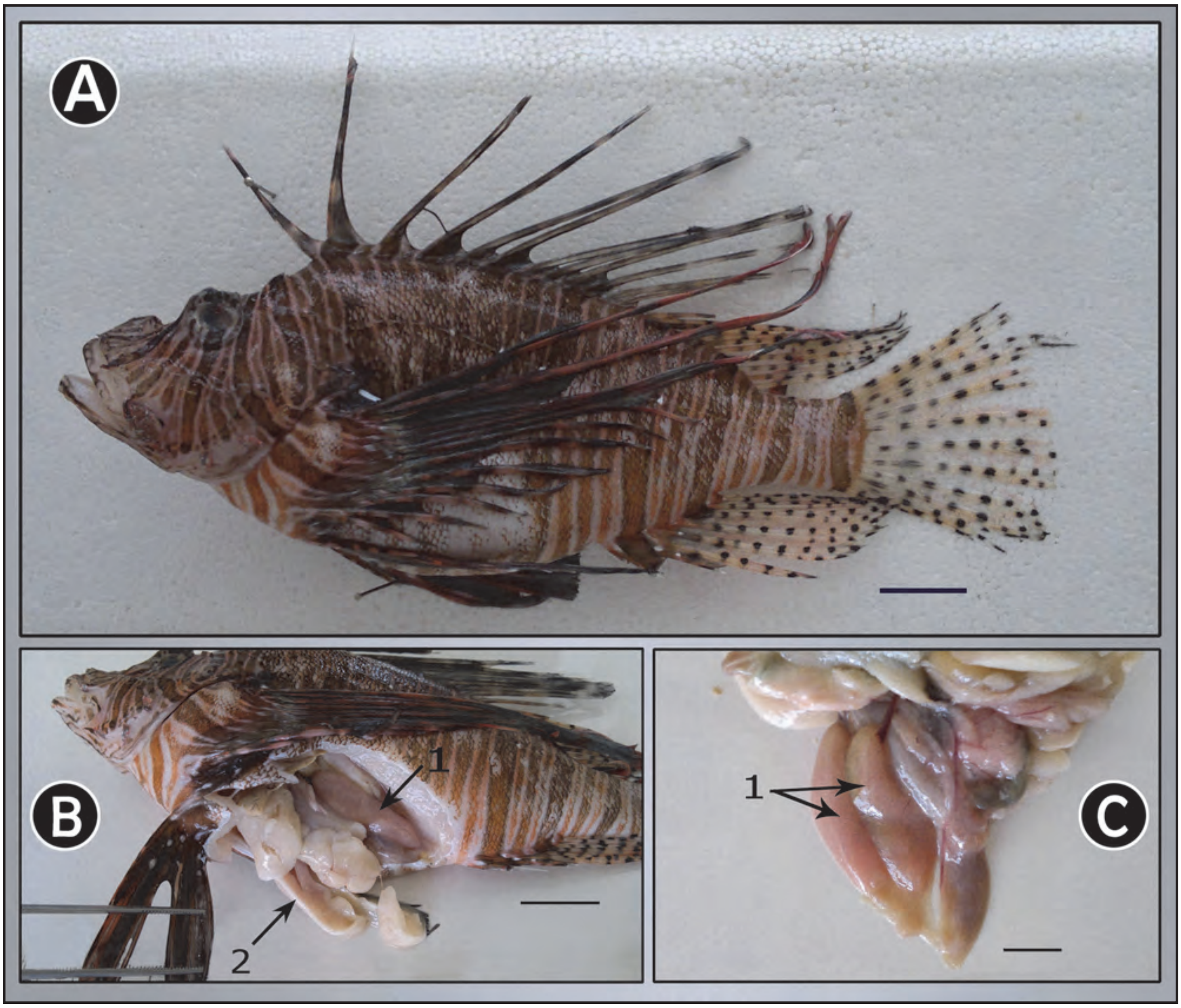

***Fig. 2: A.** Pterois miles* ***captured off the Syrian coast (Ref. 2290 M.S.L.), scale bar = 20 mm. B. 1. Sexual gland. 2. Fat surrounding the stomach, scale bar = 20 mm. C. Sexual glands, scale bar = 10 mm.***
***Sl. 2: A. Primerek plamenke (kataloška številka 2290 M.S.L.), ujet ob sirski obali, merilo = 20 mm. B. 1. Spolna žleza. 2. Plast maščobe, ki obdaja želodec, merilo = 20 mm. C. spolne žleze, merilo = 10 mm.***

consider *P. miles* a beautiful fish and are reluctant to cull this species, although it impoverishes local biodiversity and thus profoundly affects the seascape. Still, a specific monitoring of this fish is required, and the capture of this mature specimen off the Syrian coast is a signal of a huge threat, which should not be dismissed, but rather taken into consideration, as the observations made by local fishermen also confirm.

***Tab. 1: Morphometric measurements in mm and in percentages of total length (% TL), and meristic counts recorded in the specimen of** Pterois miles **(ref. 2290 M.S.L.).***

***Tab. 1: Morfometrične meritve v mm in delež glede na celotno dolžino (% TL) ter meristična štetja, ki se nanašajo na primerek plamenke (kataloška številka 2290 M.S.L.).***

| **Reference of specimen** | **M.S.L. 2290** | |
|---|---|---|
| **Morphometric measurements** | **mm** | **%TL** |
| Total length | 226.1 | 100.0 |
| Standard length | 177.1 | 78.3 |
| Head length | 69.4 | 30.7 |
| Body depth | 68.4 | 30.3 |
| Inter-orbital space | 8.6 | 3.8 |
| Eye diameter | 8.7 | 3.8 |
| Pre-orbital length | 28.1 | 12.4 |
| Dorsal fin length | 139.0 | 61.5 |
| Pectoral fin length | 129.3 | 57.2 |
| Ventral fin length | 83.2 | 36.8 |
| Anal fin length | 69.4 | 30.7 |
| Dorsal fin base | 104.5 | 46.2 |
| Pectoral fin base | 25.3 | 11.2 |
| Ventral fin base | 11.5 | 5.1 |
| Anal fin base | 30.4 | 13.4 |
| Dorsal fin height | 64.8 | 28.7 |
| Pre-dorsal length | 55.2 | 24.4 |
| Pre-pectoral length | 56.0 | 24.8 |
| Pre-ventral length | 61.7 | 27.3 |
| Pre-anal length | 125.5 | 55.5 |
| Counts | | |
| First dorsal fin rays | XIII +11 | |
| Pelvic fin rays | I + 6 | |
| Anal fin rays | III +7 | |
| Pectoral fin rays | XIV | |
| Total weight (g) | 194.2 | |
| body Weight without viscera (g) | 173.3 | |
| Sexual glands weight (g) | 1.9 | |
| Preys weight (g) | 0.6 | |

# SE JE VIABILNA POPULACIJA PLAMENKE, *PTEROIS MILES* (SCORPAENIDAE), ŽE UVELJAVILA V VODAH OB SIRSKI OBALI (VZHODNO SREDOZEMSKO MORJE)?

*Malek ALI*
Marine Sciences Laboratory, Basic Science Department, Faculty of Agriculture, Tishreen University, Lattakia, Syria

*Christian REYNAUD*
Laboratoire Interdisciplinaire de Recherche sur la Didactique, l'Education et la Formation, E.A. 3749, Composante «Didactique et Socialisation», Faculté d'Éducation, Université de Montpellier, 2, Place Marcel Godechot, B.P. 4152, 34095 Montpellier cedex 5, France

*Christian CAPAPÉ*
Laboratoire d'Ichtyologie, case 104, Université Montpellier 2, Sciences et Techniques du Languedoc, 34095 Montpellier cedex 5, France
E-mail: capape@univ-montp2.fr

## *POVZETEK*

*Na podlagi novega ulova plamenke* Pterois miles *(Bennet, 1828) in ulovov, o katerih poročajo ribiči vzdolž sirske obale v okviru lokalnega ekološkega znanja avtorji domnevajo, da se je viabilna populacija te vrste uspešno uveljavila v tem okolju. Obenem priporočajo vzpostavitev rednega monitoringa, ki bi nadzoroval invazijo te vrste, saj ta predstavlja veliko nevarnost ne samo za ožje območje, ampak tudi za celotno Sredozemsko morje.*

**Ključne besede:** Scorpaenidae, *Pterois miles*, tujerodna vrsta, vzhodno Sredozemsko morje

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# OCCURRENCE OF SLOANE'S VIPERFISH *CHAULIODUS SLOANI* (OSTEICHTHYES: CHAULIODONTIDAE) FROM THE TUNISIAN COAST (CENTRAL MEDITERRANEAN)

*Mohamed Mourad BEN AMOR & Khadija OUNIFI-BEN AMOR*
Institut National des Sciences et Technologies de la Mer, 2060 La Goulette, Tunisia

*Christian CAPAPÉ*
Laboratoire d'Ichtyologie, case 104, Université Montpellier 2, Sciences et Techniques du Languedoc 34095 Montpellier cedex 5, France
E-mail: capape@univ-montp2.fr

## *ABSTRACT*

*This paper reports the occurrence of Sloane's viperfish* Chauliodus sloani *Schneider, 1801, from the Tunisian coast. Two specimens were collected by shrimp trawl during a scientific survey carried out in the northern area of the country. The specimens were caught in deep waters, at a depth of 410 m. The lack of knowledge of this species in the Mediterranean is probably due to the fact that it occurs in deep sea areas and is of little commercial interest.*

**Key words:** description, morphometric measurements, meristic counts, distribution, deep waters

# RITROVAMENTO DI PESCE VIPERA *CHAULIODUS SLOANI* (OSTEICHTHYES: CHAULIODONTIDAE) LUNGO LA COSTA DELLA TUNISIA (MEDITERRANEO CENTRALE)

## *SINTESI*

*L'articolo riporta il ritrovamento del pesce vipera* Chauliodus sloani *Schneider, 1801, lungo la costa tunisina. Due esemplari sono stati catturati con una rete a strascico per gamberi durante una spedizione scientifica effettuata nella parte settentrionale del paese. Gli esemplari sono stati catturati a 410 m di profondità. La mancanza di dati inerenti questa specie nel mare Mediterraneo è probabilmente dovuta al fatto che vive in acque molto profonde e ha scarso interesse commerciale.*

**Parole chiave:** descrizione, misure morfometriche, conteggi meristici, distribuzione, acque profonde

## INTRODUCTION

According to Gibbs (1984), two species of the genus *Chauliodus* Schneider, 1801, occur in the FNAM area: the Dana viperfish, *Chauliodus danae* Regan and Trewavas, 1929, only known in the eastern Atlantic, and Sloane's viperfish *Chauliodus sloani* Schneider, 1801, widely distributed in the Pacific, Indian and Atlantic Oceans, but also found in the Mediterranean Sea.

*C. sloani* has been previously recorded in the western Mediterranean Basin, especially in the seas surrounding Italy (Tortonese, 1970) and the Adriatic Sea (Lipej & Dulcic, 2010). Further investigations have reported the occurrence of this species eastwards, in the Greek waters of the Aegean Sea (Papaconstantinou, 1988), southwest off Cyprus (Galil, 2004), and in the Turkish waters of the Levant Sea (Dalyan & Eryilmaz, 2008).

*C. sloani* was noted as very rare off the Algerian coast by Dieuzeide *et al*. in 1954, and no new record has been observed since (Hemida, pers. com., 2017). Bradai *et al.* (2004) reported the species occurrence off northern Tunisian coast, but no specimen was available for confirmation. The *C. sloani* specimens described herein were collected during a scientific expedition of the Hannibal vessel organized by the Institut des Sciences et Technologies de la Mer of Salammbô (Tunisia). The paper also provides comments about the species' distribution in the local area and in the Mediterranean Sea.

## MATERIAL AND METHODS

The two specimens were collected on 12 July 2017, with a shrimp trawl, at a depth of 410 m, on soft bottom, at the locality 37°33′08″ N and 09°45′49″ E (Fig. 1), together with other teleost species, the elasmobranch small-spotted catshark *Scyliorhinus canicula* (Linnaeus, 1758) and the deep-water rose shrimp *Parapenaeus longirostris* (Lucas, 1846). The fresh specimens were measured for total length (TL), standard length (SL) and all morphometric characters to the nearest millimetre, and weighed to the nearest gram, on board. They were delivered to the laboratory for morphometric measurements and counts of meristic characters, which are summarized in Table 1. Both specimens were fixed in 10 % buffered formaldehyde, preserved in 75% ethanol and deposited in the Ichthyological Collection of the Institut des Sciences et Technologies de la Mer of Salammbô (Tunisia), under the catalogue number INSTM Chau-slo 01 and INSTM Chau-slo 02, respectively, and finally described in the present paper (Fig. 2) following Bello *et al.*'s (2014) protocol.

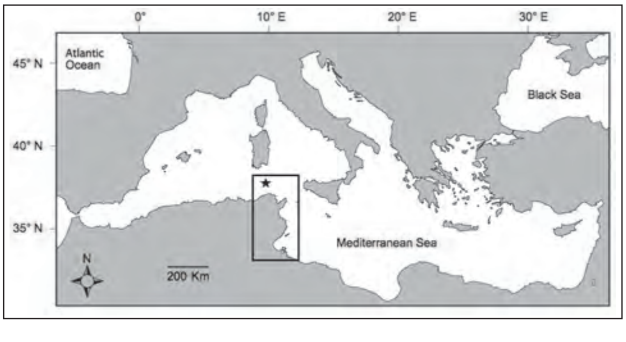

***Fig. 1: Map of the Mediterranean Sea showing Tunisia (rectangle) and indicating the capture site (black star) of the two specimens of*** *Chauliodus sloani* ***in the northern region.***
***Sl. 1: Zemljevid Sredozemskega morja z Tunizijo (pravokotnik) in označbo lokalitete (črna zvezdica), kjer sta bila ujeta primerka*** *Chauliodus sloani* ***na severu države.***

***Fig. 2:*** *Chauliodus sloani****, northern Tunisian region. A. Specimen referenced INSTM Chau-slo 01. B. Specimen referenced INSTM Chau-slo 02, scale bar for both specimens = 35 mm.***
***Sl. 2:*** *Chauliodus sloani****, Severna Tunizija. A. Primerek s kataloško številko INSTM Chau-slo 01. B. Primerek s kataloško številko INSTM Chau-slo 02, merilo za oba primerka = 35 mm.***

## RESULTS AND DISCUSSION

The specimens were identified based on the following combination of characters: body long and compressed, covered by hexagonal scales on sides, with two ventro-lateral rows of photophores; head short with a minuscule snout, jaws with long fangs, numerous large teeth on premaxilla and dentary, lower jaw longer than the upper, dorsal fin close to head, first spinous ray prolonged, its origin over the eighth photophore in lateral series, pre-dorsal length; length from snout to dorsal fin 23.8/23.9 % of standard length (SL), dorsal adipose fin present near tail, colour iridescent silver-blue. The description, colour, all measurements and counts are in total agreement with Tortonese (1970), Gibbs (1984) and Dalyan & Eryilmaz (2008). Therefore, these two findings constitute the first well-documented record of the species in Tunisian waters, and *Chauliodus sloani* can be added to the list of local ichthyofauna.

*C. sloani* can be distinguished from its co-generic species *C. danae* by its predorsal fin closer to head 17-28% (usually 21-24%) SL *versus* in *C. danae*, 24-33% (usually 26-28%) SL. The origin of dorsal fin in *C. sloani* is over the fourth to eighth photophore in lateral series, and over the ninth to eleventh photophore in lateral series in *C. danae*. Additionally, *C. sloani* reaches a larger size than *C. danae,* more than 300 mm SL *versus* about 150 mm SL (Gibbs, 1984). The size of both Tunisian specimens, 226 mm SL and 160 mm SL, respectively, strengthens our diagnosis and confirms the occurrence of *C. sloani* in local waters.

In the wake of the local ecological knowledge following Anadón *et al*. (2009), used to track the geographical distribution of rare species in their living areas (Azzurro *et al*., 2011), information was gathered through contacts with fishermen, SCUBA divers, and sea lovers, concomitantly with regular surveys of Tunisian fish markets. These investigations showed that *Chauliodus sloani* was poorly known in the region prior to this capture. This could be due to the fact that the species inhabits waters of more than 1000 m in depth, and deep bottoms are little exploited by commercial vessels or not at all. Also, the species is not appreciated by consumers and therefore of no economic interest. This means that only scientific research offers the possibility to capture such a species in Tunisia, which is probably the case throughout the Mediterranean Sea.

***Tab. 1: The morphometric measurements in mm and as percentages of standard length (SL %), meristic counts and weight recorded in the two specimens of*** *Chauliodus sloani* ***from the northern Tunisian region.***
***Tab. 1: Morfometrične meritve v mm in kot delež glede na standardno dolžino telesa (SL %), meristično štetje in teža dveh primerkov vrste*** *Chauliodus sloani* ***iz severne Tunizije.***

| **References** | **INSTM Chau-slo 01** | | **INSTM Chau-slo 02** | |
|---|---|---|---|---|
| Morphometric measurements | mm | %SL | mm | %SL |
| Total length (TL) | 251 | 111.1 | 174 | 108.8 |
| Standard length (SL) | 226 | 100 | 160 | 100 |
| Head length | 30 | 13.3 | 24 | 15 |
| Eye diameter | 6 | 2.6 | 5 | 3.1 |
| Preorbital length | 10 | 4.4 | 7 | 4.4 |
| Predorsal length | 54 | 23.9 | 38 | 23.8 |
| Preventral length | 98 | 43.4 | 83 | 51.9 |
| Preanal length | 191 | 84.5 | 147 | 91.9 |
| Prepectoral length | 40 | 17.7 | 30 | 18.8 |
| Dorsal fin base | 13 | 5.8 | 11 | 6.9 |
| Ventral fin base | 7 | 3.1 | 6 | 3.8 |
| Pectoral fin base | 4 | 1.8 | 3 | 1.9 |
| Anal fin base | 18 | 8 | 16 | 10 |
| Adipose fin base | 16 | 7.1 | 12 | 7.5 |
| **Meristic counts** | | | | |
| Dorsal fin rays | 6 | | 6 | |
| Anal fin rays | 10 | | 10 | |
| Pectoral fin rays | 12 | | 12 | |
| Pelvic fin rays | 7 | | 7 | |
| Caudal fin rays | 11 | | 11 | |
| Number of photophores | 8 | | 8 | |
| Number of teeth in upper jaw | 8 | | 8 | |
| Number of teeth in lower jaw | 14 | | 14 | |
| **Total body weight (g)** | 19.5 | | 9.5 | |

# O POJAVLJANJU MORSKEGA GADA *CHAULIODUS SLOANI* (OSTEICHTHYES: CHAULIODONTIDAE) V TUNIZIJSKIH VODAH (OSREDNJE SREDOZEMSKO MORJE)

*Mohamed Mourad BEN AMOR & Khadija OUNIFI-BEN AMOR*
Institut National des Sciences et Technologies de la Mer, 2060 La Goulette, Tunisia

*Christian CAPAPÉ*
Laboratoire d'Ichtyologie, case 104, Université Montpellier 2, Sciences et Techniques du Languedoc 34095 Montpellier cedex 5, France
E-mail: capape@univ-montp2.fr

## *POVZETEK*

*Avtorji poročajo o pojavljanju morskega gada,* Chauliodus sloani *Schneider, 1801, iz tunizijskih voda. Na strokovnih vzorčenjih sta bila na globini 410 m pri lovu kozic ujeta dva primerka na severu države. Pomanjkanje podatkov o tej vrsti je najverjetneje posledica dejstva, da se pojavlja v globokomorskem okolju, poleg tega pa ima zanemarljiv komercialni interes.*

**Ključne besede:** opis, morfometrične meritve, meristična štetja, razširjenost, globokomorsko okolje

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# DO FISH ASSEMBLAGES AT SITES FEATURING MAN-MADE CONCRETE WALLS DIFFER FROM THOSE AT NATURAL ROCKY-REEF SITES?

*Claudia KRUSCHEL & Julia HARRAS*
University of Zadar, Department of Ecology, Agronomy and Aquaculture &
CIMMAR – Center for Interdisciplinary Marine and Maritime Research, 23000 Zadar, Croatia
e-mail: ckrusche@unizd.hr

*Irmgard BLINDOW*
Biological Station Hiddensee, Ernst-Moritz Arndt University Greifswald, Biologenweg 15, 18565 Kloster/Hiddensee, Germany

*Stewart T. SCHULTZ*
University of Zadar, Department of Ecology, Agronomy and Aquaculture &
CIMMAR – Center for Interdisciplinary Marine and Maritime Research, 23000 Zadar, Croatia

## *ABSTRACT*

*The urban development of seashores is predicted to lower biodiversity. After we validated a stationary lure-assisted visual-census method, we proceeded to test the overall hypothesis that fish community structure changes when complex and heterogeneous natural rocky habitats are displaced by less complex vertical hard surfaces. Taxonomic fish community descriptors derived from pristine rocky shorelines were compared with those featuring concrete walls and to natural rocky reefs directly neighboring such developments. Fish communities differed very little between sites across the three levels of development and existing differences were not consistent across all sites within a level. We conclude that in the Croatian Adriatic, the typically small-scale concrete-wall developments do not cause major disruptions of natural near-shore fish assemblages.*

**Key words:** fish community, lure visual census, rocky reefs, seawalls, urbanization, predation intensity

# LE COMUNITÀ ITTICHE IN SITI CON PARETI ANTROPICHE DI CEMENTO SONO DIVERSE DA QUELLE IN SITI ROCCIOSI NATURALI?

## *SINTESI*

*Lo sviluppo urbano delle zone costiere contribuisce alla diminuzione della biodiversità. Dopo aver convalidato un metodo di censimento visivo assistito da un'esca, gli autori hanno verificato l'ipotesi generale che la struttura della comunità ittica subisca variazioni quando complessi habitat rocciosi naturali ed eterogenei vengono sostituiti da superfici solide verticali meno complesse. I descrittori tassonomici della comunità ittica di coste rocciose incontaminate sono stati confrontati con quelli ottenuti dal censimento su pareti di cemento e con quelli rilevati su scogliere rocciose naturali direttamente vicine a tali costruzioni. Le comunità ittiche differivano di poco tra i siti classificati secondo tre livelli di sviluppo, e le differenze esistenti non erano coerenti tra i siti all'interno dello stesso livello. Gli autori pertanto concludono che nell'Adriatico croato le costruzioni verticali in cemento su piccola scala non causano gravi disturbi nelle comunità ittiche in prossimità della costa.*

**Parole chiave:** comunità ittiche, esca censimento visivo, scogliere rocciose, costruzioni verticali, urbanizzazione, intensità di predazione

## INTRODUCTION

Urbanization of coastal areas adds artificial structures to the marine landscape (Clynick *et al.*, 2007; Bulleri & Chapman, 2010; Airoldi & Bulleri, 2011). Concrete walls within marinas and swimming enclosures are now common features of coastal environments (Chapman & Bulleri, 2003; Bulleri, 2005, 2006; Clynick *et al.*, 2008; Bulleri & Chapman, 2010; Di Franco *et al*., 2011); however, there is insufficient knowledge of their ecological effects. A better understanding about their impact is necessary to support the integration of environmental protection into coastal management plans and to reduce the magnitude of human impact (Chapman & Bulleri, 2003; Bulleri & Chapman, 2004; Bulleri, 2005; Bulleri *et al.,* 2005; Clynick *et al.,* 2007; Bulleri & Chapman, 2010; Airoldi & Bulleri, 2011; Di Franco *et al.,* 2011). Urban structures differ from natural environments in several ways. Man-made concrete walls are vertically aligned, hard surfaces that are less complex and heterogeneous than natural reefs (Clynick *et al.,* 2009). Although the existence of structure at all is enough for the recruitment of many fish species (Jenkins & Wheatley, 1998), seawall surfaces facilitate less microhabitat for colonization (Moschella *et al.,* 2005) and less refuges from predators, especially for larval fish (Kruger & Strydom, 2010). Concrete walls may not shelter viable fish population sizes (Clynick *et al.,* 2008) and steep walls in marinas may not function as fish nursery habitats due to the absence of shallow marginal water

***Tab. 1a: Locations with GPS coordinates and association with either (i) lure pre-experiment (effect of presence/absence of a lure on fish richness and abundance) or (ii) fish census to detect site difference in fish communities (natural sites vs. developed and adjacent sites).***
***Tab. 1a: Lokalitete z GPS koordinatami in oznaka, ali gre za (i) predposkus (vpliv prisotnosti/odsotnosti vabe na ribjo pestrost in abundanco) ali za (ii) opazovalni cenzus rib z namenom ugotavljanja razlik v ribji združbi med lokalitetami (naravna okolja proti razvitim oz. bližnjim okoljem).***

| Coastal Location | Northing | Easting | Lure exper. | Site difference study | | | |
|---|---|---|---|---|---|---|---|
| | | | | nat. site | adj. site | dev. site | cement wall usage |
| South of Prevlaka | 42.40571 | 18.50647 | no | yes | no | no | NA |
| **North of Prevlaka** | **42.40723** | **18.51299** | **no** | **no** | **yes** | **yes** | **as military structure** |
| **Cavtat** | **42.57954** | **18.21390** | **no** | **no** | **yes** | **yes** | **as hotel swimming area** |
| Slano | 42.77285 | 17.88325 | no | yes | no | no | NA |
| North of Slano | 42.80302 | 17.84438 | no | yes | no | no | NA |
| **Trogir** | **43.49894** | **16.21764** | **no** | **no** | **yes** | **yes** | **sheltering small boats** |
| **Murter** | **43.77535** | **15.63076** | **no** | **no** | **yes** | **yes** | **in small marina** |
| East of Tkon | 43.90845 | 15.43872 | yes | NA | NA | NA | NA |
| Tkon | 43.90845 | 15.43872 | no | yes | no | no | NA |
| U. Kablin | 44.00628 | 15.26060 | yes | yes | no | no | NA |
| Zdrelac | 44.01490 | 15.25469 | yes | NA | NA | NA | NA |
| Susina | 44.02608 | 15.23367 | yes | NA | NA | NA | NA |
| U. Lamjana Vela | 44.03677 | 15.21466 | yes | NA | NA | NA | NA |
| U. Koštanj | 44.05000 | 15.23000 | yes | NA | NA | NA | NA |
| Kolovare (Zadar) | 44.10083 | 15.23977 | yes | NA | NA | NA | NA |
| **Lukoran** | **44.10742** | **15.15363** | **no** | **no** | **yes** | **yes** | **in small marina** |
| U. Kobiljak | 44.10895 | 15.10255 | yes | yes | no | no | NA |
| Ceprljanda | 44.12577 | 15.11704 | yes | NA | NA | NA | NA |
| Borik | 44.13160 | 15.20973 | yes | NA | NA | NA | NA |
| **Muline** | **44.13468** | **15.06889** | **yes** | **NA** | **yes** | **yes** | **in small marina** |
| Sušica | 44.14572 | 15.08266 | yes | yes | NA | NA | NA |
| **Zaton** | **44.21875** | **15.16371** | **no** | **no** | **yes** | **yes** | **as breakwater** |
| Vinjerac | 44.25873 | 15.46940 | yes | NA | NA | NA | NA |
| North of Modrić | 44.27020 | 15.52350 | yes | NA | NA | NA | NA |

(Kruger & Strydom, 2010). Fish populations at natural sites may be more productive than at artificial structures, as has been shown for limpets which are larger and produce more eggs on natural rocks compared to seawalls (Moreira *et al.,* 2006). Yet, adding artificial structures as new habitat may result in overall higher species richness within the area (Connell & Glasby, 1999) but may also provide habitats for invasive species as already discovered for rocky-bottom invertebrates (Vaselli *et al,.* 2008) and algae (Bulleri & Airoldi, 2005). Currently the general prediction seems to be that artificial structures do not constitute a surrogate for natural environments even though they may accommodate a similar suite of fish species as natural habitats (Rooker *et al.,* 1997). In some studies fish assemblages indeed differed across naturally rocky shores and seawalls (Clynick *et al.,* 2008 and therein, Sala *et al.,* 2012).

Our study compares fish communities in the shallow Croatian Adriatic Sea that are associated with three different levels of shoreline development. Pristine natural sites (at least 5 km distant from any developments) contain natural rocky-algal reefs (in the following the terms “natural” or “nat” are used) that feature a habitat sequence starting at the shore line with big boulders or bedrock-cliffs followed by smaller boulders, followed by sand and eventually seagrass beds (*Posidonia oceanica* or *Cymodocea nodosa*) and/or algal meadows on sediment. At sites with man-made concrete walls (in the following the terms “developed” or “dev” are used) rocky reefs were removed and replaced by more homogeneous and less complex vertical concrete walls *e.g.* within marinas or swimming enclosures (Tab. 1a and 1b) where *C. nodosa* is more likely to consolidate the neighboring sand than *P. oceanica*. Aside from these two extremes, natural sites and concrete-wall sites, we also investigated sites which lie adjacent (within 0.5 km) to concrete-development sites but feature the natural offshore sequence of habitats (in the following the terms “adjacent” or “adj” are used).

We tested the overall prediction that fish communities differ across sites with different levels of development: natural, developed, adjacent. Fish assemblages may vary with regard to the abundance of individual fish of each species, the total abundance of fish individuals and in taxonomic richness, diversity, and evenness. We also expected to see unique taxa at each type of site due to small-scale habitat preferences or behaviors. We structured our sampling scheme to test six null hypotheses:

1. There is no difference in the abundance of individual fish taxa or in total fish abundance;
2. There is no significant difference in taxonomic richness, diversity or evenness;
3. There are no species unique to each level of development;
4. Across levels of development relative habitat coverage and habitat richness do not differ;
5. Overall fish taxonomic richness is equal comparing seawalls with natural large rocky surfaces such as large boulders and bedrock cliffs;
6. Sites belonging to the same level of development will not cluster together based on the relative abundance of the observed taxa.

## MATERIALS AND METHODS

Before testing the null hypotheses, we tested the assumption that using a visual census assisted by a stationary lure results in observing more taxa and higher fish abundance than a lure-less census (Bohnsack & Bannerot,1986; Kruschel & Schultz, 2010 a, 2010 b, 2012). The lure is a lead weight (2.5 cm long) with a double cone-shape and the largest diameter at its centre (0.75 cm). The lure was attached to a nylon fishing line.

Starting 1 m from the lure, 6 spherical lead weights with a diameter of 0.5 cm were placed in 1 m increments to allow for estimation of water depths to 7 m, the maximum depth investigated. For 50% of many replicate fish counts at each of 13 sites, one snorkeler placed the lure for 10 seconds and at 10 cm above the benthic substrate cover in the center of a 1 $m^2$ benthic area while a second snorkeler identified and counted all fish present at the benthic substrate and in the above water column for the time it took to place, present, and retrieve the lure.

The other 50% of fish counts were done without a lure but within independent 1 $m^2$ x water-depth volumes investigated for an equivalent amount of time as used for the lure assisted presentations. In both sets of observations experimental plots were selected systematically by swimming in a straight line from a random starting point while counting to 20 upon which the observer stopped. In case there was a barrier or the visibility was too low to see fish at the bottom the snorkeler randomly changed the direction and continued to count to 20 again until suitable conditions were found. We conducted the pre-experiment at a group of thirteen sites in the Zadar area (Tab. 1a). For each site we collected data on fish abundance and taxonomical richness from pooled presentations with and without a lure. Whether the application of a lure results in a difference in observed taxonomic richness and fish abundance, compared to the non-lure treatment, was tested with R (R Development Core Team 2012) with the non-parametric Wilcoxon rank sum test on untransformed response variables considering that at a large amount of experimental plots zero fish were detected for either method and that Wilcoxon does not assume normal distribution. We found a highly significant difference (Fig. 1) across the two methods in both fish abundance ($p < 0.001$) and taxonomic richness ($p < 0.001$). Based on these results, only the lure-assisted visual census was used to test all hypotheses in this study.

To study fish communities in response to shoreline-habitat differences, lure-assisted stationary visual-

***Tab. 1b: Locations names, GPS coordinates, description and illustration of the seven sites featuring cement walls (developed sites).***
***Tab. 1b: Lokalitete, GPS koordinate, opis in fotografije okolij z betonskimi stenami (razvita okolja).***

| Location name | GPS position | Description | Google image |
|---|---|---|---|
| Zaton, near Zadar | 44.218753<br>15.163708 | Concrete wall not enclosing marina, breakwater |  |
| Muline, Ugljan | 44.134680<br>15.068890 | Concrete wall enclosing small marina |  |
| Lukoran, Ugljan | 44.107420<br>15.153630 | Concrete wall enclosing small marina |  |
| Murter | 43.775350<br>15.630760 | Concrete wall enclosing small marine |  |
| Trogir | 43.498940<br>16.217640 | Concrete wall enclosing a few small boats |  |
| Cavtat | 42.579540<br>18.213900 | Concrete wall with swimming enclosure at Hotel |  |
| North Prevlaka | 42.407233<br>18.512992 | Concrete structure for former military use |  |

census events were performed at 21 sites (Tab. 1a). We investigated seven developed (concrete wall) sites (116 census events, Tab. 1b), seven matching adjacent sites (less than 0.5 km away from concrete walls, 130 census events), and seven natural rocky shore sites (at least 5 km away from any shoreline development, 117 census events). For each fish taxon, abundance was recorded to calculate total and relative abundances and taxonomic richness. Schools of fish were counted as one observation because individual fish within schools

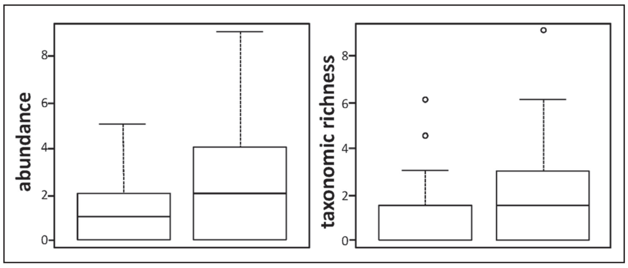

***Fig. 1: Boxplots showing the significant differences in fish abundance and taxonomical richness observed in the pre-experiment to test the hypothesis that lure-assisted stationary visual census is more powerful in detecting fish taxa and fish individuals within an observational area than lure-less stationary visual census.***
***Sl. 1: Box-plot diagram prikazuje statistično značilne razlike v ribji abundanci in taksonomski pestrosti v predposkusu, s katerim so avtorji testirali hipotezo, da lahko z metodo opazovalnega cenzusa z vabo popišemo večje število ribjih vrst in osebkov na opazovanem območju kot z metodo opazovalnega cenzusa brez vabe.***

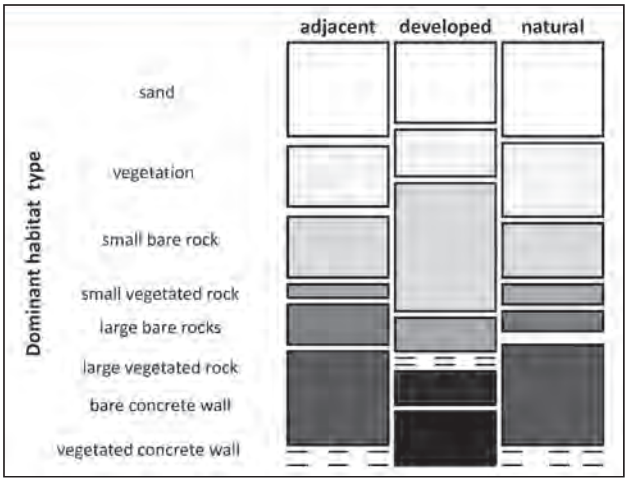

***Fig. 2: Proportions of habitat groups in different levels of development (adj = adjacent, dev = developed, nat = natural).***
***Sl. 2: Deleži skupin habitatov v različnih stopnjah razvoja (adj = bližnji, dev = razviti, nat = naravni).***

do not constitute independent observations and should therefore not be used in a study of site preference. Disregarding the lack of independence of individuals in schools, *e.g.* of *Atherina* spp., *Chromis chromis*, *Oblada melanura;* can lead to the masking of differences in fish community structure across sites that are truly based on fish individual's choices of place.

A linear mixed-model expressed each response variable in reference to the three levels of shoreline development nested inside locations. A visual census event constituted the counting of all fish, except within schools, and the estimation of relative coverage for each habitat type within a square meter above the sea bottom and within the water column above with a lure placed 10 cm above the benthic cover in the center of the resulting water volume (1 $m^2$ x water depth, maximum depth 7 m) for 30 seconds. Selection of experimental plots was again random. The lure was identical to the one used in the pre-experiment. One snorkeler placed the lure for 30 seconds of bottom time while a second snorkeler (always JH) identified and counted all fish present on the benthic substrate and in the above water column exclusively during the bottom time of the lure but not during the placement and retrieval time. Depth and relative benthic habitat cover was recorded afterwards (by JH). Unidentified fish and juvenile fish were not taken into account as taxa, thus not contributing to derived richness, diversity and evenness, except if only juvenile fish and/or if only unidentified fish had been observed. Fish identified to the genus level only (*Gobius*, *Parablennius*, *Symphodus*) were recognized as taxa (contributing to derived richness, diversity, and evenness) only if no species-level observations within the same genera were made. However, all fish contributed to the total fish abundance.

Rank abundance for each species-level taxon was calculated for natural, adjacent, developed samples using the R (R Development Core Team, 2012) packages BiodiversityR and vegan. These packages also were used to assemble a cluster dendrogram with Bray distances for taxa across all sites categorized by their level of development.

Total taxonomic richness, Shannon's diversity index, and evenness were calculated by hand in an Excel spreadsheet for each level of development according to Camargo (1995). Unique taxa were defined as taxa that were not observed at every level of development, but may have been observed at one or two level(s) of development. Observed abundance and taxonomic richness were calculated for each lure presentation. Using R (R Development Core Team, 2012), statistical analysis had been performed via ANOVA with residuals of a linear mixed-effect model for the Poisson distribution to adjust to the non-normal response variables (due to frequent zero values) abundance and taxonomic richness. The linear mixed-effect model expressed the response variable in reference to the level of development, nested inside the sites categorized by their level of development, assuming all individual lure-presentations were independent replicate experiments. The difference deviance ($\chi^2$), degrees of freedom and probability are reported in the results.

For each experimental plot all present dominant habitat groups had been recorded (Fig. 2). Using R (R

Development Core Team, 2012), the statistical tests were carried out as described above for fish taxa richness and abundance. If tested as significant a post hoc test, a pairwise t-test with Bonferroni corrections, compared each dominant habitat group pair. In R (R Development Core Team, 2012) a cross-table was created because both predictor and response variables (level of development and dominant habitat group) are categorical variables. Fisher's exact test for count data was carried out to test if the presence and relative abundance of dominant habitat groups are influenced by the level of development.

For each experimental plot habitat richness had been calculated. Two-sided Spearman's rank correlation had been used for testing the correlation across habitat richness and taxonomic richness in R (R Development Core Team, 2012), and the probabilities are reported in the results. Subsequently it was tested with the help of R (R Development Core Team, 2012), whether there is a significant difference in habitat richness due to the levels of development. Habitat richness had been transformed via boxcox transformation. The transformed data was tested on normal distribution by Shapiro-Wilk normality test and non-parametric Kruskal-Wallis rank sum test was applied. The difference deviance ($\chi^2$), degrees of freedom and probability are reported in the results.

## RESULTS

**Hypothesis 1:** There is no difference in the abundance of individual fish taxa or in total fish abundance across the three levels of shoreline development.

Total abundances were not significantly different across levels of development ($\chi^2 = 3.0$, Df = 2, p = 0.22). Across all sites four of the five most abundant species, *C. chromis*, *C. julis*, *D. annularis* and *D. vulgaris* were shared across all three levels of development (Tab. 2a). The four shared species were overall 100 to 250 times more abundant than most other species across all 21 investigated sites (Fig. 3).

The relative abundance of the four most abundant species are presented in Table 2b. While *C. julis* is clearly (3.9 x) most abundant in adjacent sites, *D. annularis* is most abundant (2.7 x) at developed sites. *C. chromis* and *D. vulgaris* are more evenly distributed across the developed and adjacent sites than the species above but are also least abundant in natural sites.

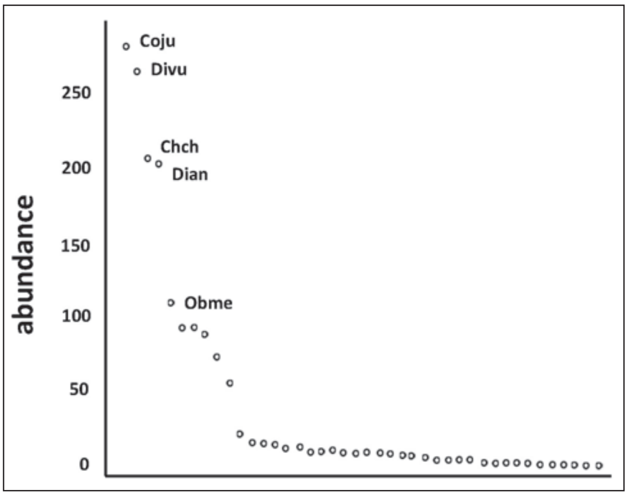

***Fig. 3: Rank abundance for taxa pooled over all sites. For abbreviations and full species names see Tab. 4.***
***Sl. 3: Rangi abundanc taksonov, združenih za vse lokalitete. Za okrajšave in polna imena rib glej Tab. 4.***

**Hypothesis 2:** There is no significant difference in taxonomic richness, diversity or evenness.

As predicted, no significant differences in taxonomic richness, diversity or evenness have been detected (Tab. 3).

**Hypothesis 3:** There are no species unique to each level of development.

Across the 21 sites 49 species and two higher taxa (unidentified species within two families) have been observed (see Tab. 4) and identified. Only five species were unique to one of the levels of development and another nine species were absent at one of the levels of development. The level of uniqueness across levels of

***Tab. 2a: Rank abundances (relative abundance) for the 5 most common taxa at each of the three levels of development.***
***Tab. 2a: Rangi abundance (relativna abundanca) za 5 najbolj pogostih taksonov rib glede na tri stopnje razvoja (naravno, bližnje in razvito okolje).***

| Rank | Natural | | Adjacent | | Developed | |
|---|---|---|---|---|---|---|
| 1 | *Diplodus vulgaris* | 0.30 | *C. julis* | 0.42 | *D. annularis* | 0.29 |
| 2 | *Chromis chromis* | 0.20 | *C. chromis* | 0.19 | *D. vulgaris* | 0.26 |
| 3 | *Coris julis* | 0.19 | *D. vulgaris* | 0.19 | *C. chromis* | 0.18 |
| 4 | *Diplodus annularis* | 0.18 | *Atherina* spp. | 0.10 | *Symphodus ocellatus* | 0.15 |
| 5 | *Gobius bucchichi* | 0.13 | *D. annularis* | 0.10 | *C. julis* | 0.12 |

***Tab. 2b: Ranking of the relative abundances of the four most abundant species according to site developmental status.***
***Tab. 2b: Rangi abundance (relativna abundanca) za 4 najbolj pogoste vrste rib glede na tri stopnje razvoja (nat - naravno, adj - bližnje in dev - razvito okolje).***

| Rank | *Coris julis* | | *Diplodus vulgaris* | | *Diplodus annularis* | | *Chromis chromis* | |
|---|---|---|---|---|---|---|---|---|
| **1** | adj | 0.66 | dev | 0.32 | dev | 0.57 | dev | 0.35 |
| **2** | dev | 0.18 | adj | 0.41 | adj | 0.21 | adj | 0.41 |
| **3** | nat | 0.16 | nat | 0.28 | nat | 0.22 | nat | 0.24 |

development was low, 73% of the observed taxa have been found within all levels of development.

**Hypothesis 4:** Across levels of development relative habitat coverage and habitat richness do not differ.

All levels of development supported six major habitat types (Fig. 1). Adjacent and natural sites feature a natural sequence of spatial distribution of the basic habitats within the investigated depth range (max. 7 m) over a larger area than at developed sites. This is because the removal of the reefs to make room for concrete walls necessitates the shortening of the littoral and sublittoral zone and all habitats investigated, large rock surfaces, small rock surfaces, vegetated sands and bare sands are confined within a more or less shorter distance from the shore.

The main and default difference in terms of presence/absence of habitats is that large boulders, bare and vegetated, are completely replaced by concrete walls, bare and vegetated, in developed sites. The total proportion of large rock-surfaces (larger than diver's body size) is about 1/3 less in developed sites than at natural and adjacent sites. Another trend is that at developed sites 1/3 of the vegetated rock surfaces are contributed by small vegetated rocks, exclusively covered in turf algae, while this proportion of small vegetated rocks is 1/6 in adjacent sites and only 1/9 at natural sites. Most of the rock-based vegetation in natural and adjacent sites is located on larger boulders and much of it supports canopy vegetation, like *Cystoseira* sp., a trend stronger in natural than adjacent sites. Another trend is that developed sites have the highest proportion of small bare rocks on the expense of vegetated and bare sediments. Overall we can conclude that: 1. tall dense vegetation on rocks is most abundant in natural, less in adjacent and substantially less in developed sites, 2. small turf-algae covered rocks and also small bare rocks are most common in developed sites and least common in natural sites, 3. vegetation on sediment is most abundant in natural sites, less in adjacent sites and least in developed sites, 4. in the benthic zone between shoreline and 7 m depth, bare sediments are most abundant in adjacent sites, less in natural sites and least in developed sites (Fig. 2).

**Hypothesis 5:** Considering all sites sampled fish taxonomic richness is equal at seawalls and natural large and small rocky surfaces.

The proportions of dominant habitats in every level of development are illustrated in Figure 2, while Figure 4 shows the significant differences in taxonomic fish richness ($\chi^2 = 21.3$, Df = 7, $p = 0.003$) across levels of development and main habitats. In detail, large vegetated rocks are less species rich than bare concrete walls ($p = 0.023$) and small vegetated rocks are less species rich than vegetated concrete walls ($p = 0.025$), small bare rocks and large vegetated rocks are more species rich than small vegetated rocks ($p = 0.032$ and $p = 0.004$). Small bare rocks are less species rich than vegetation ($p = 0.043$). Fisher's exact test for count data showed that the probability of observing dominant habitat groups is not influenced by the level of development equals $p < 0.001$.

***Tab. 3: Taxonomic richness, Shannon's diversity index, evenness and the effective number of species (richness) at each of the three levels of development.***
***Tab. 3: Taksonomska pestrost, Shannonov diverzitetni indeks, indeks enakomernosti porazdelitve in efektivno število vrst (pestrost) na vsaki razvojni stopnji.***

| Level of development | Richness | Diversity | Evenness | Effective richness |
|---|---|---|---|---|
| natural | 32 | 2.59 | 0.75 | 13 |
| adjacent | 34 | 2.43 | 0.69 | 11 |
| developed | 35 | 2.67 | 0.75 | 14 |

***Tab. 4: Listed are all taxa that have been detected within this study and if they are unique for one or two levels of development.***
***Tab. 4: Popis ugotovljenih vrst v raziskavi in njihova opredelitev, ali se pojavljajo v enem ali na dveh nivojih razvoja (DEV - razvito, ADJ – bližnje in NAT - naravno).***

| | | |
|---|---|---|
| **Apogonidae** | **Gobiidae** | **Muraenidae** |
| Apim - *Apogon imberbis* **(ADJ, DEV)** | Gobu - *Gobius bucchichi* | Muhe - *Muraena helena* **(NAT)** |
| **Atherinidae** | Goco - *Gobius cobitis* | **Pomacentridae** |
| Atbo - *Atherina* spp. | Gocr - *Gobius cruentatus* | Chch - *Chromis chromis* |
| **Belonidae** | Goge - *Gobius geniporus* **(DEV, NAT)** | **Scorpaenidae** |
| Bebe - *Belone belone* | Goni - *Gobius niger* **(DEV)** | Scno - *Scorpaena notata* **(ADJ, DEV)** |
| **Blennidae** | Poma - *Pomatoschistus marmoratus* **(NAT)** | **Serranidae** |
| Sapa - *Salaria pavo* **(ADJ)** | **Labridae** | Seca - *Serranus cabrilla* |
| Pain - *Parablennius incognitus* | Coju - *Coris julis* | Sehe - *Serranus hepatus* |
| Paga - *Parablennius gattorugine* **(ADJ, DEV)** | Lavi - *Labrus viridis* **(ADJ)** | Sesc - *Serranus scriba* |
| Paro - *Parablennius rouxi* | Syci - *Symphodus cinereus* | **Sparidae** |
| Pasa - *Parablennius sanguinolentus* **(ADJ, DEV)** | Sydo - *Symphodus doderleini* | Bobo - *Boops boops* |
| Pate - *Parablennius tentacularis* | Symed - *Symphodus mediterraneus* | Dian - *Diplodus annularis* |
| **Bothidae** | Symela - *Symphodus melanocercus* | Dipu - *Diplodus puntazzo* |
| Bopo - *Bothus podas* **(ADJ, DEV)** | Syoc - *Symphodus ocellatus* | Disa - *Diplodus sargus* |
| **Callionymidae** | Syroi - *Symphodus roissali* | Divu - *Diplodus vulgaris* |
| Capu - *Callionymus pusillus* | Syros - *Symphodus rostratus* | Limo - *Lithognathus mormyrus* **(DEV, NAT)** |
| **Centracanthidae** | Syti - *Symphodus tinca* | Obme - *Oblada melanura* |
| Spma - *Spicara maena* | Thpa - *Thalassoma pavo* | Sasa - *Sarpa salpa* |
| Spsm - *Spicara smaris* **(NAT, ADJ)** | **Muglidae (unidentified)** | Syte - *Syngnathus* sp. |
| | **Mullidae** | Syty - *Syngnathus typhle* |
| | Muba - *Mullus barbatus* | **Trachinidae** |
| | Musu - *Mullus surmuletus* **(DEV, NAT)** | Trdr - *Trachinus draco* |
| | | **Tripterygiidae (unidentified)** |

**Hypothesis 6:** Sites belonging to the same level of development will not cluster together based on the relative abundance of the observed species.

Sampling sites do not consistently cluster according to their association with one of the three developmental levels (Fig. 5). Neither do they consistently cluster by geographical closeness. Sites that are by definition geographically very close, as are developed and adjacent sites, show no clear pattern of similarity. Some adjacent/developed site-pairs are very far apart in the dendrogram, examples are adjacent and developed sites within locations Zaton and Trogir. Others are as close as predicted in Bray distance, like within the location Muline (for geographical position of all locations see Tab. 1a). Natural sites are not consistently clustering by geographical distance either. Natural sites in Slano and at U. Kobilijak are hundreds of kilometers of coastline apart but close in Bray distance, while natural sites in Tkon and U. Kablin which are both on the Island of Pašman are not close in Bray distance (Tab. 1a and Fig. 5).

## DISCUSSION

We did not detect significant differences in total fish abundance across sites of different development levels. Four species were significantly more abundant than any of the other taxa. A rank abundance curve (Fig. 2) shows that the total combined abundance of these four spe-

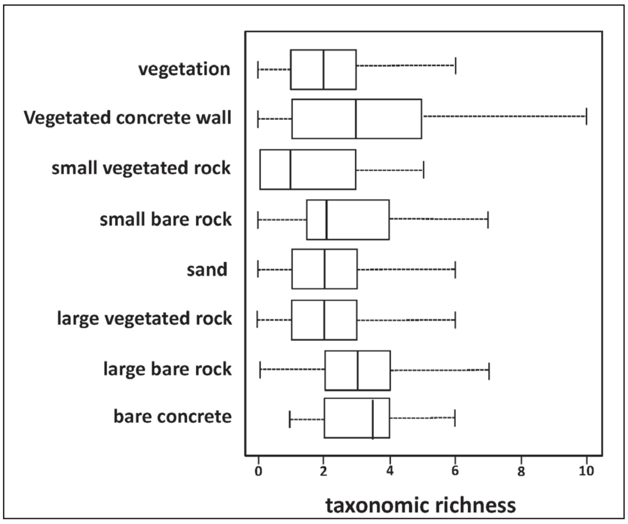

***Fig. 4: Fish taxonomic richness within major habitat types pooled across the 21 sites investigated.***
***Sl. 4: Taksonomska pestrost rib v glavnih habitatnh tipih, združenih v 21 raziskanih lokalitetah.***

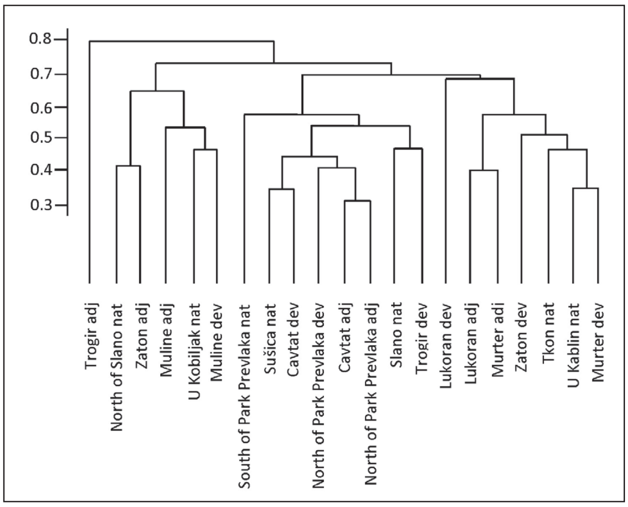

***Fig. 5: Dendrogram of taxonomical communities at the sampled sites based on pairwise Bray distances.***
***Sl. 5: Dendrogram taksonomskih združb na raziskanih lokalitetah na temelju parnih Brayevih razdalj.***

cies exceeded 900 individuals while the total combined abundance of the remaining species was lower than 700 individuals. Of these four species, *D. annularis*, *D. vulgaris* and *C. julis* are very mobile fish and known to be aggressive mesopredators (Kruschel & Schultz, 2012) while the fourth, *C. chromis,* is a schooling planktivore known to aggregate, when in the shallow waters, over transitions between rocks and sediments (Guidetti, 2000). We did discover differences in the relative abundance across developmental levels for these species. *C. julis* dominates rocky shores adjacent to developments where it is 3.9 times more abundant than in pristine and developed sites. As a wait-chase predator it may prefer the more frequent transitions from complex (vegetated rocks, vegetation) to more open habitats (bare rocks, sand) of which the adjacent sites offer more than the other two levels of development (Fig. 2). *D. annularis* is 2.7 times more abundant at developed than at natural sites. This seems surprising since developed sites offer less vegetation, especially *P. oceanica*. However, seawalls sharply border sediments, bare or vegetated, often covered with the seagrass *C. nodosa* and neighboring rocks are small. This combination should be attractive to *D. annularis,* a species attracted to vegetation and sand but rarely seen on complex rocky-reef bottoms (Bauchot & Hureau, 1990; Macpherson, 1994; Froese & Pauly, 2012). *D.vulgaris* is also more abundant at developed sites than natural sites, indicating that seawalls probably resemble rocky cliffs and larger boulder surfaces sufficiently to attract this species and may offer less competition with the ecologically similar *D. puntazzo* and *D. sargus,* which are generally in population decline, especially in developed areas with higher local fishing pressure. According to Sala and Ballesteros (1997) the three rocky-reef *Diplodus* species are known to coexist in pristine sites by differential habitat and depth preferences (*D. vulgaris* and *D. sargus*) but also by differential prey use within the same habitats and depths (*D. vulgaris* and *D. puntazzo*). It is likely that the decline of *D. sargus* and *D. puntazzo* due to overfishing has released *D. vulgaris* from its competitive restrictions. Likewise, *C. chromis* may prefer developed and adjacent sites because of the lower abundance of large piscivorous fish in heavier fished areas. Another reason for increased *C. chromis* in developed sites may be the greater availability of particulate organic matter (POM) in the water column, including plankton and anthropogenic particles, e.g. from sewage and run-off, which is typical for areas with higher human population densities (Guidetti *et al.,* 2002).

We found no significant differences in taxonomic richness, diversity or evenness across sites of different developmental level. Two obvious observations support this homogeneity across sites - they are all dominated by the same four species, resulting in similarly low evenness and very few species are unique to a particular level of development. Almost all species are everywhere but in similarly low numbers, resulting in similar species richness and number of effective species. The overwhelming dominance by four species at all sites may indicate that natural sites are similarly degraded as developed sites by factors other than development so that some taxa have effectively been excluded while a few other species dominate all sites. Another reason for a relatively similar suite of taxa could be the negative method bias against observing cryptic and epibenthic species such as Gobiidae, Blenniidae and Tryterigiidae (Lipej & Orlando-Bonaca, 2006; Kovačić *et al.,* 2012) in dense vegetation on rocks, of which there is more available in natural than in developed sites.

Increasing dominance of small predators may be due to favourable habitat changes or because of predator/competitor release and the associated trophic cascades. Trophic cascades have been extensively studied in tropical reefs with varying conclusions, e.g. Casey *et al.* (2017) found no evidence for it in the very complex Great Barrier Reef context, while Stier *et al.* (2017) found that overall abundance of taxa and the alpha diversity were reduced as a result of top-predator loss and mesopredator release, although beta diversity remained unchanged. A recent publication by Nagelkerken *et al.* (2017) clearly shows that the collapse of large predator populations combined with resource enrichment can foster behavioural changes in already common mesopredators towards more aggressive risk-taking, eventually resulting in a clear dominance by such taxa and an associated loss of biodiversity in the community. In short, predator loss and associated widespread disruptions of 'normal' species interactions significantly reduces biodiversity. The possibility of such complex scenarios has gotten less attention in the Mediterranean/Adriatic. We suspect that the three highly dominant mesopredators in our study, *C. julis*, *D. vulgaris* and *D. annularis,* may indicate a similar top-down/eutrophication mediated change in community structure in the Croatian Adriatic infralittoral belt.

Yet, fish assemblages across developmental levels were not identical, 10% of the 52 identified taxa were unique to one level of development and another 17% avoided one level of development. However, 62% of all species contributed less than 10 individuals across all sites (Fig. 2) so we must consider that detectability of all of these species is low and that total lack of observation for any one species may indicate a general under-sampling, especially considering the relatively small overall area sampled. In general stationary lure methods are positively biased towards any mobile predators and negatively biased against any sedentary fish whereas mobile lure-methods do not have that bias (Murphy & Jenkins, 2010; Kruschel & Schultz, 2010 b). Every fish-census method, including all visual ones, is biased. There is intrinsic bias because of fish traits (size, colour, behaviour) especially in the context of habitat traits (complexity, color). Extrinsic bias is due to method specifics (Edgar *et al.,* 2004; Lowry *et al.,* 2012; Kruschel & Schultz, 2012). Guidetti *et al.* (2005) compared stationary and strip-transect visual-census methods at breakwaters and concluded that in very heterogeneous habitats or at discrete structures such as seawalls and artificial reefs, point methods are more feasable. Harmelin Vivien *et al.* (1985) and Bohnsack & Bannerot (1986) concur with this preference. We generally agree with this view but recommend fish counts along short and random mobile lure-assisted transects (3-5 m) over stationary counts, because the former allow for a larger number of random and independent samples than the latter. Mobile short lure transects are less likely to result in errors due to species interferences and double counts, typical for the stationary counts. At the same time short mobile lure-transects allow the monitoring of behavioral differences e.g. in aggressivity, predation mode, and dominance. Random and short mobile lure-transects can be applied equally in homogeneous habitat patches and in heterogeneous habitat mosaics and within discrete areas (less than 100m) or across large sampling sites (> 1000 m) (Kruschel & Schultz, 2012).

We found a few significant differences in the relative proportions across the six main shared habitat types and some obvious trends across development levels (Fig. 2). All the main structural components are represented in all levels of development – large vegetated and bare rock surfaces, small vegetated and bare rock surfaces, vegetation on sand, and bare sand. The most significant proportional differences between developed sites and adjacent/natural sites are due to the removal of the rocky reef by seawalls and the associated shortening of the investigated littoral slope between the shoreline and 7 m depth. However, we also found differences between adjacent and natural sites. Adjacent sites harbor lower proportions of canopy vegetation as they have less large algae attached to rocks and boulders and less vegetation, algae and seagrass, anchored in sand. This difference between adjacent and pristine natural sites may be due to two major stressors in developed areas – higher incident of urchin barrens and low water clarity due to pollution and sediment mobilization at developed shores. In the scope of an extensive and long term visual-census throughout the Croatian Adriatic, we have observed urchin barrens everywhere at developed and pristine natural sites but we see an association with areas of high fishing pressure causing a lack of urchin predation due to declining abundances of predators, e.g. *D. sargus*, *D. putazzo*, *Sparus aurata* (Guidetti & Dulčić, 2007; Rustici *et al.,* 2017 and therein). Another trigger of urchin barrens seems to be nutrient pollution, probably due to the changes in algal composition from slow growing brown algae, e.g. *Cystoseira* species, to opportunistic fast growing algae, e.g. green algae, which may be more efficiently digested by urchins (Piazzi & Ceccherelli, 2017 and therein). Both stressors, overfishing and nutrient rich waste water are higher in developed than in pristine locations.

Fish species richness did differ across main habitat types: large vegetated boulders had significantly fewer species than bare seawalls but did not differ in species richness from vegetated seawalls (Fig. 4). Considering that canopy vegetation on rock is the preferred habitat of various cryptobenthic fish, a more detailed study addressing the difficulties of counting cryptobenthic species within vegetation in a regular visual census should be considered to further test this hypothesis but we would recommend benign methods which are non-destructive to the habitat and non-consumptive to the fishes (Orlando-Bonaca & Lipej, 2007; Kovačić *et al.,* 2012).

Overall, fish community structure based on relative abundances of all species differed very little between sites across three levels of development, and existing differences were not consistent across all sites. We also found no evidence that latitude (*contra* Guidetti & Dulčić, 2007) or smaller-scale geographical distances cause consistent similarity patterns. One reason for the lack of consistent and obvious differences between the extremes – pristine natural vs. seawall sites could be related to the fact that different communities can be observed at sheltered vs. wave exposed sides of seawalls. The landward, sheltered, seawall-side attracts different communities, including invasive species (Guidetti, 2004; Bulleri & Airoldi, 2005; Vaselli *et al.,* 2008). The communities of the seaward and exposed side of a seawall are more similar to natural reefs than at the landward side with significantly more *D. vulgaris* and less *C. chromis* and *Oblada melanura* (Clynick, 2006; Pizzolon *et al.*, 2007). A multitude of yet not investigated factors, including fishing intensity, natural predation intensities and other biological interactions, as well as details in habitat composition are possible candidates to interact, positively or negatively, with development-level effects. In this study we casually observed that the occurance and abundance of planktivorous fish, *e.g. C. chromis* and *Atherina* sp. strongly varied with wave exposure and the presence of particulate organic matter at seawall sites, while the presence of fisherman discarding fish offal into the water at developed sites caused unusually high abundances of cruising predators and substrate-dwelling fish. These observations offer direct explanations why spatially close sites such as developed and adjacent sites may be unexpectedly dissimilar or too similar. The adjacent and developed (concrete wall) sites of North Prevlaka were similar most likely due to the unusual and widespread accumulation of POM, while the adjacent and developed sites of Lukoran were distinct from each other, most likely due to regular offal feedings as a point source pollution at the seawall site only.

## CONCLUSIONS

Using a stationary lure results in observing higher fish abundance and taxonomic richness compared to the non-lure treatment, a result that corroborates the reports from previous mobile lure-assisted studies. We discovered little evidence that natural, adjacent and developed sites support different fish communities, instead there was variability within all levels. We have reason to believe, that without gathering information about the quality and quantity of many other variables at each site, true differences in fish communities due to the level of development may remain masked. We also suggest to use a long-term monitoring approach to address hypotheses related to the impact of urbanization. Nevertheless, our study does not indicate that lightly developed sites typical of the Croatian Adriatic are obviously less likely to support typical Adriatic fish communities than more natural sites. 73% of the 52 taxa observed within this relatively small scale study were present at all investigated sites, but in low numbers. We suspect that overall fish communities become lower in evenness as top predatory fish taxa decline, which allows smaller and already widespread common mesopredators to dominate and become more aggressive. This predation/competition release and associated mesopredator increase in relative abundance is likely to have far reaching top-down effects on the entire community. To understand this and other region-wide declines, the interplay between fishing pressure, habitat changes, eutrophication and interrupted species-interactions needs to be better understood in the Croatian Adriatic. Overall we conclude that the typically small scale concrete wall developments embedded into expansive undeveloped shorelines, as targeted in this study, do not directly cause major disruptions of natural near-shore fish assemblages. They can instead provide additional structure that constitutes fish habitats and their presence is, according to our study, not associated with a general local decline of fish richness in their immediate surrounding.

# ALI SE RIBJE ZDRUŽBE NA LOKALITETAH Z BETONSKIMI STENAMI RAZLIKUJEJO OD TISTIH V NARAVNEM SKALNATEM OKOLJU?

*Claudia KRUSCHEL & Julia HARRAS*
University of Zadar, Department of Ecology, Agronomy and Aquaculture &
CIMMAR – Center for Interdisciplinary Marine and Maritime Research, 23000 Zadar, Croatia
e-mail: ckrusche@unizd.hr

*Irmgard BLINDOW*
Biological Station Hiddensee, Ernst-Moritz Arndt University Greifswald, Biologenweg 15, 18565 Kloster/Hiddensee, Germany

*Stewart T. SCHULTZ*
University of Zadar, Department of Ecology, Agronomy and Aquaculture &
CIMMAR – Center for Interdisciplinary Marine and Maritime Research, 23000 Zadar, Croatia

## *POVZETEK*

*Urbani razvoj zmanjšuje biodiverziteto. Po ovrednotenju opazovalnih metod z uporabo vabe smo nadaljevali s testiranjem hipoteze, da se struktura ribje združbe spremeni, ko naravno in raznoliko skalnato okolje nadomestijo manj kompleksne navpične trdne površine. Taksonomske ribje kazalce, pridobljene z naravnega skalnatega okolja, smo primerjali s tistimi iz okolja betonskiih sten ter naravnimi skalnatimi okolji, ki mejijo na razvojno spremembo. Ribje združbe so se med lokalitetami s tremi različnimi fazami razvoja le malo razlikovale, poleg tega pa te razlike niso bile ugotovljene na vseh lokalitetah. Avtorji menijo, da v hrvaškem delu Jadrana značilne navpične betonske stene manjših razsežnosti ne povzročajo večjih motenj v obrežnih ribjih združbah.*

**Ključne besede:** ribja združba, opazovalni census z uporabo vabe, skalnato dno, betonske stene, urbanizacija, plenilski vpliv

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# DELO NAŠIH ZAVODOV IN DRUŠTEV

# *ATTIVITÀ DEI NOSTRI ISTITUTI E SOCIETÀ*

# *ACTIVITIES BY OUR INSTITUTIONS AND ASSOCIATIONS*

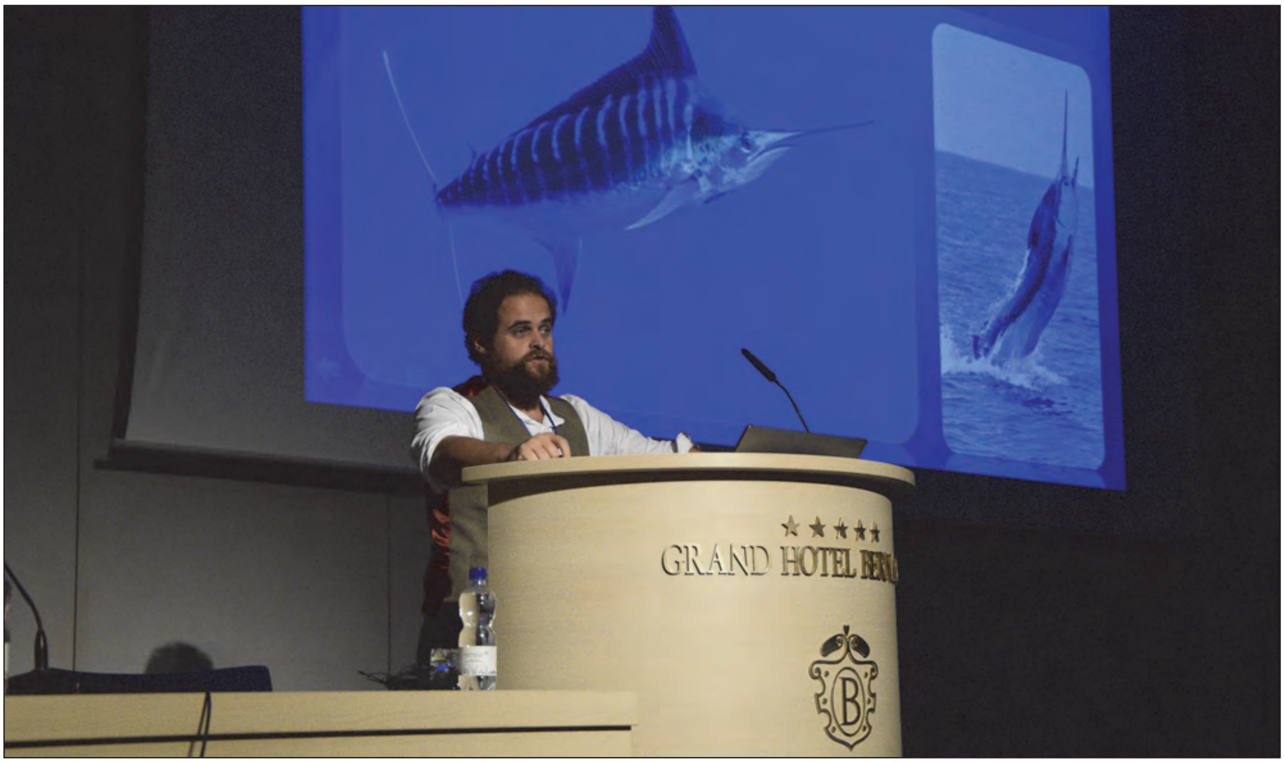
52 EMBS
EUROPEAN
MARINE BIOLOGY
SYMPOSIUM
Slovenia, Piran | 25 - 29 September 2017

## PIRAN HOSTED THE ELITE OF MARINE BIOLOGISTS

What stimulated a British citizen from the small Ascension Island, lost somewhere halfway between southern America and southern Africa, to attend the European Marine Biology Symposium (EMBS), organized in Piran in September 2017? Especially when he already knew that he would have to defeat 10 days of navigation to the Republic of South Africa and then another day or two by air and road transport to reach the venue. And then repeat the same odyssey to return home. He practically spent almost one entire month of trip for 15 minutes of lectures in the congress hall at Grand Hotel Bernardin, on the Slovenian coast. But dr. Andy Richardson is not anyone. He is a lovely British guy who gave up his life in the kingdom to explore the sea around the remote island. At the symposium he presented the results of several years of research on the Blue marlin, a sword-like fish, living in the central Atlantic. He fascinated the audience with an extraordinary presentation of his work and, of course, his enthusiasm for which there is clearly no limit, not in time nor in distance. And fortunately at the symposium in Piran, dr. Richardson was not the only such enthusiastic researcher, they were plenty.

The Marine Biology Station of the National Institute of Biology of Slovenia organized the 52nd EMBS, the largest of its kind in Europe and one of the largest for biologists in the world. A great week for socializing with European colleagues, and also with researchers from other countries, such as Australia, Canada, South Africa, Israel, China, Brazil, Mexico, USA, Algeria, Turkey, Thailand, Tunisia, Chile, Japan, Taiwan, New Zealand, Saudi Arabia, New Caledonia and Colombia, who proved that science today is a global matter. At the symposium, researchers had the opportunity to become acquainted with current contents, new approaches and techniques, and in particular to establish new cooperation in research, projects and exchanges of students.

The congress was organized in several modern thematic sections, such as marine symbiosis, imaging in marine biology, benthic-pelagic coupling, marine metagenomics, and mesophotic ecosystems. By far the most extensive part was covered by the general section.



***Dr. Andy Richardson presenting the results of several years of research on the Blue marlin living in the central Atlantic (photo: Anja Šimon).***

It would be too unfair to expose individual researchers, since there were many exceptional presentations.

The participants were received also by the mayor of Piran, Peter Bossman, with a pleasant speech about the beauties of his town and a feast, and he did not forget to promote the visit to the center and to the Sečovlje salt pans. The guests then visited the Sečovlje Salina Nature Park during an organized excursion in the only free afternoon.

The researchers of the Marine Biology Station Piran organized also the traditional Yellow Submarine competition on the beach, where younger generations competed in fun games, such as sampling of marine organisms and sediments, while the older ones cheered aloud. There was also a social dinner at the Vinakoper, where the participants had the opportunity to learn more about the tastes of local gastronomy and oenological peculiarities. This event was also used by the organizers to grant financial awards for the four best poster presentations of young researchers. The winner was a lovely biologist from Saudi Arabia.

At the symposium biologists listened to ideas for new researches, they brought new ties, basics for cooperation, and some of the lecturers also made them laugh out loud. This is the case of the Croatian professor, dr. Peter Kružić, who finished the lecture on coral bleaching in the Adriatic Sea with a photograph of the winning Slovenian national basketball team, triggering a hurricane applause in the hall and warming the heart of Slovenian researchers.

The organization of such symposium is definitely Sisyphus's work. For the first time Slovenia hosted a pan-European congress of this magnitude. For this success, the merits are of a great crowd of people, part of the organizing and scientific committees, which were preparing for the congress for almost a year and a half. Acknowledgment goes also to various local sponsors, who helped the organizers in giving to the participants of the 52nd EMBS nice memories to take home. The Marine Biology Station Piran passed the relay to the next organizer of the EMBS, the Flanders Marine Institute in Oostende, Belgium.

**Lovrenc Lipej & Martina Orlando-Bonaca**

NACIONALNI INŠTITUT ZA BIOLOGIJO
MORSKA BIOLOŠKA POSTAJA

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## LETNO SREČANJE MEDNARODNE ORGANIZACIJE ZA VODNE PTICE THE WATERBIRD SOCIETY (*WATERBIRD SOCIETY ANNUAL MEETING, REYKJAVIK, ICELAND, AUGUST 8-12 2017*)

Od 8. do 12. avgusta 2017 je v Univerzitetnem središču Askja (po istoimenskem vulkanu) v Reykjaviku na Islandiji potekalo mednarodno srečanje strokovnjakov za vodne ptice z naslovom »The Waterbird Society Annual Meeting«. Gre za že 41. Srečanje mednarodne organizacije Waterbird Society s sedežem v ZDA. Organizacija Waterbird Society je mednarodna znanstvena, nevladna organizacija, katere cilj je proučevanje in varovanje vodnih ptic.

Srečanja smo se udeležili sodelavci iz Krajisnkega parka Sečoveljske soline (KPSS) v okviru Programa Finančnega mehanizma EGP 200-2014 (SI02), CARS-OUT! Okoljsko prijazen obisk zavarovanih območij in Programa Norveškega finančnega mehanizma (SI05).

V treh delovnih dneh je bilo kar 92 predavanj, od tega dve plenarni. Predstavljenih je bilo tudi 24 prispevkov na posterjih, med njimi tudi prispevek I. Škornika »*Status, distribution and threats of five breeding species in Sečovlje Salina Nature Park: prescription of biodiversity conservation for the area*.«

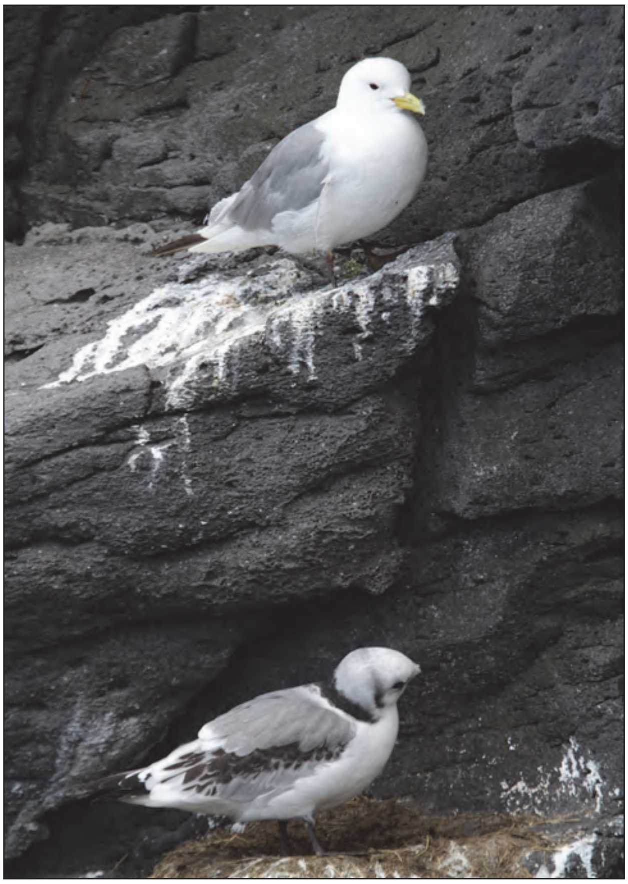
Predavanja so potekala sočasno v treh dvoranah. Odvijala so se v sklopu simpozija o galebih in simpozija o mormonu. Obenem so potekala tudi druga predavanja na temo prehranjevanja, selitve, oglašanja, kartirnih popisov, populacijskih trendov, telemetrije, itd.

Nadvse poučne so bile strokovne ekskurzije, ki so bile organizirane za udeležence simpozija. Tri ekskurzije so bile krajše, saj smo jih izvedli zgodaj zjutraj pred samim začetkom predavanj, zadnja ekskurzija pa je bila celodnevna. Prvi dan simpozija smo začeli z zgodnje jutranjo ekskurzijo v Alftanes. Drugi dan smo s čolnom odpluli do otoka Puffin Island, kjer smo občudovali kolonijo mormonov (*Fratercula arctica*) in drugih morskih ptic. Zadnja jutranja ekskurzija je bila v Seltjarnarnes, kjer je bilo kljub vetru in mrazu veliko zanimivih vrst. S celodnevno ekskurzijo na polotok Snæfellsnes pa se je končala ornitološka konferenca Waterbirds 2017. Dežela vulkanov je presenetila tudi v ornitološkem pogledu. Tisočere jate pobrežnikov, ki se pripravljajo na svoj odhod, obrežja polna rac, arktične čigre, kolonije triprstih galebov, ki se s svojimi mladiči gnetejo na ozkih skalnih policah strmega klifa tik nad fjordom so le del bogastva, ki smo ga lahko občudovali. Spoznali smo kako se Islandci spopadajo

*Sl. 1: Udeleženci srečanja so imeli priložnost spoznati posebnosti islandske biodiverzitete (Foto: I. Škornik).*

z naravovarstvenimi problemi, kaj za njihovo naravno dediščino pomeni turizem in obiskovanje ter dostopnost do zavarovanih območij.

**Iztok Škornik**
KPSS-SOLINE Pridelava soli d.o.o.



*Sl. 2: Triprsti galeb (*Rissa tridactyla*) (Foto: I. Škornik).*

# OCENE IN POROČILA

# *RECENSIONI E RELAZIONI*

# *REVIEWS AND REPORTS*

**Book review:** TOURISM IN PROTECTED AREAS
OF NATURE IN SERBIA AND SLOVENIA
Editors: Dejan Filipović, Anton Gosar,
Miha Koderman, Snežana Đurđić
University of Belgrade – Faculty of Geography,
2017, 183 pp.
ISBN 978-86-6283-053-1

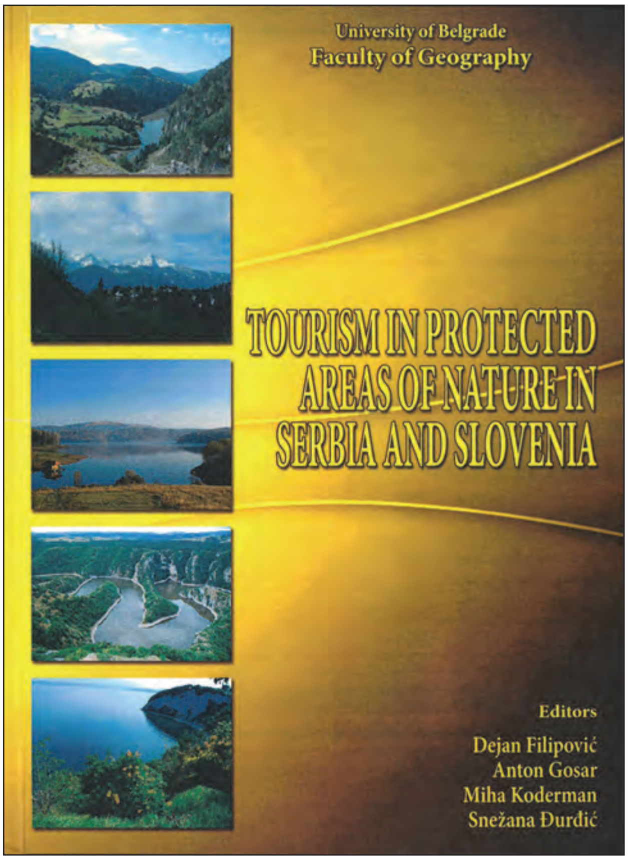
Just recently I had a conversation with one of my former colleagues at the Triglav National Park - where I used to work - and he complained to me saying »I'm really fed up with this schizophrenic situation«. I needed no further explanation of his »anxiety« as I know the reason for it lies in problems common to the majority, if not all, of protected areas (PA) management authorities (MA). The main purpose and task of the protected areas MA is of course to safeguard ecosystems, species and habitats, and to preserve the traditional cultural landscape of the area. But on the other hand (with an exception of the IUCN category 1), the majority of them also have the duty to promote the sustainable development of local communities. Sustainable agriculture and sustainable tourism are in this regard the most common denominators of protected areas development strategies. Sustainable tourism in protected areas - what a "fancy" idea - so easy to imagine, so difficult to realize. The green dots and areas on tourist maps attract a rapidly increasing number of visitors every year (e.g. 40% of visitors to Slovenia), and tourism and recreation-related industries take advantage of this growth. New types of visits are being introduced so rapidly that MAs can hardly follow. Consequently, they are in continuous search of the »right« balance between nature conservation, management of tourist flows, and sustainable development of the areas involved. Anything but a simple task.

The book presented here tackles also some of the above mentioned issues. It is a collection of scientific papers delivered as a result of bilateral project »Integrated approach to tourism development in protected areas - Experiences of Serbia and Slovenia« between the University of Belgrade - Faculty of Geography and the University of Primorska, Faculty of Tourism Studies - Turistica. The first three papers deal with general arrangements on the level of legislation, international standards, and national planning systems, with a special attention on tourism-related activities, regulations and spatial planning. Andrej Sovinc presents a general overview of the management objectives of protected areas of different categories as defined by the IUCN international standards. More specifically, his focus is on limitations, possibilities, and ambiguities in planning tourism activities in National (IUCN 2) and Landscape parks (5). Anton Gosar presents the selected cases of differences and (in)consistencies between the objectives of protected areas as set by IUCN standard classification and Slovenian general Law of Nature Protection. Moreover, he illustrates two cases where even in the central area of the Triglav national park strict rules are not followed: the case of second homes, and the »famous« case of the Pokljuka Sport Center. As for the Serbian side, Dejan Filipović presents the spatial planning system in the Republic of Serbia, with special emphasis on integration of protected areas objectives in the spatial planning documents at different hierarchical levels. As he concludes, it is necessary to increase the coverage of protected areas by planning documents with a long-term perspective in order to achieve more efficient measures of protection.

The majority (6) of other papers deal with issues of the current state of tourism development, most of them focusing on potentials, constraints, and prospects of sustainable tourism development in the selected protected areas of Slovenia and Serbia. Although the paper delivered by Dobrica Jovičić doesn't exactly fit into this book, it nevertheless brings a valuable insight into general socio-economic aspects of the recent tourism development trends in Serbia (including few notes on ecotourism potentials). Her conclusion is that tourism in Serbia is not yet developing in line with general sustainable development objectives and guidelines. Snežana Đurđić on the other hand explores potentials and constraints of ecotourism development in four macro-regions (»clusters«) of Serbia: Vojvodina, Belgrade area, Western and Eastern Serbia. Ecotourism as a particularly suitable form of tourism offer in protected areas is only in the initial phase of organization, but the ambiguous and even »conflict« relations between tourism development and nature conservation have already emerged. Marija Belij highlights potentials of cultural tourism development in Serbian national parks, mainly by describing cultural heritage assets of the three NP under consideration. She points out that many of them have not yet undergone adequate protective measures. The next two papers present interesting case-studies from the Slovenian coast. Dane Podmenik and Simon Kerma explore potentials for ecotourism development in the Dragonja valley, an area in the hinterland where a nature park is to be (?) established. After interpreting the selected extensive field work data, the authors summarize conclusions in the form of a SWOT analysis table. Lovrenc Lipej and Simon Kerma present an overview of the existing and potential tourist offer linked to biodiversity in the protected areas on the Slovenian coast. They point out some of the nature and history-based potentials that have so far been overlooked in the current coastal tourism offer. Maja Sevšek and Irma Potočnik Slavec examine local inhabitants' involvement in the tourism related development of the Landscape Park Kum. Drawing upon a field survey, they conclude that a more participative management of the PA is necessary in order to stimulate stronger sustainable development of the area. Last but not the least, two papers dealing with the issue of the second home phenomenon in the protected areas in Serbia and Slovenia are included. Sanja Pavlović presents an overview of the development of the so-called »weekend cottages« and settlements in eight protected areas in Serbia, while Miha Koderman focuses on the Triglav national park, and the settlement Goreljek that lies within it. The presence of »weekend cottages« can and does have positive impacts on socio-economic development and landscape preservation in protected areas, however, at the same time it can and it does bring negative effects to those areas as well.

To conclude, the book is a very valuable collection of papers dealing with diverse topics and perspectives on the development of tourism in protected areas in general, and in the selected case-study areas. The majority of contributions focus on unrealized potentials of tourism development in PA. Hopefully, in the near future an opportunity will arise to examine the existing tourist offer and flows, and the related question of how to or how not to deal with them. Beside the wish of the editors of this collection to foster communication between diverse actors and stakeholders, this could also serve as an effective pill for the above mentioned PA managers' "schizophrenia".

**Matej Vranješ**
Nova Gorica, Slovenia



## NAVODILA AVTORJEM

**1.** Revija ANNALES (*Anali za istrske in mediteranske študije* Series historia naturalis) objavlja **izvirne znanstvene** in **pregledne članke** z naravoslovnimi vsebinami, ki obravnavajo posebnosti različnih podpodročij sredozemskega naravoslovja: morska biologija in ekologija, ihtiologija, geologija s paleontologijo, krasoslovje, oljkarstvo, biodiverziteta Slovenije, varstvo narave, onesnaževanje in varstvo okolja, fizična geografija Istre in Mediterana idr. Vključujejo pa tudi **krajše** znanstvene prispevke o zaključenih raziskovanjih., ki se nanašajo na omenjeno področje.

**2.** Sprejemamo članke v angleškem, slovenskem in italijanskem jeziku. Avtorji morajo zagotoviti jezikovno neoporečnost besedil, uredništvo pa ima pravico članke dodatno jezikovno lektorirati.

**3.** Članki naj obsegajo do 48.000 znakov brez presledkov oz. 2 avtorski poli besedila. Članek je mogoče oddati na e-naslov annales@mbss.org (zaželjeno) ali na elektronskem nosilcu (CD) po pošti na naslov uredništva.

Avtor ob oddaji članka zagotavlja, da članek še ni bil objavljen in se obvezuje, da ga ne bo objavil drugje.

**4. Naslovna stran** članka naj vsebuje naslov članka, ime in priimek avtorja (avtorjev), ime in naslov inštitucije, kjer je (so) avtor(ji) zaposlen(i) oz. domači naslov in naslovom elektronske pošte (samo prvi oz. korespondenčni avtor).

**5.** Članek mora vsebovati **povzetek** in **izvleček.** Izvleček je krajši (cca. 10 vrstic) od povzetka (cca. 30 vrstic).

V *izvlečku* na kratko opišemo namen, metode dela in rezultate. Izvleček naj ne vsebuje komentarjev in priporočil.

*Povzetek* vsebuje opis namena in metod dela ter povzame analizo oziroma interpretacijo rezultatov. V povzetku ne sme biti ničesar, česar glavno besedilo ne vsebuje. V povzetku se avtor ne sklicuje na slike, tabele in reference, ki so v članku.

**6.** Avtorji naj pod izvleček članka pripišejo ustrezne **ključne besede** (največ 6). Zaželjeni so tudi angleški (ali slovenski) prevodi izvlečka, povzetka, ključnih besed, podnapisov k slikovnemu in tabelarnemu gradivu. V nasprotnem primeru bo za prevode poskrbelo uredništvo.

**7. Glavni del besedila** naj vključuje sledeča poglavja: Uvod, Material in metode, Rezultati, Razprava ali Rezultati in razprava, Zaključki (ali Sklepi), Zahvala (če avtor želi), Literatura. Dele besedila je možno oblikovati v podpoglavja (npr. Pregled dosedanjih objav v Uvodu, Opis območja raziskav v Material in metode). Podpisi k slikam so priloženi posebej za poglavjem Literatura.

**8. Tabele** avtor priravi posebej na ločenih straneh v programu Word, tako kot rokopis, jih zaporedno oštevilči in opremi z naslovom – kratkim opisom. V glavnem delu besedila se sklicuje na tabele tako, da jih na ustreznem mestu označi z npr. "(Tab. 1)".

**9. Slikovno gradivo** (grafi, zemljevidi, fotografije, table) avtor posreduje v ločenih datotekah (jpeg, tiff) z najmanj 300 dpi resolucije pri želeni velikosti. Največja velikost slikovnega gradiva je 17x20 cm. Vsa potrebna dovoljenja za objavo slikovnega gradiva (v skladu z Zakonom o avtorski in sorodnih pravicah) priskrbi avtor sam in jih predloži uredništvu pred objavo članka. Slike je potrebno tudi podnasloviti in zaporedno oštevilčiti (glej točko 7). V glavnem delu besedila se avtor sklicuje na slike tako, da jih na ustreznem mestu označi z npr. "(Sl. 1)".

**10**. Bibliografske opombe, s čimer mislimo na **citat** – torej sklicevanje na druge publikacije, sestavljajo naslednji podatki v oklepaju: *avtor* in *leto izida*; npr. (Novak, 2007). Če sta dva avtorja, se izpišeta oba (Novak & Kranjc, 2001), če so trije ali več pa se izpiše samo prvi, ki mu sledi okrajšava *et al.* (Novak *et al.*, 1999). Več citatov je med seboj ločenih s podpičjem in si sledijo kronološko - z naraščajočo letnico izdaje, npr. (Novak *et al.*, 1999; Adamič, 2001; Kranjc & Zupan, 2007). Osebno informacijo (ustno, pisno) izpišemo prav tako v oklepaju z navedbo kratice imena in priimka posredovalca informacije, za vejico pa dodamo "osebno sporočilo", npr. (J. Novak, *osebno sporočilo*).

**11.** Celotni **bibliografski podatki** so navedeni v poglavju Literatura v abecednem vrstnem redu. Pri tem avtor navede izključno dela, ki jih je v članku citiral. Če ima isti avtor več bibliografskih podatkov, se najprej kronološko izpišejo tisti, kjer je edini avtor, sledijo dela v soavtorstavu še z enim avtorjem in dela v soavtorstvu z več avtorji. Imena revij, v katerih so izšla citirana dela, se izpišejo okrajašano (splošno priznane okrajšave revij). Članki, ki še niso bili publicirani, se lahko citirajo le, če so bili dokončno sprejeti v tisk, pri čemer se na koncu bibliografskega podatka doda beseda "v tisku". Člankov, ki so šele bili poslani v recenzijo, se ne sme citirati.

Primeri navajanje različnih tipov bibliografskih podatkov:

*članki v revijah:*

**Klock, J.-H., A. Wieland, R. Seifert & W. Michaelis (2007):** Extracellular polymeric substances (EPS) from cyanobacterial mats: characterisation and isolation method optimisation. Mar. Biol., 152, 1077-1085.

*Knjige in druge neserijske publikacije (poročila, diplomska dela, doktorske disertacije):*

**Wheeler, A. (1969):** The fishes of the British Isles and North-West Europe. McMillan, London, 613 p.

*Poglavje v knjigi:*

**McEachran, J. D. & C. Capapé (1984):** Myliobatidae. In: Whitehead, P. J. P., M. L. Bauchot, J.-C. Hureau, J. Nielsen & E. Tortonese (eds.): Fishes of the North-eastern Atlantic and the Mediterranean, Vol. 1. Unesco, Paris, pp. 205-209.

**12. Drugo:** latinski izrazi kot npr. *in vivo*, *in situ*, *e.g.*, *i.e.*, ter rodovna (*Myliobatis* sp.) in vrstna (*Myliobatis aquila*) imena se izpišejo v fontu italic. Kadarkoli je možno, se uporabljajo enote iz sistema SI (Système international d'unités).

**13.** Prvi odtis člankov uredništvo pošlje avtorjem v **korekturo.** Avtorji so dolžni popravljeno gradivo vrniti v enem tednu. Besedilo popravljamo s korekturnimi znamenji, ki jih najdemo na koncu Slovenskega pravopisa (2001), Ljubljana, ZRC SAZU, 24–25.

Širjenje obsega besedila ob korekturah ni dovoljeno. Druge korekture opravi uredništvo.

**14.** Za dodatna pojasnila v zvezi z objavo člankov je uredništvo na voljo.

UREDNIŠTVO

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**1.** La rivista ANNALES (*Annali per gli studi istriani e mediterranei*, Series historia naturalis) pubblica **articoli scientifici originali** e **compendii** dai contenuti scientifici relativi ai vari settori della storia naturale e pertinenti l'area geografica del Mediterraneo: biologia marina, ecologia, ittiologia, geologia, paleontologia, carsologia, olivicoltura, biodiversità della Slovenia, tutela della natura, inquinamento e tutela dell'ambiente, geografia fisica dell'Istria e del Mediterraneo ecc. La rivista pubblica anche articoli scientifici **brevi** relativi a ricerche concluse pertinenti a tali settori.

**2.** La Redazione accetta articoli in lingua inglese, slovena e italiana. Gli autori devono garantire l'ineccepibilità linguistica dei testi, la Redazione si riserva il diritto di una revisione linguistica.

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**4.** Ogni articolo deve essere corredato da: **titolo**, nome e cognome dell'autore (autori), denominazione ed indirizzo dell'ente di appartenenza o, in alternativa, l'indirizzo di casa, nonché l'indirizzo di posta elettronica (solo del primo autore o dell'autore di corrispondenza).

**5.** I contributi devono essere corredati da un **riassunto** e da una **sintesi**. Quest'ultima sarà più breve (cca. 10 righe) del riassunto (cca 30 righe).

Nella *sintesi* si descriveranno brevemente lo scopo, i metodi e i risultati delle ricerche. La sintesi non deve contenere commenti e segnalazioni.

Il *riassunto* riporterà in maniera sintetica lo scopo, i metodi delle ricerche e l'analisi ossia l'interpretazione dei risultati. Il riassunto non deve riferirsi alle tabelle, figure e alla bibliografia contenuta nell'articolo.

**6.** Gli autori sono tenuti ad indicare le **parole chiave** adeguate (massimo 6). Sono auspicabili anche le traduzioni in inglese (o sloveno) della sintesi, del riassunto, delle parole chiave, delle didascalie e delle tabelle. In caso contrario, vi provvederà la Redazione.

**7. Il testo principale** deve essere strutturato nei seguenti capitoli: Introduzione, Materiali e metodi, Risultati, Discussione o Risultati e discussione, Conclusioni, Ringraziamenti (se necessari), Bibliografia. Il testo può essere strutturato in sottocapitoli (ad es. sottocapitolo Rassegna delle pubblicazioni nell'Introduzione; sottocapitolo Descrizione dell'area di ricerca nel capitolo Materiali e metodi). Le didascalie devono essere presentate separatamente, a seguito del capitolo Bibliografia.

**8. Le tabelle** saranno preparate in forma elettronica come il manoscritto (formato Word) e allegate in fogli separati alla fine del testo. Gli autori sono pregati di contrassegnare ogni tabella con un numero e il titolo ossia una breve descrizione. Nel testo la tabella viene richiamata come segue: (Tab. 1).

**9. Il materiale grafico** (grafici, carte geografiche, fotografie, tavole) va preparato in formato elettronico (jpeg o tiff) e consegnato in file separati, con una definizione di 300 dpi alla grandezza desiderata, purché non ecceda i 17x20 cm. Prima della pubblicazione, l'autore provvederà a fornire alla Redazione tutte le autorizzazioni richieste per la riproduzione del materiale grafico (in virtù della Legge sui diritti d'autore). Tutto il materiale grafico deve essere accompagnato da didascalie (vedi punto 7) e numerato.. Nel testo i grafici vengono richiamati come segue: (ad es. Fig. 1).

**10. I riferimenti bibliografici (citazioni)** richiamano un'altra pubblicazione (articolo). La nota bibliografica, riportata nel testo, deve contenere i seguenti dati tra parentesi: *cognome dell'autore*, *anno di pubblicazione*, ad es. (Novak, 2007). Se gli autori sono due, verranno indicati entrambi (Novak & Kranjc, 2001), nel caso di tre o più autori verrà indicato soltanto il primo, seguito dall'abbreviazione *et al.* (Novak *et al.,* 1999). Vari riferimenti bibliografici in una stessa nota vanno divisi dal punto e virgola e segnalati in ordine cronologico, ad. es. (Novak *et al.*, 1999; Adamič, 2001; Kranjc & Zupan, 2007). La testimonianza (orale, scritta) verrà indicata tra parentesi con l'abbreviazione del nome e con il cognome di chi l'ha trasmessa, seguiti dalla virgola e la dicitura "informazione personale", ad es. (J. Novak, *informazione personale*).

**11. La bibliografia** completa va inserita in ordine alfabetico nel capitolo Bibliografia. L'autore indicherà esclusivamente i lavori e le edizioni citati nell'articolo. Se si citano più lavori dello stesso autore, verranno indicati prima in ordine cronologico i lavori in cui l'autore appare solo, poi quelli in cui l'autore compare assieme ad un secondo coautore, seguiti infine da quelli in cui egli compare tra più coautori. I nomi delle riviste in cui sono pubblicati i lavori citati saranno indicati nella forma abbreviata (abbreviazioni ufficialmente riconosciute). Gli articoli inediti si possono citare soltanto se sono in corso di pubblicazione, facendo loro seguire la dicitura "in corso di pubblicazione". Gli articoli, non ancora recensiti non possono essere citati.

Esempio di lavoro bibliografico:

*Articoli in riviste:*

**Klock, J.-H., A. Wieland, R. Seifert & W. Michaelis (2007):** Extracellular polymeric substances (EPS) from cyanobacterial mats: characterisation and isolation method optimisation. Mar. Biol., 152, 1077-1085.

*Libri ed altre pubblicazioni non periodiche (relazioni, tesi di laurea, dissertazioni di dottorato):*

**Wheeler, A. (1969):** The fishes of the British Isles and North-West Europe. McMillan, London, 613 p.

*Capitoli di libro:*

**McEachran, J. D. & C. Capapé (1984):** Myliobatidae. In: Whitehead, P. J. P., M. L. Bauchot, J.-C. Hureau, J. Nielsen & E. Tortonese (eds.): Fishes of the North-eastern Atlantic and the Mediterranean, Vol. 1. Unesco, Paris, pp. 205-209.

**12. Altro:** Le espressioni latine come ad es. *in vivo*, *in situ*, *e.g.*, *i.e.*, i nomi dei generi famiglie (*Myliobatis* sp.) e delle specie (*Myliobatis aquila*) si scrivono con il carattere italic. Quando possibile saranno utilizzate le unità del sistema SI (*Système international d'unités*).

**13.** Gli autori ricevono le **prime bozze** di stampa per la revisione. Le bozze corrette vanno quindi rispedite entro una settimana alla Redazione. In questa fase, i testi corretti con segni adeguati (indicazioni in merito si trovano alla fine della pubblicazione "Slovenski pravopis" (2001), Ljubljana, ZRC SAZU, 24-25, non possono essere più ampliati. La revisione delle bozze è svolta dalla Redazione.

**14.** La Redazione rimane a disposizione per eventuali chiarimenti.

LA REDAZIONE

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**2.** The articles submitted can be written in the English, Slovene or Italian language. The authors should ensure that their contributions meet acceptable standards of language, while the editorial board has the right to have them language edited.

**3.** The articles should be no longer than 48,000 characters (spaces excluded) or 32 typewritten double-spaced pages. They can be submitted via e-mail annales@mbss.org (preferably) or regular mail, with the electronic data carrier (CD) sent to the address of the editorial board.

Submission of the article implies that it reports original unpublished work and that it will not be published elsewhere.

**4.** The **title page** should include the title of the article, the name and surname of the author(s), their affiliation (institutional name and address) or home address, and e-mail address (of the first author or the corresponding author only).

**5.** The article should contain the **summary** and the **abstract,** with the former (c. 30 lines) being longer than the latter (c. 10 lines).

The *abstract* contains a brief description of the aim of the article, methods of work and results. It should contain no comments and recommendations.

The *summary* contains the description of the aim of the article and methods of work and a brief analysis or interpretation of results. It can contain only the information that appears in the text as well. It should contain no reference to figures, table and citations published in the main text.

**6.** Beneath the abstract, the author(s) should supply appropriate **keywords** (max 6) and, if possible, the English (or Slovene) translation of the abstract, summary, keywords, and captions to figures and tables. If unprovided, the translation will be provided by the editorial board.

**7.** The **main text** should include the following chapters: Introduction, Material and Methods, Results, Discussion or Results and Discussion, Conclusion, Acknowledgement (not obligatory), References. Individual parts of the text can form a sub-chapter (e.g. Survey of Previous Studies under Introduction; Description of Research Area under Material and Methods). Captions to figures should appear on a separate page beneath References.

**8.** Each **table** should be submitted on a separate page in Word programme (just like the main text). It should be numbered consecutively and supplied with the title – brief description. When referring to the tables in the main text, use the following style: (Tab. 1).

**9. Illustrative matter** (diagrams, maps, photographs, plates) should be submitted as separate files (in jpeg or tiff format) and saved at a minimum resolution of 300 dpi per size preferred, with the maximum possible publication size being 17x20 cm. Prior to publication, the author(s) should obtain all necessary authorizations (as stipulated by the Copyright and Related Rights Act) for the publication of the illustrative matter and submit them to the editorial board. All figures should be captioned and numbered consecutively (cf. Item 7). When referring to the figures in the main text, use the following style: (Fig. 1).

**10**. **Bibliographic notes** or **citations** – i.e. references to other articles or publications – should contain the following data: *author* and *year of publication,* e.g. (Novak, 2007). If there are two authors, include both surnames (Novak & Kranjc, 2001); if there are more than two authors, include the surname of the first author followed by a comma and the abbreviation *et al.* (Novak *et al.*, 1999). If there is more than one reference, separate them by a semicolon and list them in ascending chronological order, e.g. (Novak *et al.*, 1999; Adamič, 2001; Kranjc & Zupan, 2007). When citing information obtained through personal communication (oral, written), provide the initial letter of the name and full surname of the informant followed by a comma and the phrase *personal communication,* e.g. (J. Novak, *personal communication*).

**11.** The entire list of **bibliographic data** should be published under References in alphabetical order. The author(s) should list only the works cited in the article. If you are listing several works by the same author with some of them written in co-authorship, first list those written by the author him/herself, then those written in co-authorship with another author, and finally those written in co-authorship with more than one author, with the entries listed in chronological order. The names of journals in which the works cited were published should be abbreviated (cf. list of official journal abbreviations). Unpublished articles can be cited only if they have been approved for publication, which should be indicated by adding the phrase *in press* to the end of the relevant bibliography entry.

Some examples of how to cite different types of bibliographical data:

*Articles published in serial publications:*

**Klock, J.-H., A. Wieland, R. Seifert & W. Michaelis (2007):** Extracellular polymeric substances (EPS) from cyanobacterial mats: characterisation and isolation method optimisation. Mar. Biol., 152, 1077-1085.

*Books and other non-serial publications (reports, diploma theses, doctoral dissertation):*

**Wheeler, A. (1969):** The fishes of the British Isles and North-West Europe. McMillan, London, 613 p.

*Chapters published in a book:*

**McEachran, J. D. & C. Capapé (1984):** Myliobatidae. In: Whitehead, P. J. P., M. L. Bauchot, J.-C. Hureau, J. Nielsen & E. Tortonese (eds.): Fishes of the North-eastern Atlantic and the Mediterranean, Vol. 1. Unesco, Paris, pp. 205-209.

**12. Miscellaneous:** Latin phrases such as *in vivo*, *in situ*, *e.g.*, *i.e.*, and names of genera (*Myliobatis* sp.) and species (*Myliobatis aquila*) should be written in italics. Whenever possible, use the SI units (Système international d'unités).

**13.** The authors are sent the **first page proofs**. They should be returned to the editorial board within a week. When reading the proofs, the authors should use the correction signs listed at the end of the book Slovenski pravopis (2001), Ljubljana, ZRC SAZU, 24–25.

It is not allowed to lengthen the text during proof-reading. Second proof-reading is done by the editorial board.

**14.** For additional information regarding article publication contact the editorial board.

EDITORIAL BOARD

## KAZALO K SLIKAM NA OVITKU

SLIKA NA NASLOVNICI:
Hobotnice (*Octopus vulgaris*) so pridneni plenilci, ki imajo pomembno vlogo tako v morskem ekosistemu kot tudi v morskem ribištvu. Manj znano pa je, da so zelo uporabni tudi za pridobivanje zdravilnih učinkovin. (Foto: L. Lipej)
Sl. 1: V zadnjih desetletjih smo priča vse pogostejšemu pojavljanju tujerodnih vrst v slovenskem delu Jadrana. Tokrat raziskovalci poročajo o opazovanju orjaškega gološkrgarja vrste *Melibe viridis* v naravnem spomeniku Rt Madona. (Foto: M. Mandić)
Sl. 2: Že od nekdaj so peptidi iz črnila glavonožcev znani kot uporabne učinkovine proti raku. Oligopeptidi iz črnila sipe (*Sepia* sp.) dokazano zavirajo rast celic in so uporabni pri zdravljenju raka. (Foto: L. Lipej)
Sl. 3: V zadnjih letih so biogene formacije v Tržaškem zalivu pritegnile pozornost raziskovalcev. V teh izjemnih življenjskih okoljih, ki jih tvorijo mrtvi internet koraliti sredozemske kamene korale (*Cladocora caespitosa*), so našli nekatere zanimive vrste koraligenih alg, kot je vrsta *Neogoniolithon mamillosum* na sliki. (Foto: S. Kaleb)
Sl. 4: Tokratno mednarodno srečanje strokovnjakov za vodne ptice je potekalo avgusta v Reykjaviku na Islandiji. Udeleženci so imeli priliko seznaniti se z izjemno pestrostjo ptičjih vrst. Na sliki arktične čigre (*Sterna paradisea*). (Foto: I. Škornik)
Sl. 5: Z metodo opazovalnega cenzusa z uporabo vabe so ihtiologi v hrvaškem delu Jadranskega morja ugotovili, da se ribja združba v umetno preoblikovanem okolju manjših razsežnosti ne razlikuje občutno od tistih v naravnem okolju. (Foto: L. Lipej)
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