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## QUANTIFYING ABUNDANCE OF PREDATORS USING EXPERIMENTAL HOOK AND LINE FISHING: COMPARISONS INSIDE AND OUTSIDE A MARINE PROTECTED AREA IN THE CENTRAL ADRIATIC

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### Summary

A fundamental question in ecology is the role of predators in limiting their prey populations. This question can be approached for shallow marine fish communities by comparison of abundance of species inside and outside marine protected areas (MPAs), where fishing restrictions may enforce large abundance differences in large (top) predators. The first step in demonstrating differences in these populations is the development and testing of methods capable of estimating abundance of fast-swimming and alert species which generally are difficult to quantify with traditional net-based sampling or visual census. That the method of experimental fishing with hook and line is capable of sampling predatory fish of a wide range of sizes, from 15 to 125 cm, inside and outside an MPA in the central Adriatic Sea, Kornati National Park, Croatia, was demonstrated in this preliminary study. Evidence of significantly more abundant top predators inside the MPA and a significantly higher mean size of fish overall was found. A total of 11 species of fish were sampled across several benthic habitats, of which six were found only in the MPA. It is concluded that the MPA represents a natural experiment with greater abundance of larger predators than outside, and that experimental fishing can take advantage of these differences to test the hypothesis of top-down regulation of fish communities.

**Key words:** experimental fishing, MPA, top-down regulation, top predators, mesopredators

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## INTRODUCTION

A fundamental question in fish ecology and fisheries science is how fish communities are organized and to what extent predation influences community structure. Top-down control of a community is the limitation of populations by their consumers (Baum and Worm, 2009). Fisheries, especially if intense and size-selective, may cause low abundance or even absence of top predators and an interruption of top-down community control (Russ, 1991; Jennings and Lock, 1996). The absence of top predators, here defined as individual predatory fish of 30 cm in length or more, can in theory cause cascading effects across the food web (Paine and Vardas, 1969, Witman and Dayton 2001, Guidetti and Dulcic, 2007), as their prey and smaller sized competitors increase in abundance and in turn reduce the abundance of prey at the next position in the food web. Jukić-Peladić et al. (2001), comparing trawl surveys from 1948 and 1998 in the Adriatic Sea, were able to detect (despite methodological inconsistencies) a plausible loss of top predator abundance, especially of those belonging to the elasmobranchs. A more detailed study by Krstulović-Šifner et al. (2009) showed a significant loss in geographical distribution area, decreased abundance and decreased total length at first sexual maturity for the top predator, *Raja clavata* – all signs of overexploitation. Such losses of top predators may cause changes in the entire fish community, resulting in large shifts in fish diversity and productivity. Using an ecosystem model tested against biomass and catch data, Coll et al. (2009) found that trophic interactions and direct fishing exploitation were important variables in predicting the observed decline in biomass for several fish species in the Northern-Central Adriatic.

One way to test the hypothesis that top predators influence fish community structure is to compare communities inside marine protected areas (MPAs) and outside (open sites), since large chase predators are likely to be present in the former and absent from the latter. Hence MPAs represent a natural experiment that can test the role of large top predators on the local fish community.

One important challenge is to determine an appropriate field method for sampling and quantifying fish communities, as each method has its unique limits and biases. Netting methods are biased against fish that hide and wait in three dimensional structure, bury themselves in the substrate, escape with rapid and alert movement, and pass freely through the net mesh (Harmelin-Vivien and Francour, 1992). The repeated use of passive trammel nets along the Croatian Adriatic by Stagličić has shown to be effective in catching the majority of the expected species (100+) and apparently there was little bias against top predators. Yet, the species identified as those that were driving the observed overall increases in abundance and biomass within the study period did not represent top predators. Visual census is primarily biased against large fast moving fish that can detect and actively avoid the diver or snorkeler carrying out the survey (Watson et al., 2009; Bozec et al., 2011). In an ongoing three-year visual census study of fish in the Croatian Adriatic by Kruschel (see Kruschel et al. (2012) herein for methodology), very few of the 35000+ individually observed fish were of top predator size. However, Kruschel's survey which is matching the geographical scale of Stagličić et al. (2011) also detected 100+ species (unpublished data). One comparative method that may be less biased against top predator sized fish is experimental hook and line fishing (Cardona, 2007), especially when bait

is presented under the physical absence of humans. This method can target predators of a wide size range through the use of various bait types and hook sizes, and can be applied equally efficiently in diverse habitats ranging from flat, unconsolidated sediments to structurally complex ground such as rocky reefs and seagrass meadows. Also, hooks can be displayed at known or even preselected depths.

Here we report on the use of experimental fishing in the summer of 2011 in the Central Croatian Adriatic for estimating the relative abundance of top (30+ cm) versus meso (15-30 cm) predators in an MPA (Kornati National Park, Croatia) and adjacent areas open to fisheries (along the outer coast of Pašman Island). We predicted that experimental fishing would allow us to fish across the predator community with the caught fish presenting a large range of sizes. We further predicted that the ratio of top predators to mesopredators would be higher inside the MPA than outside (open sites), that consequently predatory fish are on average smaller outside the MPA at sites open to fisheries, and that top predator richness is higher inside the MPA.

## *MATERIALS AND METHODS*

### *Study sites*

Experimental fishing was carried out inside the boundaries of the Kornati National Park (further referred to as “MPA sites”) where fisheries is restricted, and outside the national park boundaries in an area open to fisheries (further referred to as “open sites”). Within each of these macroregions, several fishing sites were selected (Figure 1).

### *Experimental Fishing*

Open-site fishing was conducted in MPA sites with the same effort and within three habitat types: *Posidonia oceanica* beds, unconsolidated sediments and rocky reefs. At each of these macroregions, 72 vertical lines locally called “samica” (Figure 2) which carried hooks of three different sizes (“big” - loop diameter 35 mm, “medium” - 13 mm loop diameter and “small” - 8 mm loop diameter) were installed and the same variety of bait types were offered: seaworm, whole fish or fish pieces, octopus and holothurians. All bait types used had shown in pre-trials to be the best baits for top predators and mesopredators and they are the preferred baits of fishers in the area. Hooks were placed either directly on the sea bottom or 1.5 m above in all targeted habitats. The individual line locations represented a range of depths from very shallow (2-3 m) to as deep as 30 m. Lines were laid out during the day for approximately six hours for a total of 42 hours and at night for approximately 7 hours for a total of 49 hours. Upon retrieval of the lines, hooks were recorded as retrieved, cut or not found. Retrieved hooks were identified as not having caught fish vs. having caught fish. Hooked fish were identified by species and the standard length of each individual was measured. According to length, fish were categorized into top predators (> 30 cm) and mesopredators (15-30 cm), regardless of species.

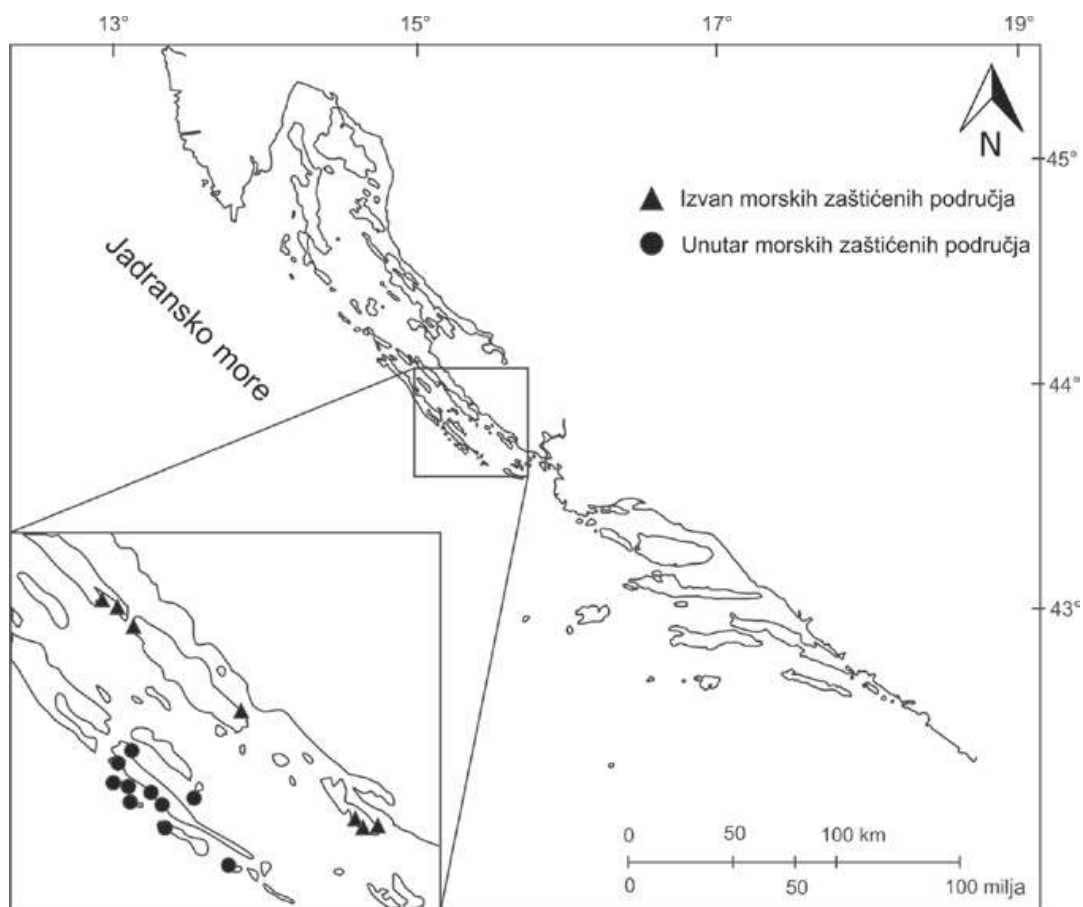


Figure 1. Map of the fishing sites visited in summer 2011. Black triangles represent the sites in an unprotected area open to fisheries (UA sites). Black circles represent the sites inside the boundaries of the Kornati National Park (MPA sites).

Slika 1. Karta lokacija s mjestima na kojima je obavljen ribolov u ljetu 2011. godine. Crni trokuti predstavljaju lokacije izvan zaštićenog područja gdje je dozvoljen ribolov (UA). Crni krugovi predstavljaju lokacije unutar granica Nacionalnog parka Kornati, Hrvatska (MPA).

#### Data analysis

Pearson chi-square tests with Yates' continuity correction were used to compare the number of fish caught within different predator categories inside and outside the MPA. The Kruskal-Wallis test was used to test MPA effect on caught fish size, as the residuals of the comparison were not normally distributed (Shapiro-Wilk test).

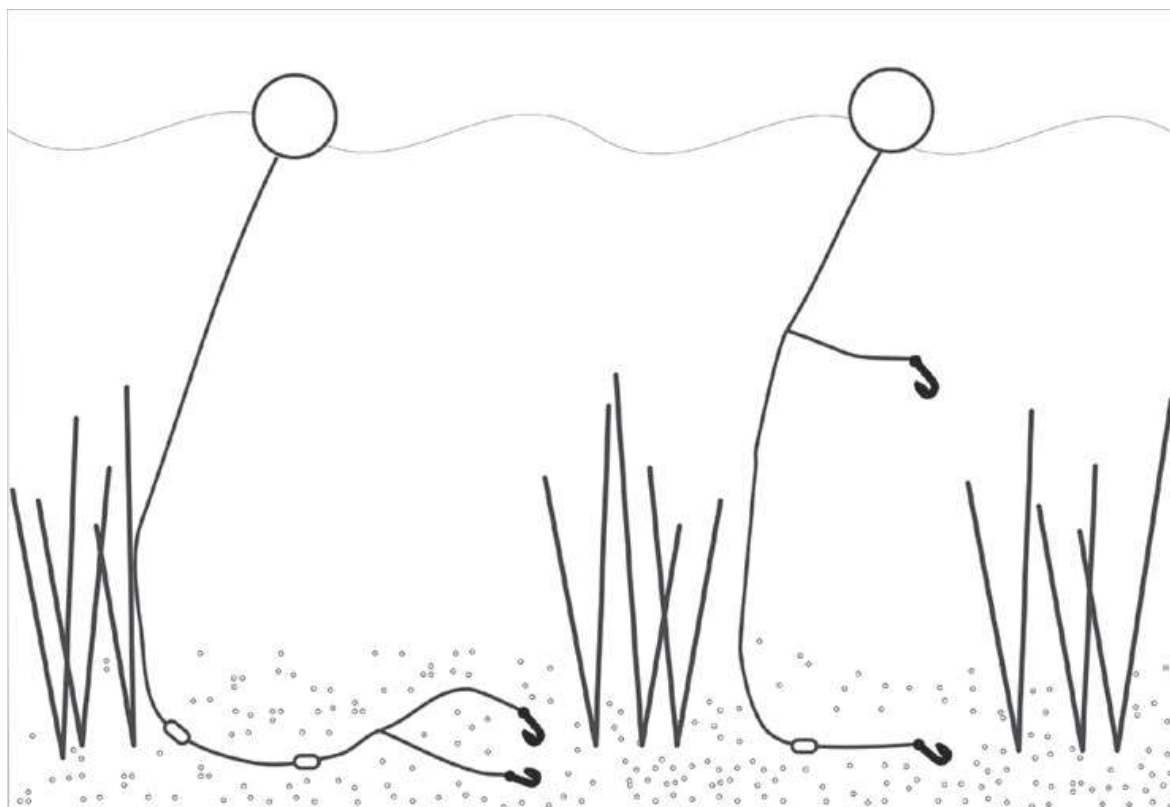


Figure 2: Illustration of a fishing tool locally called “samica”. It is a single fishing line which carries hooks of two different sizes placed directly on the sea bottom or above, in all targeted habitats.

Slika 2: Ilustracija ribolovnog alata koji je poznat pod nazivom “samica” ili samolovka. Radi se o alatu s jednom niti najlona na kojemu su dvije udice različite veličine postavljene na morsko dno ili u neposrednoj blizini dna, na svim ciljanim staništima.

## RESULTS

At MPA and open sites respectively, 91.5% and 97.9% of the hooks were retrieved. At MPA sites, 8.3% of the hooks were cut and 1% lost, while only 2.2% were cut at open sites (Table 1). There were significantly more cut hooks inside the MPA than at open sites ( $X^2 = 9.04$ ,  $df = 2$ ,  $p = 0.0109$ , Pearson’s chi-square test).

At MPA sites, 79% of the retrieved hooks caught no fish, 8.3% caught mesopredators and 5.4% caught top predators. In open sites, 89.7% of the retrieved hooks caught no fish, 8.7% caught mesopredators and 1.6% caught top predators, which indicates no significant differences in relative number of top predators vs. mesopredators when comparing MPA and open sites ( $X^2 = 1.97$ ,  $df = 1$ ,  $p = 0.160$ , Pearson’s chi-square test).

*Table 1: Number of hooks laid out in MPA sites and open sites. There were significantly more cut hooks at MPA sites than at open sites ( $X^2 = 9.04$ ,  $df = 2$ ,  $p = 0.0109$ , Pearson's chi-square test) and also a significantly higher top predator to mesopredator ratio in MPA sites as compared to open sites ( $X^2 = 4.95$ ,  $df = 1$ ,  $p = 0.0261$ , Pearson's chi-square test).*

*Tablica 1: Broj udica postavljenih unutar i izvan morskih zaštićenih područja. Značajno više udica je otkinuto unutar zaštićenog područja nego izvan njega ( $X^2 = 9.04$ ,  $df = 2$ ,  $p = 0.0109$ , Pearson's chi-square test). Također je i značajno veći omjer vršnih predatora i mezopredatora unutar zaštićenog područja u usporedbi s nezaštićenim područjima ( $X^2 = 4.95$ ,  $df = 1$ ,  $p = 0.0261$ , Pearson's chi-square test).*

MPA sites/ Morska zaštićena područja	Open sites/ Izvan zaštićenih područja	Number of hooks/ Broj udica
224	188	Laid out/ Postavljeno
205	184	Retrieved/ Pronađeno
17	4	Cut/ Otkinuto
2	0	Not found/ Nije pronađeno
177	165	Without caught fish/ Bez ulovljene ribe
17	16	With caught mesopredators/ S ulovljenim mezopredatorima
11	3	With caught top predators/ S ulovljenim vršnim predatorima
28	7	Combined: cut hooks and hooks with top predators/ zajedno: otkinute udice i udice s ulovljenim vršnim predatorima

However, when cut hooks are interpreted as indicators for the action of large fish (like top predators), 8.3% of the caught fish in MPAs were mesopredators and 13.7% were top predators, while 8.7% of the fish caught in open sites were mesopredators and 3.8% were top predators. This indicates a significantly higher top predator to mesopredator ratio in MPA sites as compared to open sites ( $X^2 = 4.95$ ,  $df = 1$ ,  $p = 0.0261$ , Pearson's chi-square test).

The 47 fish caught with our hook and line experimental fishing method represent a wide range of predator sizes, ranging from 15 to 125 cm. The fish were caught across all three targeted habitats within MPA sites and open sites.

The 28 fish caught at MPA sites had a mean standard length of 27.5 cm and were significantly larger than the 19 fish caught at open sites with a mean standard length of 23 cm (Figure 3) ( $X^2 = 5.56$ ,  $df = 1$ ,  $p = 0.0183$ , Kruskal-Wallis chi-square test).

The 47 fish caught belong to 11 species of which three were caught in both MPA and open sites, two were unique to open sites and six were unique to MPA sites (Table 2).

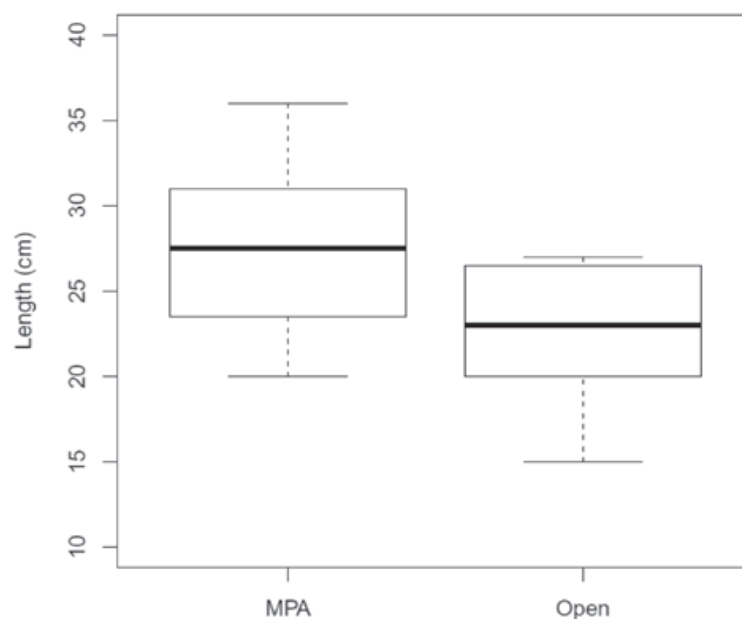


Figure 3: Boxplot of the number of fish caught at MPA sites and fish caught at open sites. The 28 fish caught at MPA sites were significantly larger than the 19 fish caught at open sites ( $X^2=5.56$ ,  $df=1$ ,  $p=0.0183$ , Kruskal-Wallis chi-square test).

Slika 3: Boxplot dijagram koji prikazuje broj riba ulovljenih unutar morskih zaštićenih područja i broj riba ulovljenih izvan morskih zaštićenih područja. 28 riba ulovljenih unutar zaštićenih područja značajno su veće nego 19 riba ulovljenih izvan zaštićenih područja ( $X^2=5.56$ ,  $df=1$ ,  $p=0.0183$ , Kruskal-Wallis chi-square test).

## DISCUSSION

The method used in this study, experimental hook and line fishing using a variety of hook sizes and numbers, was shown to sample a wide range of predator species (11) and sizes (15 - 125 cm) in unconsolidated sand habitats, complex rocky reefs and seagrass beds. This method can therefore be used to compare MPA sites and open sites with respect to the relative abundance and sizes of top predators and mesopredators of these species.

Our finding of larger predator size within the MPA is in agreement with the published studies in the Mediterranean and elsewhere (Harmelin, 1995; Jennings & Kaiser, 1998). Francour (1994), in a study in the NW Mediterranean, attributed these differences to a “refuge effect” characterized by larger fishes and a greater variety of species of predatory fish. Halpern et al. (2002) reviewed 80 reserves worldwide and concluded that MPAs as compared to outside locations consistently harbored fish of larger size (relative to control) across the majority of species regardless of the age of the reserve.

Table 2: Fish species caught at MPA (a) and UA sites (b). Names given in bold represent the species exclusive to MPA sites or open sites. Names indicated by an asterisk (\*) indicate species found in either sites.

Tablica 2: Vrste riba ulovljene unutar morskih zaštićenih područja a) i izvan morskih zaštićenih područja b). Podebljani nazivi predstavljaju vrste koje su zabilježene isključivo unutar ili izvan morskih zaštićenih područja. Imena kraj kojih je zvjezdica (\*) predstavljaju vrste zabilježene i unutar morskih zaštićenih područja i izvan njih.

Species/vrsta	Predtype/tip predatora	
	Meso/mezo	Top/vršni
a) MPA sites/Morska zaštićena područja		
<b><i>Conger conger</i></b>	0	<b>3</b>
<b><i>Diplodus sargus</i></b>	<b>4</b>	<b>4</b>
<i>Diplodus vulgaris</i> *	7	0
<b><i>Epinephelus guaza</i></b>	0	<b>1</b>
<i>Mustelus mustelus</i>	0	0
<b><i>Pagellus erythrinus</i></b>	<b>2</b>	0
<b><i>Scorpaena scorfa</i></b>	<b>1</b>	0
<i>Scyliorhinus stellaris</i>	0	0
<i>Serranus scriba</i> *	1	0
<i>Sparus aurata</i> *	2	2
<b><i>Spondyliosoma cantharus</i></b>	0	<b>1</b>
b) Open sites/Izvan morskih zaštićenih područja		
<i>Conger conger</i>	0	0
<i>Diplodus sargus</i>	0	0
<i>Diplodus vulgaris</i> *	3	0
<i>Epinephelus guaza</i>	0	0
<b><i>Mustelus mustelus</i></b>	0	<b>2</b>
<i>Pagellus erythrinus</i>	0	0
<i>Scorpaena scorfa</i>	0	0
<b><i>Scyliorhinus stellaris</i></b>	0	<b>1</b>
<i>Serranus scriba</i> *	7	0
<i>Sparus aurata</i> *	5	0
<i>Spondyliosoma cantharus</i>	0	0

Matić-Skoko (2012) compared total fish abundance in 2011 at the Lastovo Nature Park to baseline data from 2010 and reported a 9% increase in abundance associated with a 19% increase in biomass in marine protected areas, and a 12% lower total abundance and 24% lower biomass in fishing zones. This indicates that the protection of fisheries results in more and larger fish, whereas continued fishing results in the opposite – less and smaller fish. The reported data also indicate that enforced protection from fisheries can result in increased fish size on a short-term temporal scale.

While the relative abundance of top predators was higher in MPA sites than in open sites, this result was only significant when using a cut line as a proxy for a strike by a top



predator. Assuming only larger fish have the strength to break these lines, by our definition, top predators (> 30 cm) are primarily responsible for line breakage. However, we cannot exclude the possibility that hooks on the cut lines first caught smaller fish that then attracted top predators, which then cut the lines. If this were true, our abundance estimates of the smaller fish would be biased slightly downwards. We saw no evidence of this possibility in the caught top predators, which ingested only our bait. The methodological inconsistencies in the trawling data comparison by Jukić-Peladić et al. 2001 unfortunately prevent a quantitative analysis of the data with respect to relative top predator vs. mesopredator abundances following increases in top predator fisheries within the 60 years in between experimental trawling events. However, the reported relative increase in abundance of *Mullus barbatus* and *Serranus hepatus*, two mesopredator species with common length of 10-20 cm (Vrgoč et al., 2004) and 15 cm (Froese and Pauly, 2000) respectively may indicate such a predicted shift. Stagličić et al. (2012) observed, in experimental trammel net experiments over 16 years (1983-2009) in the shallow (< 40 m) Croatian Adriatic, a significant increase in four mesopredator species (all of which have, according to Froese and Pauly (2000), common length lower than 30 cm): *Symphodus tinca*, *Pagellus erythrinus*, *Mullus surmuletus*, and *Scorpaena porcus*, and interpret this increase as a result of changes in fishing gear use and restriction comprehensively reviewed in Matić-Skoko et al. (2011). However, such long-term and temporary consistent unidirectional changes could also be the result of ongoing decreases in top predators that are either predators or competitors of these four species. Plausible candidates for such interactors are *Labrus viridis* and *L. merula*, *Pagrus pagrus*, *Dentex dentex*, *Dicentrarchus labrax*, and *Scorpeana scrofa* (all of which, according to Froese and Pauly (2000), have common lengths higher than 30 cm and share similar habitats with at least one of the four species).

A greater number of species were caught in MPA compared to outside sites. Similar results, estimated as higher richness and diversity, were obtained in many other locations (e.g. Polunin and Roberts, 1991). However, considering the small total number of predators caught in this study, larger fishing effort is necessary to increase the confidence and generality of this result and to estimate and compare diversity indices.

Given that lure-assisted visual census has shown to be an effective method for sampling a range of fish species concentrated in the smaller size ranges, including hidden species, we recommend this method to be used to supplement experimental fishing to provide independent and probably more extensive sampling of smaller size classes. Other methods such as traps, tethering and baited cameras might also prove useful as supplementary independent assays, depending on the specific habitats, topographic constraints and behavior of the sampled species.

We conclude that the method of experimental fishing is capable of quantifying relative size and abundance of a wide range of predatory fish, including top predators, and is a valuable method for testing hypotheses of fish community organization using MPAs as experimental treatments that provide sharp spatial variation in size and abundance of top predators.

*Sažetak***ODREĐIVANJE BROJNOSTI PREDATORA KORIŠTENJEM  
EKSPERIMENTALNOG RIBOLOVA: USPOREDBE UNUTAR  
I IZVAN MORSKIH ZAŠTIĆENIH PODRUČJA U SREDNJEM  
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Temeljno pitanje u ekologiji je uloga predatora u kontroli populacije plijena. Za zajednice riba u plitkom moru, na ovo pitanje može se odgovoriti uspoređujući brojnost vrsta unutar morskih zaštićenih područja (MPA) i izvan njih, gdje regulacija ribolovnih aktivnosti može značajno utjecati na brojnost velikih (vršnih) predatora. Prvi korak u dokazivanju razlika u navedenim populacijama je razvoj i testiranje metoda uz pomoć kojih je moguće procijeniti brojnost brzih i opreznih vrsta, koje je obično teško kvantificirati korištenjem tradicionalnih metoda poput lova mrežama ili vizualnim cenzusom. U ovom preliminarnom istraživanju je prikazano da se korištenjem metode eksperimentalnog ribolova mogu uzorkovati predatorske vrste riba raznih veličina, od 15 do 125 cm, unutar i izvan morskih zaštićenih područja u srednjem Jadranu (na području Nacionalnog parka Kornati, Hrvatska). Dokazala se značajno veća brojnost vršnih (top) predatora unutar zaštićenog područja kao i značajno veća prosječna veličina riba. Uzorkovano je ukupno 11 različitih vrsta riba na raznim pridnenim staništima, od kojih je 6 zabilježeno isključivo unutar zaštićenog područja. Može se zaključiti da su morska zaštićena područja svojevrsni prirodni eksperiment gdje je brojnost predatorskih vrsta veća nego izvan zaštićenog područja. Osim toga, eksperimentalni ribolov je učinkovita metoda uz pomoć koje se na temelju uočenih razlika može testirati hipoteza o top-down regulaciji zajednica riba.

**Ključne riječi:** eksperimentalni ribolov, MPA, top-down regulacija, top-predatori, mezo-predatori

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