Struktura ribljih naselja na okomitim koraligenim staništima u Hvarskom kanalu

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Poslijediplomski sveučilišni studij
Primijenjene znanosti o moru

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STRUKTURA RIBLJIH NASELJA NA OKOMITIM KORALIGENIM STANIŠTIMA U HVARSKOM KANALU

Igor Glavičić

Rad je izrađen na Sveučilišnom odjelu za studije mora, Sveučilišta u Splitu

Sažetak

Ova disertacija se sastoji od pet objedinjenih radova i zasniva se na istraživanjima ribljih naselja na podvodnim vertikalnim koraligenim grebenima tehnikom podvodnog vizualnog cenzusa (metoda kvadrata i metoda transekta) i ulovnim neribolovnim tehnikama. Podaci su se prikupljali na četiri lokacije u Hvarskom kanalu tijekom razdoblja od pet godina. Najveća dubina ronjenja prilikom istraživanja je bila 65 m, a uz korištenje tehnike dubokih dekompresijskih zastanaka osim komprimiranog zraka za disanje korišteni su i Nitrox50 i Trimix mješavine plinova. Prvi rad obuhvaća kvantitativno uzorkovanje metodom kvadrata od površine do 45 m dubine te je prikupljeno 220 kriptobentoskih i 61 epibentoska jedinki. Kriptobentoskim naseljima riba dominirala je obitelj glavoča, kako po biološkoj raznolikosti (> 60% svih vrsta) tako i po brojnosti (> 90% svih primjeraka). Vrste koje su se ranije smatrale rijetkim bile su prisutne, neke čak i brojne u ovom istraživanju. Drugi rad opisuje prvi put korištenu kvantitativnu metodu uzorkovanja za duboku kriptobentosku i epibentosku ihtiofaunu pomoću Trimix mješavina plinova koja je provedena na dubini od 57 do 62 m. Prikupljeno je 16 primjeraka iz četiri vrste riba, tj. tri vrste glavoča i jedna babica. Dvije od sakupljenih vrsta, *Thorogobius macrolepis i Vanneaugobius dollfusi* su do tada smatrane rijetkima, usljed veoma malo zabilježenih nalaza u svijetu. U trećem radu testirane su dnevne razlike u sastavu naselja epibentoskih i kriptobentoskih riba. Ukupno je zabilježeno 27 kriptobentoskih i epibentoskih vrsta riba. Kombinirani rezultati sugeriraju da su kriptobentoska riblja naselja većinom sastavljena od stalnih stanovnika skrivenih prostora s veoma malim dnevnim promjenama. Četvrti rad opisuje novu metodu podvodnog vizualnog cenzusa prikladnu za dublje vertikalne podvodne grebene.

Nazvana je Deep Vertical Transect (DVT) i rađena je na dubinama od površine mora do 50 m. U istraživanju je ukupno zabilježena 51 vrsta riba, pri čemu je 41 vrsta kategorizirana kao stalni stanovnik koraligenog grebena, dok se 10 vrsta smatra povremenim stanovnicima. U transektima ispod termokline opažen je najmanji broj vrsta što ukazuje na temperaturu kao jedan od najvažnijih parametara koji utječe na dubinsku raspodjelu ribljih naselja u ljetnom razdoblju. Peti rad opisuje metodu podvodnog vizualnog cenzusa zasnovanu na korištenju transekta te podvodnog skutera uz uporabu ronilačkih aparata zatvorenog kruga disanja. Tri varijable okoliša, dubina, životinjski pokrov i istočni položaj grebena, identificirane su kao vrlo značajne za pojavljivanje vrsta, ali značajne varijable bile su i nagib, morsko dno s pukotinama i šupljinama (prostorna heterogenost) te podloga s više ili manje glatkom površinom.

Ključne riječi: podvodni vizualni cenzus, metoda vertikalnog transekta, metoda kvadrata, riblja naselja, koraligeni grebeni, kriptobentoske vrste.

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STRUCTURE OF FISH COMMUNITIES ON VERTICAL CORALLIGENOUS HABITATS IN THE HVAR CHANNEL

Igor Glavičić

Thesis performed at: University Department of Marine Studies, University of Split

Abstract

This dissertation consists of 5 combined papers and is based on research of fish communities on underwater vertical coralligenous reefs using the underwater visual census technique (square method and vertical transect method) and catching non - fishing techniques. Data were collected at 4 locations in the Hvar Channel over a five years period. The maximum diving depth during the research was 65 m, and with the use of the technique of deep decompression stops, in addition to compressed breathing air, Nitrox50 and Trimix gas mixtures were also used. The first paper includes quantitative sampling by the method of squares from the surface to a depth of 45 m, and 220 cryptobenthic and 61 epibenthic individuals were collected. Cryptobenthic fish communities were dominated by the gobiid family, both in terms of biological diversity (> 60% of all species) and in terms of abundance (> 90% of all samples). Species previously considered as rare were present, some of them even numerous in this locality. The second paper describes the first-used quantitative sampling method for deep cryptobenthic and epibenthic ichthyofauna using Trimix gas mixtures conducted at a depth of 57 to 62 m. Two of the collected species, *Thorogobius macrolepis* and *Vanneaugobius dollfusi*, were previusly considered rare in the Mediteranean, due to the very few reported records. In the third paper, daily differences in the composition of cryptobenthic and epibenthic fish populations were tested. A total of 27 cryptobenthic and epibenthic fish species were recorded. The results suggest that cryptobenthic fish settlements are mostly composed of permanent residents with very little daily change. The fourth paper describes a new method of underwater visual census suitable for deeper vertical underwater reefs. We named it as Deep Vertical Transect (DVT) and we performed it at depths of up to 50 m. The study recorded a total of 51 fish species, with 41 species categorized as permanent inhabitant of the coralligenous reef, while 10 species are considered occasional residents. In the transects below the thermocline, the smallest number of species was observed, which indicates temperature as one of the most important parameters influencing the depth distribution of fish settlements in the summer period. The fifth paper describes the method of underwater visual census based on the use of a transect and an underwater scooter with the use of closed-circuit diving apparatus - CCR rebreather. Three environmental variables, depth, zoocover and eastern reef position, were identified as very significant for species occurrence, but significant variables were also slope, seabed with cracks and cavities, and substrate with more or less smooth surface.

Thesis deposited in National and University Library in Zagreb and Split University Library

Keywords: underwater visual census, vertical transect method, square method, fish communities, coralligenous reefs, cryptobenthic species..

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1. SADRŽAJ

2. PREGLED OBJAVLJENIH RADOVA

Ova doktorska disertacija temelji se na objedinjenim sljedećim znanstvenim radovima:

1. Glavičić, I., Paliska, D., Soldo, A., Kovačić, M. (2016*)*. A quantitative assessment of the cryptobenthic fish assemblage at deep littoral cliffs in the Mediterranean. *Scientia Marina*, 80, 329-337. http://dx.doi.org/10.3989/scimar.04307.23A
2. Glavičić, I., Kovačić, M., (2016). A quantitative sampling method for assesment of deep cryptobenthic ichthyofauna using trimix diving. *Acta Ichthyologica and piscatoria*, 46 (1), 43–47. http://dx.doi.org/10.3750/ AIP2016.46.1.06
3. Glavičić, I., Kovačić, M., Soldo, A., Schliewen, U. (2020). A quantitative assessment of the diel influence on the cryptobenthic fish assemblage of the shallow Mediterranean infralittoral zone. *Scientia Marina*, 84(1), 49-57. https://doi.org/10.3989/scimar.04994.21A
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5. Glavičić, I., Kovačić, M., Paliska, D., Laslo, D. (2020*)*. Reef fish assemblage changes along depth gradient revealed by video on underwater scooter method. *Acta Adriatica 61 (2)*, 145-162.

3. UVOD

3.1. Metode istraživanja riba

Za primjereno upravljanje i kontrolu stanja ribljih populacija i zajednice u cjelini neophodno je skupiti podatake o kvalitativnom i kvantitativnom sastavu ribljih naselja u nekom području kao i o ekološkim čimbenicima koji oblikuju taj sastav. Još je Brock (1954) ustvrdio da ako ne postoji metoda koja može mjeriti rezultate upravljanja ili regulacije, bilo koje opravdanje za upravljanje ili reguliranje nestaje. Bez takvih podataka te njihove analize ne može se krenuti u procjenu stanja i izradu planova upravljanja. Upravo stoga metode za prikupljanje podataka moraju osigurati što točniji i precizniji prikaz trenutne stvarne situacije na izabranom području kao i trendove u usporedbi s ranijim podacima. Postoje različite metode prikupljanja podataka o stanju populacija riba i zajednice u cjelini, a sve se mogu svrstati u tri različite skupine: ulovne metode, kombinirane i neulovne (Soldo & Glavičić, 2011). Pod ulovnim metodama uglavnom smatramo sve one metode u kojima se istraživani organizmi love različitim ribolovnim alatima ili se love posebnim metodama koje nisu uobičajene u ribolovu. Kombinirane metode imaju sličnosti i sa ulovnim i neulovnim metodama jer koriste određene tehnike iz obje grupe. Primjerice, tipična kombinirana metoda prikupljanja podataka bi bila metoda označavanja, pri čemu se određeni organizam lovi te označava različitim oznakama ili uređajima, zatim pušta, da bi se nakon nekog zadanog protoka vremena skupili podaci dobiveni označavanjem. Ovakve metode su manje primjenjive za sakupljanje kvalitativnih i kvantitativnih podataka o strukturi zajednica i strukturi i brojnosti populacija, ali su veoma učinkovite u istraživanjima starosti, rasta, kretanja i ponašanja morskih organizama. Neulovne metode uključuju hidroakustična istraživanja raspodjele populacija riba te podvodne vizualne cenzuse ribljih zajednica i vrsta, a glavna im je odlika da nisu štetne te ne dolazi do uništavanja niti organizama niti okoliša te se mogu primijeniti u zaštićenim područjima (Soldo & Glavičić, 2011).

3.1.1. Ulovne istraživačke metode primjenom ribolovnih alata

Istraživačke metode primjenom ribolovnih alata pripadaju ulovnim metodama i najčešće uključuju upotrebu različitih ribolovnih alata i opreme koji primarno ciljaju istraživane resurse, a zasnovane su na analizi ostvarenog ulova po jedinici ribolovnog napora te se on postavlja kao referentni indeks na osnovu kojeg će se procjenjivati gustoća populacije određene vrste. Dobra strana im je da se mogu koristiti tijekom cijele godine, na različitim dubinama. S tehničke i organizacijske strane gledišta ovakve metode ne zahtijevaju visoko znanstveno obrazovano osoblje kod prikupljanja podataka. Loša strana ovih metoda je selektivnost ribolovnih alata, njihova konstrukcija i izbor mamaca koji uvjetuju da pouzdanost ovih metoda varira od veoma male do umjerene, ograničenost ribolova u zaštićenim područjima te štetnost po istraživane resurse. Također, ulovne metode se ne mogu koristiti na svim vrstama dna jer ne daju cjelovitu sliku stanja, npr. totalni cenzus koćom nije moguć na stjenovitom dnu, a alati koji su mogući na takvom dnu, npr. mreže stajačice, su jako selektivni u sakupljanju i samim time rezultiraju nedovoljno točnim i preciznim podacima (Labrosse i sur., 2002) također ove metode ne daju nikakve podatke o stanju na dnu.

3.1.2. Ulovne neribolovne metode

Riblja zajednica koja živi na podvodnim grebenima sastavljena je od dvije komponente, vidljivog dijela riba: bentopelagičnih (žive na dnu, ali i iznad dna u zoni otvorenog mora), hiperbentoskih (žive u vodenom stupcu u blizini dna) i epibentoskih (koje žive na samom dnu) te od kriptobentoskih (La Mesa, 2006) pa se samim vizualnim cenzusom ne može u potpunosti obuhvatiti njena cjelina (Brock, 1982; Beldade & Goncalves, 2007). Kriptobentoska riblja zajednica općenito obuhvaća male vrste, kao što su npr. neke ribe iz obitelji babica (Blennidae) i glavoča (Gobiidae), koje su vizualno teško uočljive i drže se u neposrednoj blizini ili unutar samog dna. Unatoč tome što je važnost kriptobentskih riba u funkcioniranju ekosustava infralitorala prepoznata (Depczynski & Bellwood, 2003), relativno malo istraživanja usredotočeno je na te, manje vidljive, članove pridnene riblje zajednice. Kao što ističu različiti autori (Brock, 1982; Ackerman & Bellwood, 2000; Depczynski & Bellwood 2003), standardni vizualni cenzusi korišteni za proučavanje ribljih naselja nisu prikladni za uočavanje, identifikaciju i detaljan popis kriptobentoskih vrsta. Upravo zbog toga, mnogi autori smatraju da bez ulovnih metoda nije moguće u potpunosti opisati riblju zajednicu na određenom području (Ackerman & Belwood, 2000; La Messa, 2006). Da bi se steklo puno razumijevanje o tome kakav je sastav kriptobentoskih i epibentonskih ribljih naselja na tvrdom morskom dnu, potrebno je koristiti i ulovne neribolovne tehnike. Nažalost njihova primjena u zaštićenim područjima nije moguća. Ove tehnike, po definiciji, imaju štetan učinak na ribe. Često se koriste zajedno s vizualnim cenzusima radi što potpunijih popisa vrsta ili procjene relativne brojnosti (Ackerman & Bellwood, 2000; Willis, 2001). Tri glavne ulovne neribolovne metode međusobno se razlikuju obzirom na sredstvo koje se koristi pri sakupljanju uzoraka, eksplozivi, ihtiocidi i anestetici. Eksplozivi su neselektivni i razorni, danas se veoma malo, ako uopće koriste i omogućuju prikupljanje većine zajednice s određenog područja. Ipak, za ribe bez plivajućih mjehura (npr. neke vrste glavoča) koje ostaju u rupama ta metoda nije prikladna, a također osim ubijanja riba na mjestu primjene, razaraju i ostale životne zajednice kao i stanište na kojem one obitavaju (Kulbicki, 1990; Ackerman & Bellwood, 2000). Ihtiocidi, kao primjerice rotenon, općenito se koriste za kvalitativne metode prikupljanja uzoraka, npr. za zbirke muzeja ili istraživanja ihtiofaune. Nekoliko studija koristile su rotenon na kvantitativan način (Randall, 1963; Brock, 1982; Kulbicki, 1990), ali njegovi nedostaci su u tome što je relativno skup i nepraktičan jer mu je djelovanje veoma sporo, odnosno nakon nanošenja potrebno je pričekati 20-ak minuta te ga kod dubokih zarona nije moguće primjenjivati zbog vremenski ograničenog boravka na dnu (Smith, 1973; Robertson & Smith-Vaniz, 2008). Anestetici kao quinaldin koriste se kao sredstvo za prikupljanje uzoraka još od 60-tih godina prošlog stoljeća (Gibson, 1969; Sayer i sur., 1994). Njegova primjena pod vodom je uglavnom vezana za istraživanja kriptičkih i bentoskih ribljih vrsta (Kotrschal, 1983). Obzirom da nije topljiv u vodi, najčešće se miješa s 95% alkoholom u omjeru 1:4. Postaje učinkovit već nakon 1-3 min nakon čega većina riba počinje izlaziti omamljena iz svojih skrovišta (Sayer i sur., 1994).

3.1.3. Neulovne istraživačke metode podvodnog vizualnog cenzusa

Metoda podvodnog vizualnog cenzusa (UVC od engl. Underwater Visual Census) se razvila iz potrebe da se prikupe podaci na mjestima problematičnima za standardne načine uzorkovanja, primjerice koraljnim grebenima. Prvi ju koristi Brock (1954) na Havajima za procjenu stanja ribljih naselja na koraljnim grebenima. Od tada je metoda podvodnog vizualnog cenzusa široko prihvaćena i korištena za različita istraživanja, primjerice u istraživanjima ekologije morskih organizama (Edgar i sur., 2004), strukturi naselja (Prochazka, 1998), raznim ekološkim procesima (Nanami & Nishihira, 2003) te biogeografskim uzorcima u okolišu (Gasparini & Floeter, 2001). Sama metoda se koristi na veliki broj načina, a obzirom na subjekte u istraživanju najčešće je koriste ronioci na dah te ronioci s autonomnom ronilačkom opremom (ARO) (English i sur., 1994). Može se također obavljati i upotrebom podvodnih vozila sa ili bez ljudske posade. Što se tiče načina uzimanja podataka, metoda podvodnog vizualnog cenzusa se općenito dijeli na metodu kvadrata, metodu točke i metodu transekta. Postoje različiti čimbenici koji ograničavaju pouzdanost podvodnog vizualnog cenzusa, a među njima su najvažniji vidljivost, stanje površine mora i dubina provođenja istraživanja koja je u izravnoj korelaciji s osposobljenošću ronilaca (Soldo & Glavičić, 2011).

Vidljivost mora biti dovoljna da bi se mogle zabilježiti potrebne korisne informacije. U slučaju da je voda mutna, npr. na ušćima rijeka i kod drugih miješanja slatke i slane vode, nakon olujnog vremena u priobalnom pojasu ili u razdoblju nedovoljne količine svjetlosti, kao primjerice kod ronjenja u sumrak ili noću, vizualni cenzus je veoma teško provediv jer će točnost i preciznost dobivenih rezultata biti uvelike umanjena.

Stanje površine mora ne smije biti previše uzburkano, jer kod ronioca na dah potpuno onemogućava rad i prikupljanje informacija, a kod ronjenja s ARO ugrožava ronioca i dodatno ga izlaže mogućim ozljedama.

Dubina provođenja istraživanja kod ronilaca na dah je vrlo ograničena, a kod ronjenja s ARO-m strogo su određene granice dubine i vremena boravka pod vodom, koje ukoliko su prekoračene mogu lako dovesti ronioce u opasnost. Uz sve navedeno ronioci su ograničeni i dužinom boravka u relativno hladnoj vodi te intenzitetom fizičkog rada.

Zbog svih ovih razloga provođenje UVC koji uključuje ronioce na dah ili one s autonomnim aparatom najlakše je provoditi u bistroj, toploj i mirnoj vodi i na dubinama koje nisu veće od 30 metara (Soldo & Glavičić, 2011).

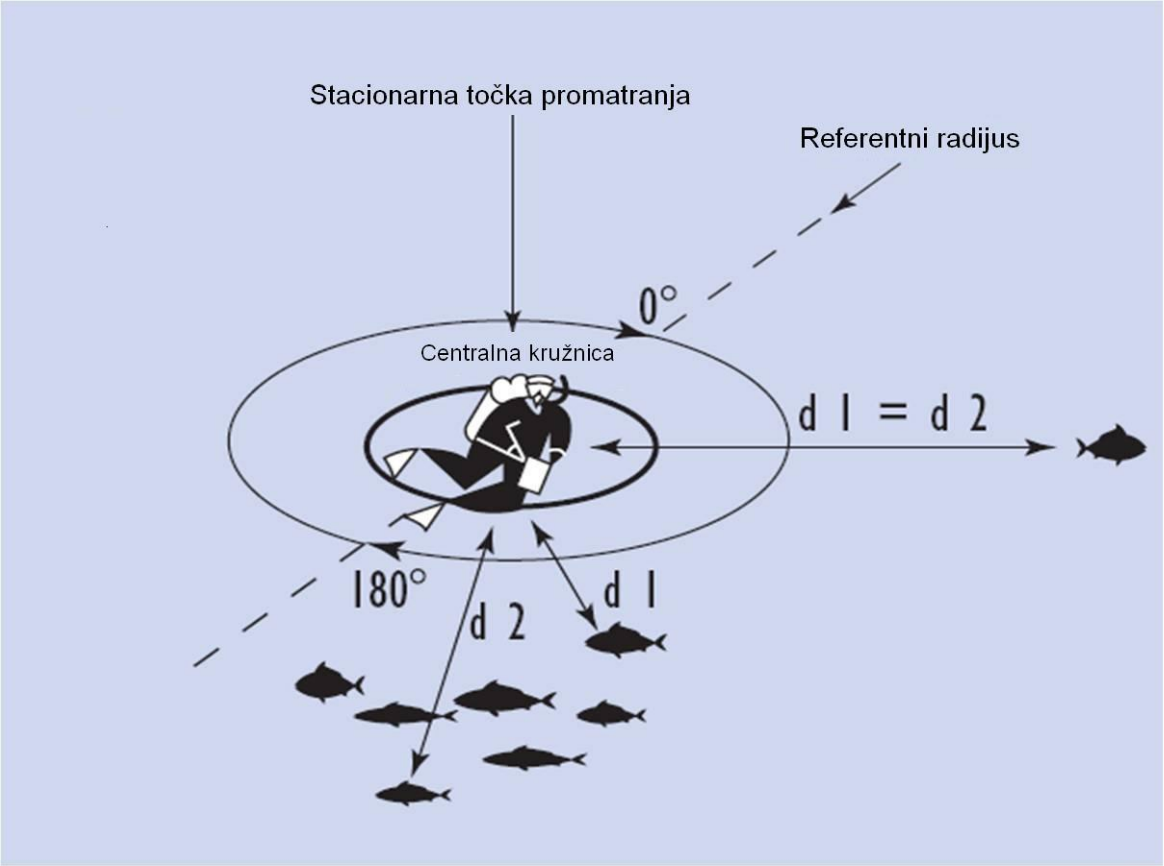
Podvodni vizualni cenzus dijeli se prema obliku promatrane površine i načinu njenog osmatranja na nekoliko metoda:

Metoda uzorkovanja kvadratom je jedna od najstarijih klasičnih metoda uzorkovanja koja se i dan danas koristi ne samo u biološkim i ekološkim istraživanjima već i u drugim područjima podvodnih istraživanja, primjerice u arheologiji. Općenito, metoda se sastoji od smještanja niza kvadrata određene veličine (površine) u područje istraživanja nakon čega se sve unutar kvadrata popisuje i bilježi. Sama metoda je pasivna i ne zahtijeva uklanjanje organizama iz obilježenog područja, a podaci se uzorkuju na dva različita načina, manualno ili fotografski.

Kod manualnog uzrokovanja istraživač se nalazi ispred kvadrata te determinira i bilježi svaki pojedini organizam.

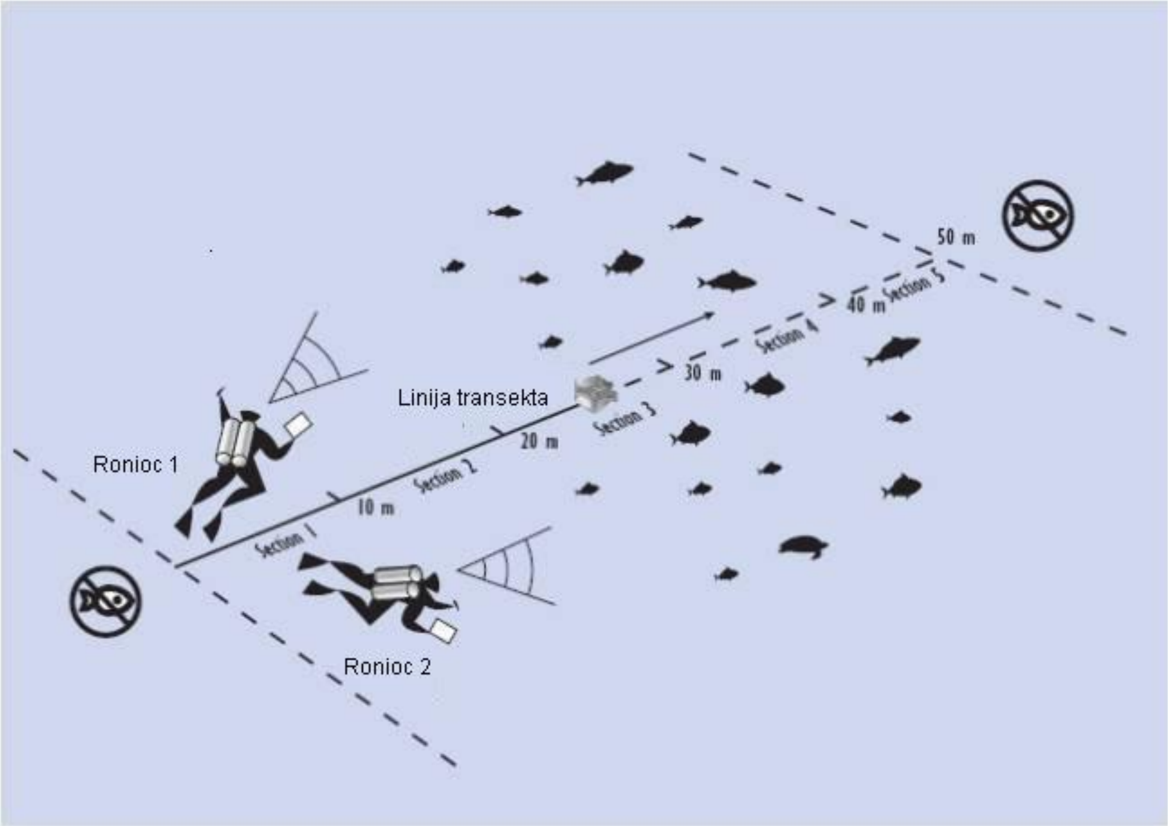
Fotografsko uzorkovanje podrazumijeva da istraživač snima fotografiju kvadrata koja se kasnije analizira. Količina organizama nađena na istraživanom području kvadrata kasnije se izračunava kao gustoča, to je broj organizama nađenih po kvadratu, tj. površini kvadrata. Ovo je i intervalna metoda, tj. može se koristiti niz fotografija slikanih unutar određenog vremenskog razdoblja. Ova metoda je najviše prigodna za obalna područja gdje je pristup istraživanom okolišu najjednostavniji.

Metoda točke (slika 1) se zasniva na nasumičnom odabiru određene točke istraživanja na koju se spušta ronilac te u odabranom radijusu (npr. uobičajenih 10 m pri dobroj vidljivosti, što daje površinu od 314 m^2), identificira vrste, bilježi njihovu brojnost i veličinu. U zavisnosti od unaprijed određenog vremenskog ograničenja, zadnji dio vremenskog razdoblja ronilac se pokreće i u istom radijusu bilježi male i kriptičke vrste koje nisu bile zabilježene u prvom razdoblju promatranja.



Slika 1 – Metoda točke

Metoda transekta (slika 2) koristi pravokutno područje točno definiranih granica, a cenzus se izvodi unutar granica transekta koje su najčešće određene fleksibilnom vrpcom ili konopom razvučenim po morskome dnu. Vrpca može imati oznake svaki metar ili više metara, sukladno potrebi istraživanja. Kod metode transekta prvo se odabire područje istraživanja, zatim nekoliko manjih lokacija podijeljenih na transekte. Lokacije su općenito udaljena više od 250 m jedna od druge, a transekti su obično duljine 20-50 m (Labrosse i sur., 2002). Kod autonomnih ronilaca transekti su uglavnom dužine 25-50 m i širine 5-10 m, u zavisnosti od vidljivosti. Dubina položenog transekta obično varira 3-5 m. Idealno bi bilo korištenje dva ronioca za svaki transekt.



Slika 2 - Transekt

Informacije dobivene istraživanjima pomoću UVC-a prvenstveno služe da bi se mogle izvoditi usporedbe između pojedinih područja obzirom na promjene ribolovnog napora u pojedinim zonama tijekom nekog vremenskog razdoblja ili da bi se definirale korelacije između pojedinih resursa i njihove okoline. Veoma često koriste se i u nacionalnim parkovima i drugim zaštićenim morskim područjima, jer ulovne metode u tim područjima nisu dozvoljene.

Prilikom podvodnih istraživanja najčešće se prikupljaju i analiziraju slijedeći podaci:

a) identifikacija i bogatstvo vrsta

Ovaj podatak govori nam o procjeni broja vrsta na određenom području, a može biti ograničen samo na dio populacije riba koji je iskoristiv za prehranu, tj. gospodarski važne vrste, ili se može provoditi s ekološke točke gledišta i obuhvatiti sve vrste uočene ovom metodom. To je vrlo važan parametar za promatranje jer nedostatak/uništenje jednog organizma može uvjetovati i smanjenje brojnih drugih vrsta koje su s njim neraskidivo povezane u hranidbenom i ekološkom lancu.

b) **brojnost vrste**

Jedinke se prebrojavaju da bi se procjenila brojnost pojedine vrste i njena gustoća (broj riba po jedinici površine). To su osnovni indikatori ribolovnog napora tako da u nekim slučajevima mogu biti i pokazatelji njegovog intenziteta.

Prebrojavanje se može izvoditi unutar transekta ili kružnog područja (metoda točke) s tim da su dužina, širina i radijus područja unaprijed definirani. Istraživač prebrojava ribe unutar striktno definiranog područja širine **d** i dužine **L**. Procijenjena srednja gustoća riba unutar datog područja označava se **D**.

$$D = \frac{\sum_{i=1}^{p} ni}{Ld}$$

Gdje je

- ***n*****:** broj opaženih riba
- ***i = 1,…,*p**

Korektno procijenjena gustoća riba ovom metodom podrazumijeva da:

- istraživač ne previdi niti jednu ribu na promatranom području,
- se svaka ribu broji samo jednom.

Metoda transekta posebno je pogodna za prebrojavanje populacija sedentarnih vrsta (gdje je manja mogućnost greške usljed brzog pomicanja jedinke). Prilikom istraživanja ovom metodom ronilac treba biti dobro obučen u prepoznavanju i identifikaciji ribljih vrsta jer procjena može imati sklonost prikazivanja gustoće populacije i biomase niže od stvarne. Uz to i širina transekta također može imati utjecaja na rezultat. Što je transekt širi, procijenjena gustoća je manja, jer je riba dalje od istraživača, čime je i manja šansa da je ovaj zamijeti. Omjer varira od vrste do vrste i značajan je za veće jedinke i pokretnije vrste. Metoda podvodnog vizualnog cenzusa ribljih naselja u Mediteranu većinom je rađena u njegovom zapadnom dijelu (Bell, 1983; Harmelin, 1987, 1990). Što se tiče Jadrana istraživanja ovom metodom, a koja su se odnosila na ribe, rađena su na zapadnoj (Fasola i sur., 1997; Guidetti, 2000; Guidetti i sur., 2005) te istočnoj jadranskoj strani (Lipej i sur., 2003; Kovačić & Arko

Pijevac, 2008), a ista metoda je korištena i za istraživanje koraligenskih zajednica (Casellato & Stefanon, 2008).

Tradicionalno, ronioci tijekom obavljanja podvodnog transekta bilježe podatke na plastičnu tablicu koju imaju kod sebe tijekom ronjenja (Brock, 1954; English i sur., 1994). Tablice su jeftine i jednostavne za upotrebu, ali imaju svoja ograničenja: područje istraživanja mora se subjektivno procijeniti, postoji varijabilnost među promatračima, moguće je preopterećenje istraživača i nema referentnih podataka (poput fotografskih ili video zapisa) (Brock 1982; Bortone i sur., 1991). Ova ograničenja sugeriraju da bi UVC pomoću tablice mogao biti neprikladan za dugoročne programe praćenja kakvi se rade u zaštićenim morskim područjima (Irigoyen i sur., 2013). Novije digitalne medijske tehnologije, kao što su fotografija ili videozapisi, mogu eliminirati mnoga ograničenja plastične tablice (Watson i sur., 2005, 2010; Langlois i sur., 2010). Digitalni video i fotografski transekti ne zahtijevaju stručnjake za identifikaciju riba tijekom obavljanja terenskih radova te omogućuju procjenu dodatnih varijabli poput strukture podvodnog grebena ili prevladavajućeg pokrova, a mogu ih po potrebi pregledavati i više znanstvenika (Costello i sur., 2005; Preuss i sur., 2009; Pelletier i sur., 2011). Digitalne foto transekte prvi su proveli Bortone i sur. (1986), ali unatoč određenim prednostima, ova tehnika transekta nije značajnije implementirana.

Video transekti se koriste češće i brzo su postali prihvaćeni standard za ovakvu vrstu istraživanja (Tessier i sur., 2005; Langlois i sur., 2010; Pelletier i sur., 2011). Jedna od prednosti videozapisa je ta što su objekti snimljeni u pokretu, što može pomoći u prepoznavanju i smanjenju subjektivnosti promatrača (Bortone i sur., 1986; Harvey i sur., 2001, 2004). Digitalna fotografija i video transekti daju kao krajnji produkt digitalne snimke, koje se, međutim, moraju analizirati u post produkciji za obradu podataka i to troši dodatno vrijeme koje u slučaju rada s tablicom nije potrebno.

3.2. Povijest istraživanja ribljih naselja na okomitim koraligenskim grebenima u Mediteranu i u svijetu općenito

Prvotno je UVC razvijen za upotrebu u istraživanjima riba na tropskim koraljnim grebenima (Brock, 1954). U današnje vrijeme primjena UVC-a široko je raširena u umjerenim vodama poput Mediterana te u mnogim morskim zaštićenim područjima (MPA), kao i u drugim nezaštićenim, ali važnim morskim staništima, npr. stjenovitim grebenima, podmorskim špiljama i livadama morskih cvijetnica (Tunesi i sur., 2006; Bussotti & Guidetti, 2009). Razvijene su razne UVC metode, ali one koje se fokusiraju na grebenske ribe uglavnom se temelje na roniocima s autonomnom ronilačkom opremom, zbog bolje mogućnosti pretraživanja tako kompleksnih staništa. Glavni nedostatak ronjenja s ARO je ograničenje dubine zbog sigurnosnih razloga ronjenja. Također, nakupljanje zaostalog dušika u krvi ronilaca nalaže kratko maksimalno vrijeme zarona. Povezano s gore navedenim, kako se dubina ronjenja povećava, dopušteno vrijeme ronjenja se smanjuje. Stoga je većina istraživanja bila ograničena na dubine od 3-25 m, rijetko prelazeći 30 m (Fasola i sur., 1997; Gül i sur., 2011; Quimpo i sur., 2018). Slijedom toga, tradicionalne UVC metode nisu primjenjive na dublje, strme i okomite padine mediteranskih stjenovitih grebena (Warnock i sur., 2016) koje karakterizira složenost staništa i heterogenost (Bussotti & Guidetti, 2009). Stoga postoji stalna potreba za metodom uzorkovanja ribljih naselja na većim dubinama koja je učinkovita, ali i sigurna za ronioce.

Transekti su najčešće korištena metoda istraživanja UVC-a (Lam i sur., 2006.) i temelje se na uzorkovanju vodoravnih područja, umjesto vertikalnih. Slijedom toga, trenutno ne postoje opisne ili usporedne studije o ribljim naseljima povezanim s dubljim okomitim grebenima na Mediteranu. Razlog ograničenja dubine u konvencionalnom autonomnom ronjenju proizlazi iz ograničenja u postojećoj tehnologiji. Većina ronilaca širom svijeta koristi ronilačku opremu otvorenog kruga s komprimiranim zrakom kao plinom za disanje. Tijekom posljednjih godina, sve veći broj ronilaca eksperimentirao je s tehnologijom ronjenja s plinskim mješavinama, kako bi proširili dubinske granice konvencionalnog ronjenja. Rebreatheri (ronilački aparati zatvorenog kruga disanja) s plinskim mješavinama i ranije su se koristili za uzorkovanje riba i staništa povezanih s koraljnim grebenima na velikim dubinama (Pyle i sur., 2008). Međutim, takva se tehnika može više klasificirati kao slučajna metoda nego široko korištena i usporediva metoda transekta, jer se do danas većina rezultata koji su proizašli iz ovih istraživanja dubokih grebena (mezofotičkih), bazirala na istraživanja isključivo biološke raznolikosti, uključujući prikupljanje vrsta, umjesto, linjskih transekata za opisivanje zajednica, koje koriste UVC metode. Pored toga, nedostaci upotrebe rebreathera sa zatvorenim krugom su visoka cijena opreme i zahtjevna obuka, što ograničava mogućnosti istraživanja samo na malu skupinu visoko obučenih znanstvenih ronilaca.

Podvodni strmi stjenoviti grebeni presudni su čimbenici koji djeluju na ekosustav jer je fizička složenost takvih grebenskih staništa pozitivno povezana s bogatstvom vrsta i brojnošću riba (Garcia-Charton & Perez-Ruzafa, 2001). Na Jadranu, kao i na Mediteranu, postoje dugački dijelovi stjenovite obale koju karakterizira prisutnost mnogih strmih i okomitih padina prekrivenih koraligenim formacijama. Definirani su kao tvrdi supstrat biogenog podrijetla, uglavnom nastao nakupljanjem vapnenastih alga koje se razvijaju u uvjetima slabog osvjetljenja (Ballesteros, 2006). Prethodna istraživanja potvrđuju ulogu stjenovitog staništa na raznolikost obalnih ribljih naselja (Piazzi i sur., 2014 koja imaju ključnu ulogu u obalnim staništima zbog svoje ekonomske vrijednosti i ekološke važnosti. Štoviše, dubina se zajedno s morfološkim karakteristikama staništa, smatra važnim čimbenikom za određivanje raznolikosti ribljih naselja (Lorance i sur., 2002; Piazzi i sur. 2012). Strmi i okomiti stjenoviti grebeni obično se protežu do dubine veće od 30 m.

Kvantitativne studije o kriptobentoskim ribljim naseljima su iznenađujuće rijetke u svijetu i ograničene na obalna područja s dubinama uglavnom od 15 do 20 m (Prochazka, 1998; Ackerman & Bellwood, 2000; Willis, 2001; Smith-Vaniz i sur., 2006; Beldade & Gonçavles, 2007). Do istraživanja u ovom doktorskom radu nije bilo kvantitativnih istraživanja kriptobentoskih riba na ili dublje od 40 metara (Kovačić i sur., 2012). Osim toga kvantitativna istraživanja ribljih naselja na dubokim stjenovitim grebenima, tj. UVC pomoću ronjenja s rebreatherom, vrlo su rijetka i ograničena na tropska mora (Brokovich i sur., 2008). Istraživanja koje uključuju destruktivne metode podvodnih grebena idu dublje, tj. ispod 40 m u tropskim morima, ali su pri tome korištene samo kvalitativne metode (Feitoza i sur., 2005; Pyle i sur., 2008). Značaj kriptobentoskih ribljih zajednica i njihovu brojčanu dominaciju u usporedbi s epibentonskim ribljim zajednicama za područje Mediterana pokazali su Kovačić i sur., (2012). Niti jedno drugo kvantitativno istraživanje ne postoji na kriptobentoskim ribama za Mediteran do radova u ovoj disertaciji, uključujući podatke o mogućim dnevnim promjenama staništa kriptobentoskih ribljih naselja.

Niz je istraživanja provedeno u svijetu kako bi se utvrdio utjecaj čimbenika okoliša na kriptobentosku zajednicu riba te kako bi se riješilo pitanje različitih staništa kriptobentoskih vrsta, odnosno odredilo ulogu kompleksnosti staništa i utjecaj čimbenika okoliša na sastav ribljih naselja (Roberts & Ormond, 1987; Garcia Charton & Perez Ruzafa, 2001; Gratwicke & Speight 2005). Jednako kao i u tropskim područjima, i na stjenovitim grebenima u umjerenim zemljopisnim širinama, gustoće epibentoskih i kriptobentoskih naselja su često pozitivno povezane s kompleksnošću staništa (Connell & Jones, 1991; Garcia Charton & Perez Ruzafa, 2001; Willis & Anderson, 2003). U umjerenim područjima, glavni čimbenici okoliša koji su pokazali utjecaj na distribuciju vrsta obitelji babica (Blennidae) i glavoča (Gobiidae), jesu dubina, složenost i heterogenost dna i tip pokrova (Harmelin, 1987; Guidetti, 2000). Jednako tako, istraživanja dnevne i noćne aktivnosti epibentoskih i hiperbentoskih naselja riba započela su prije pola stoljeća (Hobson, 1965), ali do danas dnevne i noćne varijacije ribljih naselja obalnih područja, ostaju uglavnom neistražene (Azzurro i sur., 2007). U Mediteranu je provedeno samo nekoliko objavljenih istraživanja o dnevnim varijacijama epibentonskih i hiperbentoskih riba na mekim sedimentima do dubine nekoliko metara (Dulčić i sur., 2004,

2005) ili na stjenovitom dnu vizualnim cenzusom uz korištenje autonomne ronilačke opreme do 3 m (Azzurro i sur., 2007, 2013). Objavljeni rezultati jasno pokazuju dnevne varijacije naselja epibentonskih i hiperbentonskih riba. Dulčić i sur., (2004) utvrdili su izrazito noćne i izrazito dnevne vrste riba i velike razlike u ukupnom broju riba i biomasi. Azzurro i sur., (2007) pronašli su jasno razdvajanje između dnevnih i noćnih naselja riba s značajnom razlikom u sastavu vrsta, bogatstvu vrsta i brojnosti. Nažalost, u Mediteranu ne postoje podaci o ekološkom značaju malih kriptičnih vrsta. S druge strane, tropske studije kriptičkih prostora, iako su vrlo rijetke i teško izvodljive, procijenile su da volumen vrsta kriptičkih prostora može činiti do 30-75% ukupnog volumena ribljih naselja koraljnih grebena (Scheffers i sur., 2003) i da su kriptobentoski organizmi koji žive u tim prostorima odgovorni za nevjerojatnih 22% metabolizma cijelog grebena u zajednici (Richter i sur., 2001).

3.3. Ciljevi cjelovitog istraživanja ribljih naselja na okomitim koraligenskim grebenima u Hvarskom kanalu

Prva zadaća ove studije bila je opisati riblja naselja u Hvarskom kanalu na i uokolo okomitih koraligenskih staništa i pri tome odrediti sastav vrsta, raznolikost i relativnu gustoću. Dobiveni kvantitativni podaci mogu biti od koristi za zaštitu i upravljanje podmorjem, kao i za praćenje učinka ribolova u tom području. U radovima koji su dio ove disertacije cilj je bio dobiti cjelovitu sliku sastava ribljih naselja te su zato korištene komplementarne metode, tj. korištene su ulovne i neulovne metode te su implementirane neke nove tehnike koje su omogućile dublje zarone (Nitrox 50 kao dekompresijski plin za ubrzavanje dekompresije, Trimix plinska mješavina, CCR rebreatheri - kompjuterski kontrolirani uređaji zatvorenog kruga disanja koji značajno produžuju boravak na većim dubinama te podvodni skuteri za brže pretraživanje terena).

Za cjeloviti opis ribljih naselja bilo je važno istražiti i kriptobentosku komponentu zajednice riba te upotpuniti kvantitativna istraživanja kriptobentoskih ribljih naselja u Mediteranu. Navedene istraživačke metode primjenjivane su u području okomitih koraligenskih staništa, ali i u njihovom podnožju te na plitkom morskom dnu iznad njega kako bi se dobila potpuna slika priobalnih ribljih naselja.

4. MATERIJALI I METODE

Istraživanja su se provodila u području Hvarskog kanala koji predstavlja morski prostor između otoka Hvara i Brača. U tom području izrazito je izražen ribolov od strane lokalnog stanovništva, posebno u ljetnom dijelu godine. Nadalje, u Hvarskom kanalu nalaze se turističke destinacije koje spadaju u najznačajnije na Jadranu: Bol, Jelsa, Starigrad i Vrboska, a koje su u sezoni izrazito turistički opterećene.

Uzorkovanje je provedeno na dubini od površine pa do 65 m, ovisno o istraživanju koje se provodilo. Da bi se produžilo vrijeme koje ronioci imaju na raspolaganju te povećala sigurnost, kod dubljih ronjenja koristile su se plinske mješavine Nitrox i Trimix. Nitrox je mješavina dušika i kisika, ali za razliku od zraka postotak kisika u njoj je znatno povećan. Najčešće je taj udio između 30 i 50%. Nitrox se u ovim radovima koristio isključivo kao mješavina za ubrzanu dekompresiju, odnosno za brže izbacivanje dušika iz organizma nakon boravka u području povećanog tlaka (Šegrt Ribičić i sur., 2019).

Trimix je mješavina dušika, kisika i helija koja se koristi za ronjenje na dubinama većim od 50 m da bi se smanjio narkotični učinak dušika koji na tim dubinama uvelike utječe na kognitivne sposobnosti ronioca. U radovima koji su sastavni dio ove disertacije koristio se Trimix 20/30 (odnosno mješavina koja sadrži 20% kisika, 30% helija i 50% dušika).

Prilikom svih istraživanja na uronu su bila dva ronioca, oba iskusna u ronjenju i prepoznavanju riba i drugih morskih vrsta.

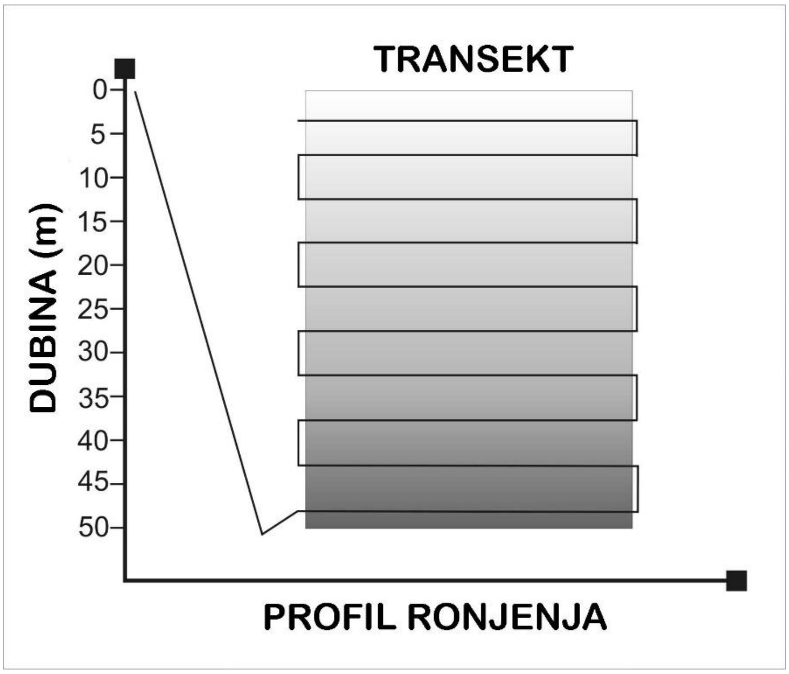
Protokol za uzorkovanje korištenjem metode kvadrata, koji se koristio kod prikupljanja uzoraka u prvom, drugom i trećem radu, usvojen je i modificiran od Kovačić i sur. (2012) i zasniva se na obavljanju radnji prema slijedećem redosljedu: (1) vizualni cenzus epibentoskih riba, (2) postavljanje referentnog okvira (1x1 m) na stijenu (vidi sliku 3), (3) fotografiranje kvadrata od 1 m², (4) određivanje temperature pomoću ronilačkog računala, (5) raspršivanje anestetika po kvadratu, hvatanje ribe mrežicama i čekanje oko 2 min, (6) uklanjanje biopokrova i pomičnih dijelova strukture dna. Korišteni anestetik bio je quinaldin, razrijeđen 1:15 s 96% -tnim etanolom i zatim pomiješan u omjeru 1:5 u bočicama od 750 ml s morskom vodom. Dvije bočice obično su korištene za jedan kvadrat s ukupnim volumenom upotrijebljene otopine quinaldine-etanol po kvadratu od oko 300 ml / m².

Slika 3- Postavljanje kvadrata na stijenu

Prilikom istraživanja mjerene su još: "Dubina" (ovisno o radu, od 0,5 m najpliće pa do 65 m kada se ronilo s plinskim mješavinama). "Nagib terena", kao pet procijenjenih razreda (blago 0-19° = 1, srednje 20-59° = 2, strmo 60-74°= 3, vrlo strmo do okomito 75-90° = 4, nadvis = 5). Svi prikupljeni primjerci riba ubijeni su nakon zarona anesteziranjem quinaldinom, a potrebne dozvole ishodovane su na vrijeme od nadležnih institucija. Primjerci su pohranjeni u u 65% -tnoj otopini etanola. Preliminarne identifikacije vrsta kriptobentskih riba kao i mjerenja standardne duljine obavljene su kasnije u laboratoriju.

Vizualni cenzus koji je rađen u četvrtom i petom radu ove studije obavila su dva ronioca, (primarni i prateći ronilac), od kojih je jedan uvijek snimao s video kamerom.

Metoda podvodnog vizualnog cenzusa (DVT) koja se koristila u četvrtom radu je, obzirom na lokacije istraživanja, metoda koja na najbolji način može prikupiti najtočnije podatke o ribljim naseljima na koraligenskim zajednicama koje su se istraživale. U četvrtom radu transekt je definiran kao pravokutno područje granica dužine 50 m (Ordines i sur., 2005) te 5 m dubine, (s obzirom da se radi na okomitom grebenu, onda je ta dubina, u ovom slučaju 5 m, ujedno i širina/visina transekta), a cenzus se izvodi unutar granica transekta koje su određene unaprijed postavljenim oznakama, dok se dubina konstantno prati dubinomjerom (slika 4).



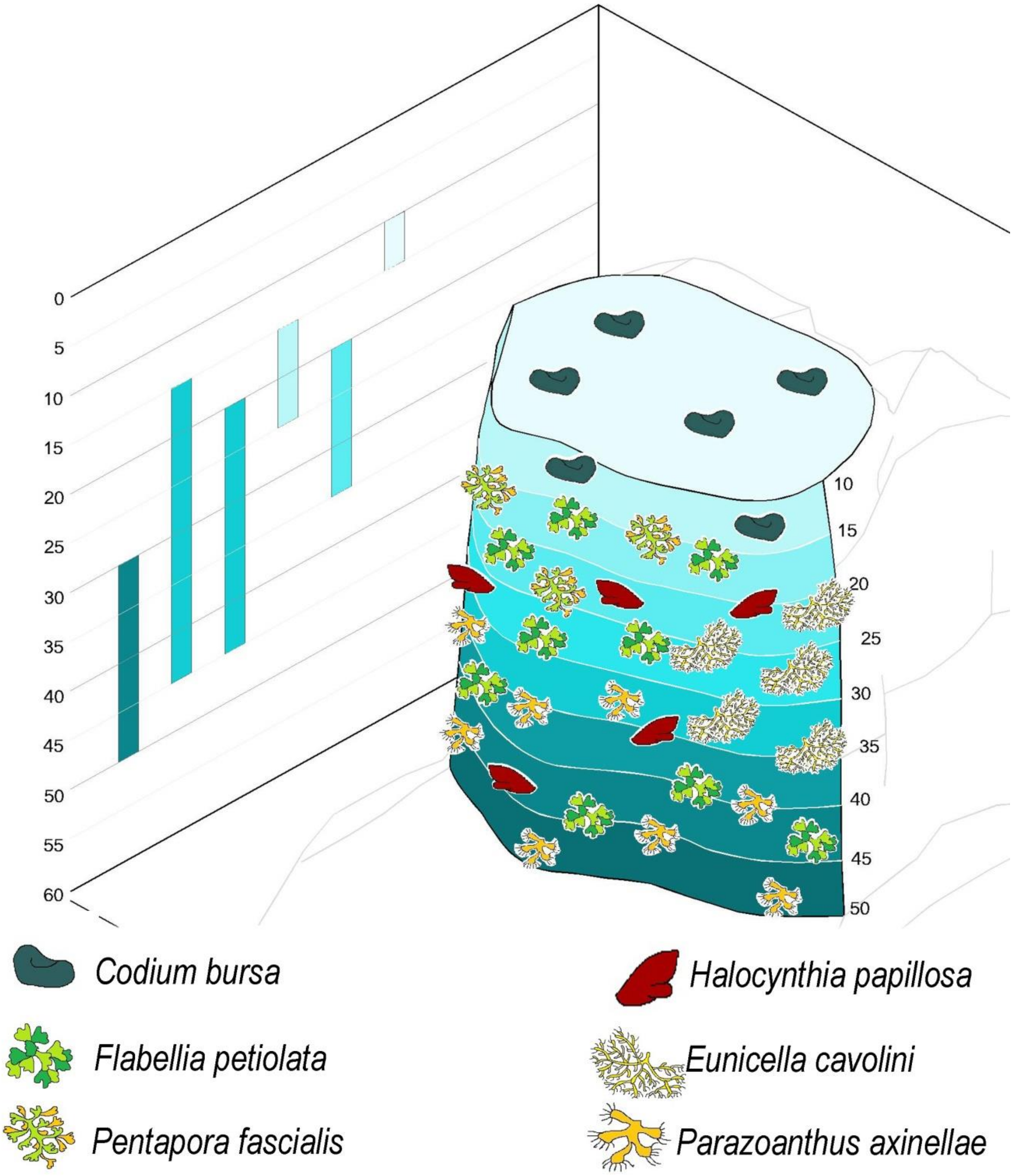
Slika 4- Shematski prikaz transekta.

Odabrano područje istraživanja za četvrti rad bio je Rt Smočiguzica (φ - 43 14,124N, λ - 16 34,393E), koje se na osnovu prethodno dostupnih informacija koristilo i za neke od prethodno navedenih radova u sklopu ovog doktorata (prikaz grebena na slici 5).

Oba ronioca koja su obavljala istraživanje koristila su identična ronilačka računala s mogućnošću pohrane podataka o dubini i temperaturi. Provedena su ukupno 24 zarona. Prva tri početna zarona korištena su za mjerenje geometrije grebena. Nakon mapiranja područja istraživanja izvedeno je 18 zarona s površine do 50 m i fokusirano na uzorkovanje ribe u svakom od 10 pojedinačnih transekata. Ronioci su se odmah spustili s površine na 50 m i započeli uzorkovanje od najdubljeg transekta prema površini (slika 4). Budući da je područje ispitivanja bilo podijeljeno na 10 okomitih transekata, svaki je transekt uzorkovan 18 puta pomoću Full HD Sony FX-1 video kamere. Uz to, izvedena su još tri zarona na kojima se koristio fotoaparat visoke rezolucije (Olympus E-P3 s pripadajućim kućištem) za fotografije malih vrsta riba kako bi se dobila točna identifikacija na razini vrste. Čest problem različitih UVC metoda je ta što se izravna usporedba podataka koje su prikupila dva promatrača koja provode metodu transekta oslanjala na pretpostavke 1) neovisnosti podataka, 2) da su ubrojene samo ribe unutar područja istraživanja, 3) da su različite vrste točno identificirane (Bernard i sur., 2013). Da bi prevladao takve pristranosti, prvi ronilac koristio je video kameru visoke razlučivosti (Full HD) i umjesto konvencionalnih transekta I tipa (plivajući u ravnoj liniji konstantnom brzinom) plivao je transektom krivudajući u njemu (S tip transekt) i varirajući u dubini, kutu i zumiranju kada je to potrebno da bi se opazile i male, odnosno kriptobentoske vrste, koje se obično propuste ako se koriste transekti I-tipa (Pelletier i sur., 2011). Zbog razine slabog osvjetljenja na dubinama zarona, korištena su posebno izrađena LED svjetla 2x25 W, proizvodeći temperaturu svjetlosti od 6400° K.

Kako je razdoblje uzorkovanja za svaki dubinski transekt bilo 5 minuta, korištene su 2 minute dubokog dekompresijskog zastanka na jednom od plićih transekata. To nije ometalo protokol uzorkovanja jer je zastanak ugrađen u cjelokupno trajanje transekta od 5 minuta. Stoga je svaki ronilac promijenio plin za disanje na dubini od 18 m, što odgovara parcijalnom tlaku kisika u smjesi Nitrox50 od 1,4 bara. Na temelju stečenog iskustva preporučuje se upotreba najmanje 18-litrenog spremnika za ronjenje za komprimirani zrak i 11-litarskog spremnika za Nitrox.

Post-analiza snimljenog videozapisa provedena je na 55-inčnom ekranu, koristeći standardni softver (MPC-HC 1.7.10), koji omogućuje pauziranje, usporeno gledanje i zumiranje slike. Video analiza provedena je u roku od 24 sata nakon zarona (kako bi se po potrebi koristile još uvijek svježe informacije u sjećanju ronilaca) sa svim identificiranim i prebrojanim ribama po vrstama. Za svaku promatranu vrstu analizirana je učestalost pojavljivanja f (broj zarona kada je vrsta zabilježena) klasificirajući ih kao 1) vrlo česte ($f\geq70\%$), 2) česte ($40\%\leq f\leq70\%$), 3) uobičajene ($15\%\leq f\leq40\%$) i 4) povremene vrste ($f\leq15\%$) (García -Charton i sur., 2004.). Dubina i temperatura tijekom zarona pohranjene su u ronilačko računalo uz podatke o profilu zarona s preciznošću od 10 cm po dubini i 0,1°C temperature.



Slika 5- Prikaz lokacije podvodnog okomitog grebena s dominantnim pokrovom.

U petom radu ove studije vizualni cenzus obavila su dva ronioca, primarni promatrač i prateći ronilac, s video kamerom na podvodnom skuteru (slika 6). Ronioci su koristili rebreather JJ-CCR (elektronski kontroliran ronilački aparat zatvorenog kruga disanja) i podvodni skuter Suex Model: XJOY7. Kamera je bila Canon EOS 5D MK II. Korištena leća Canon EF 17-40 / 4L USM, 17 mm, ima dijagonalni kut gledanja od 104 stupnja.

Slika 6- podvodna kamere i skuter

Sva video snimanja snimljena su u istom dnevnom vremenskom periodu, odnosno između 11 i 16 sati, od 8 do 50 m dubine. Veličina transekta bila je 25x2 m. DSOV (Diver Scooter Operated Video) transekti napravljeni su u seriji od nekoliko njih u jednoj video snimci. Video isječci traju 36 sekundi, dugački su 25 m. Video snimke analizirane su na 27 inčnom ekranu koristeći softver za pregled koji omogućuje usporeni pregled i zumiranje (MPC-HC 1.7.10). Koristilo se i dodatno usporavanje, premotavanje unatrag i ponavljajuća reprodukcija kad god je to bilo potrebno (Wartenberg & Booth, 2015).

Tijekom dva tjedna razdoblja predtestiranja isprobavale su se razne opcije udaljenosti kamere od dna i kuta kamere u odnosu na podlogu koja se snima. Obzirom na kvalitetu video materijala koji je dobiven, zaključak je bio da snimatelj treba upravljati podvodni skuter 1,5 m iznad dna i držati video kameru mirnom ispred sebe s kutom od 45 stupnjeva prema dnu kako bi osigurao vidljivu širinu transekata od 2 m.

5. SAŽETI PREGLED REZULTATA OBJEDINJENJH RADOVA

5.1 **Rad Br. 1**.

A quantitative assessment of the cryptobenthic fish assemblage at deep littoral cliffs in the Mediterranean

Istraživanje je provedeno u području Hvarskog kanala uz sjevernoistočnu obalu otoka Hvara tijekom toplijeg dijela godine, od lipnja do listopada. Sva uzorkovanja su rađena tijekom dana, između 12 i 16 sati. Proučavani lokalitet bio je strmi podvodni greben pokraj Rta Smočiguzica (43° 14.124 'N, 16° 34.393'E) (slika 5), koji je više od 200 m udaljen od obale otoka. Greben je strmih i okomitih litica s nekoliko relativno velikih špilja, a baza mu je okružena pjeskovitim dnom. Oblikovan je kao krnji eliptični konus. Najviša točka grebena je 9,1 m dubina, a dubina baze grebena varira od 43 do 61 m. Glavni pokrov grebena je koraligenska zajednica.

Cilj istraživanja bio je:

1. nastaviti istraživanja u neistraženom infralitoralnom staništu koje može sadržavati kriptobentoske vrste riba;

2. provesti prvu kvantitativnu procjenu kriptobentoske raznolikosti vrsta riba na tvrdom dnu ispod 20 m dubine, proučavanjem dubokih vertikalnih stijena Jadrana;

3. identificirati varijable okoline koje najbolje objašnjavaju raznolikost i relativnu gustoću vrsta, u ovom staništu, i zbog čega se očekuje da oblikuju kriptobentosku strukturu ove zajednice riba.

Ovo istraživanje daje prvu kvantitativnu procjenu raznolikosti i brojnosti kriptobentoskih vrsta riba mediteranskih podvodnih grebena ispod 20 m dubine. Kvantitativno uzorkovanje izvedeno je na dubinama do 45 m i prikupljena je ukupno 281 jedinka, od toga 220 kriptobentoskih i 61 epibentoska. Protokol za odabir kvadrata usvojen je iz Kovačića i sur. (2012). Rezultati rada ukazuju na visoku raznolikost i brojnost ovog neistraženog dijela bentoske zajednice riba. Kod kriptobentoskih ribljih naselja dominiraju ribe iz obitelji glavoča (Gobiidae) koje čine preko 60% svih vrsta, a dominiraju također i po brojnosti jer čine preko 90% svih uzoraka. U ovom istraživanju uzorkovane su i vrste koje su ranije smatrane rijetkim u Mediteranu, a neke od njih bile su i brojne, primjerice Splechtnin glavočić, *Didogobius splechtnai, (*Ahnelt i Patzner, 1995), Kolombatovićev glavoč (*Gobius kolombatovici,* Kovačić i Miller, 2000), Dollfusijev glavočić *Vanneaugobius dollfusi, (*Brownell, 1978). U radu je analizirano četrnaest registriranih varijabli staništa, od čega su tri (dubina, površina stjenovite litice nasuprot dnu litice i prisutnost pijeska kao donjeg supstrata) identificirani kao značajni za pojavu vrsta.

5.2 **Rad Br. 2.**

A quantitative sampling method for assesment of deep cryptobenthic ichthyofauna using Trimix diving

Proučavani lokaliteti bili su dva podvodna strma grebena, već opisani na Rtu Smočiguzica te Zala Luka (43.3068º N, 16.441517ºE), koji su dio podvodne obale otoka Hvara, a općenito udaljeni više od 200 m od obale. Površine oba grebena sastoje se od strmih do okomitih vapnenačkih stijena prekrivenih koraligenskom zajednicom i okruženi pješčanim dnom. Baza oba grebena je u rasponu od 50 do 62 m dubine, tako da je upravo ona odabrana za područje istraživanja kao najdublji dio grebena.

U radu je opisana nova metoda uzorkovanja za duboku kriptobentosku i epibentosku ihtiofaunu upotrebom Trimix ronjenja. Istraživanje je provedeno na dubini od 60 m, na dvije lokacije u Hvarskom kanalu tijekom početka jesenskog razdoblja. Sigurna, učinkovita i relativno jednostavna tehnika ronjenja korištenjem Trimix mješavine plinova kombinirana je s metodom prikupljanja uzoraka quinaldinom (Kovačić i sur., 2012). Ukupno je skupljeno 16 primjeraka iz četiri vrste riba, tj. tri vrste glavoča (Gobiidae) i jedna vrsta iz porodice babica (Blenniidae), a sakupljeni su metodom kvadrata na ukupnoj površini od 6 m^2. Ovaj radpredstavlja prvo učinkovito uzorkovanje kriptobentoskih i epibentoskih naselja riba na tim cirkalitoralnim staništima. Dvije od prikupljenih vrsta, glavoč narančasti, *Thorogobius macrolepis* (Kolombatović, 1891) i *V. dollfusi*, smatraju se rijetkim s vrlo malo prijavljenih zapisa u Mediteranu. Nadalje, zabilježeno je i najdublje stanište vrsta babica prugasta *Parablennius rouxi* (Cocco, 1833), glavoč žutac *Gobius auratus* (Risso, 1810), i glavoč narančasti *T. macrolepis*.

Istraživanje je također potvrdilo i da je uporaba Trimix ronjenja relativno jednostavna za kvantitativne studije kriptobentonskih i epibentonskih naselja cirkalitoralne zone Mediterana, posebice u kombinaciji s metodom kod koje ronilac koristi anestetik ili ihtihocid, što je inače i jedina metoda koja može osigurati učinkovito prikupljanje kriptobentoskih vrsta (Kovačić i sur., 2012). Također, to je jedina učinkovita metoda koja se može koristiti za prikupljanje malih i skrivenih primjeraka na stjenovitom i miješanom dnu.

5.3 **Rad Br. 3**.

A quantitative assessment of the diel influence on the cryptobenthic fish assemblage of the shallow Mediterranean infralittoral zone

Istraživanje je provedeno u Hvarskom kanalu na jugozapadnoj strani otoka Brača, oko 1,5 km zapadno od mjesta Bol, u (43° 15.543'N, 16° 38.607'E). Morsko se dno na istraživanoj dubini sastojalo od pijeska i raznih mješovitih stjenovitih tipova dna (čvrsta stijena, valutice, šljunak) prekrivenog sesilnom biotom, uključujući alge i spužve. Na mjestu nema jakih strujanja, horizontalna vidljivost obično iznosi 20-25 m na dubini vode plićoj od 5 m, a područje je svakodnevno izloženo popodnevnim valovima maestrala, dok su najjači valovi na toj poziciji od juga.

Osnovna ideja istraživanja bila je:

1. ispitati ukupne razlike u sastavu kriptobentonskim ribljih naselja između: dana / noći / sumraka, koje bi se moglo tumačiti kao vremenski ovisne promjene;

2. identificirati varijable okoline ili njihovu kombinaciju koja značajno pridonosi promjeni kriptobentoske ribljih naselja u plitkom infralitoralu;

3. ispitati razlike između epibentoskog i kriptobentoskog sastava riba;

4. testirati dnevne pomake infralitoralnih vrsta između kriptobentoskih i epibentoskih staništa.

Podatke i uzorke su prikupili dva autonomna ronioca tijekom tri tjedna provedenih istraživanja. Pri istraživanju se koristila metoda kvadrata, a broj uzorkovanih kvadrata u jednom ronjenju varirao je od 3 do 5, ovisno o dijelu dana i drugim uvjetima, s dva zarona u prosjeku dnevno. Uroni su obavljeni tijekom dnevnog svjetla, sumraka i noći, od toga: 26 dnevnih, 25 u sumrak i 27 noćnih kvadrata, tj. ukupno 78 kvadrata površine 1 m^2.

Protokol za odabir kvadrata usvojen je iz Kovačića i sur., (2012). Uzorkovalo se na dubini: 0,5, 1, 2 i 3 m. Kvadrati su birani na način kako bi se postigao maksimalni mogući učinak na svakom od dominantnih tipova supstrata na svakoj od izobata. Izbor je bio polu-slučajan, tj. izobata je praćena do sljedećeg nedovoljno uzorkovanog dominantnog tipa supstrata, najmanje 10 m od prethodnog kvadrata (Kovačić i sur., 2012).

Kriptobentoski su uzorci u velikoj mjeri nadmašivali epibentoske uzorke, s omjerom u brojnosti primjeraka od 7,5 : 1, odnosno u broju vrsta 22 naprema 13 epibentoskih vrsta uz osam ambivalentnih vrsta (vrste koje žive skrivene u strukturama dna, ali i u stupcu vode sve do površine). Ukupni sastav kriptobentoskih i epibentoskih ribljih naselja se razlikovao, pogotovo u odnosu na tri brojčano dominantne i stenotopske (usko prilagođene vrste koje žive samo u određenim staništima) kriptobentoske vrste: glavočić korčulanski *Corcyrogobius liechtensteini* (Kolombatović, 1891), glavočić zebrasti*, Zebrus zebrus,* (Risso 1827*)* i glavočić kamenjarčić*, Chromogobius zebratus,*(Kolombatović, 1891). Obzirom na nedostatak podataka o dnevnim varijacijama kod kriptobentoski zajednica općenito, opći cilj ovog istraživanja bio je popuniti ovu prazninu, što bi moglo otkriti važne aspekte dinamike ovih zajednica.

5.4.**Rad Br 4**.

Underwater Visual Census of Deeper Vertical Rocky Reefs

Istraživanje je provedeno u Hvarskom kanalu na već opisanom podvodnom grebenu kraj Rta Smočiguzica. Cilj rada bio je opisati:

1. novu metoda podvodnog vizualnog cenzusa dizajniranu za dublja i vertikalna područja, nazvanu Deep Vertical Transect (DVT), koja je ekonomski prihvatljiva i tehnički nije zahtjevna za izvođenje;

2. brojnost i raznolikost ribljih naselja na vertikalnim koraligenim grebenima Jadrana.

Sesilne vrste koje pokrivaju ovaj greben varirale su po dubini i uključivale su i biljni i životinjski pokrov, ali dominantne su bile zelena alga *Codium bursa* (C. Agardh, 1817) (na 10 do 15 m) i *Flabellia petiolata* (Turra) (Nizamuddin, 1987) (od 15 do 40 m), mahovnjak *Pentapora fascialis* (Pallas, 1766) (od 15 do 25 m), mješčićnica *Halocynthia papillosa* (Linnaeus, 1767) (od 20 do 45 m) i koralji *Eunicella cavolini* (Koch, 1887) (od 20 do 35 m) i *Parazoanthus axinellae* (Schmidt, 1862) (od 30 do 50 m).

Prilikom istraživanja ukupno je zabilježena 51 vrsta riba iz 21 porodice. Prema učestalosti pojavljivanja, 41 vrsta iz 15 porodica kategorizirana je kao stalni stanovnik grebena, dok se 10 vrsta iz 6 porodica smatra povremenim posjetiteljima.

Unutar vrsta povezanih s grebenom, taksonomski su bili domininantni pripadnici porodice Sparidae (9 vrsta), Labridae (6 vrsta), Gobidae (6 vrsta), Serranidae (5 vrsta), Scorpaenidae (3 vrste) i Centracanthidae (3 vrste), dok je ostalih 9 porodica bilo zastupljeno sa samo jednom vrstom. Prosječna brojnost riba (broj zabilježenih riba) po zaronu bilo je 1144,3 ribe. Najčešće zabilježene vrsta su bile fratar *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817), zatim crnej *Chromis chromis* (Linnaeus, 1758), bukva *Boops boops* (Linnaeus, 1758), glavoč žutac *Gobius auratus* (Risso, 1810) i gira oštrulja *Spicara flexuosa* (Rafinesque, 1810). *B.boops*, *C. chromis* i *S. flexuosa* bile su ujedno i jedine vrste koje su opažene u svim transektima od površine do 50 m dubine, a slijede ih knez *Coris julis* (Linnaeus, 1758), špar *Diplodus annularis* (Linnaeus, 1758) i fratar *D. vulgaris*, koji su bili prisutni u svim transektima osim od površine do 5 m dubine te glavoč žutac *G. auratus* koji nije bio uočen samo u vodenom stupcu.

Temperatura mora kretala se od 17,3 C na dnu do 27,1 C u blizini površine. Termoklina se javljala od 13 do 16 m. U transektima ispod termokline opažen je najmanji broj od vrsta. To ukazuje na temperaturu kao jedan od najvažnijih parametara koji utječu na dubinsku raspodjelu vrsta u ljetnom razdoblju. Istraživanje je pokazalo da termoklina djeluje kao svojevrsna fizička barijera jer je 6 vrsta opaženo isključivo iznad nje, a 18 vrsta samo ispod nje. Multivarijantna analiza na temelju broja zabilježenih jedinki pokazala je statistički značajne razlike u sastavu vrsta između svih dubina, osim između 20-25 i 25-30 dubinskih pojaseva. Nadalje, primijećena je i statistički značajna razlika između različitih temperaturnih zona (iznad i ispod termokline) (ANOSIM, R = 0,667, p = 0,001).

Analiza sličnosti (SIMPER) pokazala je da su glavoč žutac *G. auratus* i fratar *D. vulgaris* najviše pridonijeli razlikama između između različitih temperaturnih zona.

5.5. Rad Br. 5

Reef fish assemblage changes along depth gradient revealed by video on underwater scooter method

Istraživanje je provedeno na dvije lokacije u Hvarskom kanalu i to pokraj Rta Smočiguzica i Rta Glavna. Lokaliteti predstavljaju grebene širine od oko 200 m i na sličan način su izloženi prevlađujućim vjetrovima koji dolaze iz SSI, JI i JZ kvadranta. Vizualni cenzus rađen je uz pomoć podvodnog skutera (DPV) Suex: XJO Y7 na kojem je bila postavljena video kamera. Ronioci su koristili JJ-CCR rebreather (aparat za ronjenje zatvorenog kruga disanja upravljan pomoću ronilačkog računala) .

Transekti su bili dugački 25 m i široki 2 m, s ciljem da se postigne oko 30 ponovljenih replika na svakoj istraživanoj dubini (5-10 m, 15-20 m, 25-30 m, 35-40 m, 45-50 m). Transekti su rađeni u nizu jedan iza drugoga duž željene dubine. Svaki transekt uzorkovan je ravnom linijom, približno slijedeći izobate. Tijekom urona, glavni ronilac uskladio je konstantnu brzinu podvodnog skutera i vozio ga snimajući video zapis. Uloga drugog ronioca bila je: na početku svakog transekta provjeriti je li horizontalna vidljivost dovoljno dobra; uspostaviti polazište transekta; upotrijebiti štap na početnoj točki transekta za kalibraciju visine kamere i za provjeru širine transekta na videu. Drugi ronilac je također zabilježio *in situ* temperaturu mora, dubinu i vrijeme trajanja svakog transekta.

Cilj ovog rada bio je opisati riblja naselja na koraligenskim staništima dubine od 5 do 50 m i testirati hipotezu da se sastav i raznolikost vrsta značajno ne razlikuju između različitih dubina. Drugi, jednako bitan cilj je bio je i uvesti metodu vizualnog cenzusa u kombinaciji s rebreatherom, podvodnim skuterom (DPV) i video kamerom visoke rezolucije. Prednost ove metode je da se na ovaj način troši manje vremena uz zadovoljavajuću kvalitetu zapisa (zahvaljujući većoj brzini DPV-a i visokokvalitetnoj video kameri) i zadovoljene sigurnosne parametre vezane za ronioce koji su izloženi znatno većem riziku prilikom rada na takvoj dubini te se na ovaj način mogu provoditi studije UVC-a u područjima dubljim od 40 m, čak i do 100 m dubine.

Analizom podataka prikupljenih DSOV (diver scooter operated video) metodom vidljivo je da brojnost i raznolikost vrsta kod ribljih zajednica nisu pokazale značajne razlike u dubinama, nagibima terena i orijentaciji u odnosu na strane svijeta. Međutim, suprotno rezultatima ranijih studija (Letourneur i sur., 2003; Pais i sur., 2004; Renones i sur., 1997), ovo je istraživanje permutacijskom multivarijantnom analizom varijance utvrdilo značajnu razliku u sastavu ribljih naselja na različitim dubinama. To je u skladu s ostalim istraživanjima koji čine ovu disertaciju, a koja potvrđuju dubinu kao vrlo značajnu varijablu okoliša koja utječe na sastav vrsta.

6. RASPRAVA

Unatoč ograničenom broju staništa istraživanih u prvom radu, u kojem je fokus bio na područjima donjeg infralitorala i početka cirkalitorala, jedna od tri značajne okolišne varijable koje su najviše doprinijele varijaciji u pojavljivanju vrsta je bila dubina. Prisutnost pijeska bila je druga značajna varijabla okoliša koja je objasnila raspodjelu i brojnost, s nekoliko vrsta glavoča koji pokazuju sklonost mješovitim dnima te nekoliko vrsta koje izbjegavaju pijesak. Treća značajna varijabla bila je dno područja grebena, gdje su isključivo zabilježene uvijek iste vrste. Uz to, čini se da su veličina i količina špilja i šupljina, pa čak i biopokrov kao drugi izvor skloništa, bili bez značajnog utjecaja na raspodjelu i brojnost vrsta.

U prethodnom sličnom istraživanju (Kovačić i sur., 2012) tri su varijable bile vrlo značajne za varijacije u pojavi vrsta: dubina, vrste podloge i prisutnost algi s kratkim talusima na čvrstom dnu. Dubina je, kao i u ovom radu, varijabla koja najviše pridonosi varijacijama u pojavi vrsta. Razlike u drugim značajnim objašnjenjima varijabli između dvije studije mogu biti rezultati razlike u staništu, jer nisu sve varijable bile prisutne u obje studije, npr. područje na dnu grebena u Kovačić i sur. (2012). Drugo istraživanje koje se bavilo kvantitativnim proučavanjem utjecaja varijable dubine na sastav ribljih naselja također je provedeno u umjerenim vodama, u Južnoj Africi, na dubini od 0-20 m (Prochazka, 1998) i također je identificiralo dubinu kao drugu najvažniju varijablu okoliša koja objašnjava sastav kriptobentoskih ribljih naselja.

Među vrstama uključenim u kvantitativnu analizu podataka, jedinke glavočić zebrasti *Z. zebrus,* babica prugasta *P. rouxi,* glavočić balearski *Odondebuenia balearica,* glavočić kamenjarić *C. zebratus,* splechtnin glavočić *D. splechtnai* i glavočić korčulanski *C. liechtensteini* su pronađene isključivo na kriptobentoskoj poziciji, isto kao i kod Kovačić i sur., (2012), što predstavlja još jedan dokaz o kriptobentoskom karakteru tih vrsta. Nalaz vrste *P. rouxi* u ovom radu bio je iznenađujući u usporedbi s prethodnim podacima (Kovačić i sur. 2012) gdje je pretežno zabilježen kao epibentoska vrsta. Vrste prisutne na stijenovitoj podlozi (kolombatovićev glavoč *G. kolombatovici,* glavoč narančasti *T. macrolepis,* dollfusijev glavoč *V. dollfusi*) preferiraju epibentoske pozicije blizu skloništa. Druga skupina riba prisutna duž cijele površine grebena (*G. auratus,* glavoč crnobok *G. vittatus i* bodeč crveni *S. notata*) je epikriptobentoska (vrste koje često nalazimo i na površini, dnu i skrivene u šupljinama samog dna) prema Kovačić i sur. (2012), što se i u ovom radu također potvrdilo. Isključivo kriptički uzorak *C. chromis* u ovom radu je bio premalen za izvođenje preciznijeg zaključka, iako su poznate kriptičke preferencije ove hiperbentoske vrste (uglavnom za juvenilne primjerke) tijekom noći (Harmelin, 1987), a također i za vrijeme dnevnog svjetla (Kovačić i sur., 2012).

Poznato je da je metoda transekta superiornija kod bilježenja ukupnog broja ribljih vrsta koje obitavaju na nekom području. (La Mesa i sur., 2004; Jokiel i sur., 2005). Međutim, iako se metodama UVC uočava čak i mnogo malih primjeraka, u prvobitnim istraživanjima značajno veliki postotak kriptobentonskih i epibentoskih vrsta propušteno je tom metodologijom. (Ackerman i Bellwood, 2000; Kovačić i sur., 2012). Upravo stoga se u doktoratu pokušalo objediniti više različitih metoda, kako bi se riblja naselja istraživanog područja što bolje opisala.

Biološke zajednice koje su bile prisutne u kvadratima proučavanim u drugom radu pripadaju cirkalitoralu. Jednim dijelom rađeno je na identičnoj lokaciji (Rt Smočiguzica-otok Hvar) kao i prvi rad, ali na većim dubinama (50-65 m). Hiperbentoske ribe, i veći dio epibentoskih, na ovim dubinama su bile rijetke te su pronađeni samo *C. chromis,* tabinja mrkulja *Phycis phycis* (Linnaeus, 1766), škarpina, *Scorpaena scrofa* (Linnaeus, 1758) i kokot *Trigloporus lastoviza* (Bonnaterre, 1788). *V. dollfusi* bio je jedini među četiri zabilježene vrste koje se pojavljuju isključivo u cirkalitoralu do dubine 160 m (Ahnelt & Dorda 2004). Trenutno prijavljen nalaz na 60 m predstavlja najdublji zapis o *P rouxi*, *G. auratus* i *T. macrolepis* te pokazuje po prvi put da su ove vrste prisutne u velikom dubinskom rasponom, tj. infralitoralu i cirkalitoralu. *T. macrolepis* je do sada bio poznat kao infralitoralna vrsta s rasponom dubine od 6-45 m (Ahnelt & Kovačić 1997, Francour i sur. 2007.). Također, činilo se da je *G. auratus* ograničen na infralitoralnu zonu s maksimalno iskazanim dubinama od 35 m (Herler i sur., 2005) i 40 m (Francour i sur., 2007.), dok je *P. rouxi* bio zabilježen na 42 m (Zander 1986). *V. dollfusi* je poznat iz samo nekoliko zapisa. Opisan je na atlantskoj obali Maroka, i prošlo je dva desetljeća bez novog zapisa. Pronađen je ponovno na istočnom Jadranu (Pallaoro i Kovačić, 2000) i u Egejskom moru (Ahnelt i Dorda, 2004). *T. macrolepis* je poznat iz samo nekoliko zapisa iz Jadranskog mora (Ahnelt i Kovačić, 1997; Guidetti i sur., 2006), iz Francuske i Baleara u zapadnom Mediteranu (Ahnelt & Patzner, 1996), a u istočnom Mediteranu ima nalaz iz Egejskog mora i istočne Levantinske obale (Francour i sur., 2007). Ova disertacija dala je kvantitativne podatke o velikoj raznolikosti vrsta dna i uvela je, u ovom radu opisanu, novu i relativno jednostavnu i djelotvornu metodu Trimix ronjenja za prikupljanje podataka ispod dosega ronilaca s komprimiranim zrakom.

Kada se promatraju dnevne varijacije ribljih naselja plićeg dijela grebena koje su obrađene u trećem istraživanju, vidljivo je da su glavoči (14 vrsta) i babice (šest vrsta) najviše zastupljeni, dok su druge obitelji bile prisutne samo s jednom ili dvije vrste. Oko 70% vrsta pronađenih na kriptobentoskom području isključivo su kriptobentske. Polovica uzoraka u ovom istraživanju pripada trima najčešćim vrstama, sva tri glavoča (Gobiidae). Među pet najčešćih vrsta, *C. liechtensteini*, *Z. zebrus* i *C. zebratus* već su identificirani kao strogo kriptobentični i kod Kovačić i sur., (2012), dok je milerov glavočić *M. macrocephalus* zbog malih dubina, bio odsutan ili slabo zastupljen u ovim istraživanjima. Iznenađujuće, četvrta najčešća vrsta je *C. chromis*, čije su odrasle jedinke dobro poznate kao epibentoske, ali i hiperbentoske (Abel, 1961). Ovo istraživanje potvrđuje prethodna otkrića o korištenju kriptičnih prostora ove inače hiperbentoske vrsta barem za mlade jedinke tijekom noći (Harmelin, 1987), ali i za vrijeme dnevnog svijetla (Kovačić i sur., 2012). Uzorci svih pet dominantnih vrsta riba činili su otprilike 75% svih uzorkovanih primjeraka, dok ostalih oko 25% primjeraka predstavljaju 22 vrste.

Podaci o prisutnosti ili odsutnosti ili preklapanju između kriptobentoskih i epibentoskih staništa pomažu nam pronaći odgovor je li kriptobentoska zajednica riba, zajednica koja svoja skloništa koristi samo za dnevne migracije i dio svog dnevnog ritma te kao zaštitu od grabežljivaca tijekom dana ili alternativno, živi skrivena i izolirana u ovim kriptičnim prostorima i na njima provodi najveći dio životnog ciklusa.

Analiza rezultata sugerira da su kriptobentoske vrste zabilježene tijekom ovog istraživanja stalni stanovnici kriptobentskih mikrostaništa i stoga se najvjerojatnije tamo hrane i razmnožavaju tvoreći specijaliziranu kriptobentosku zajednicu riba u kriptobentoskim okruženjima obalnih morskih staništa.

Dublji djelovi grebena istraživani su dodatno u četvrtom i petom radu ove disertacije te je bez obzira na homogenost staništa i odsutnost plitkog supstrata, ukupan broj vrsta zabilježenih u četvrtom istraživanju na 10 vertikalnih transekata do 50 m dubine metodom DVT (Deep Vertical Transect) znatno veći od broja vrsta zabilježenih na 32 vodoravna transekta do 42 m dubine tradicionalnom UVC metodom koju su u Jadranu koristili Fasola i sur. (1997). Postoje UVC istraživanja na Mediteranu koja su rezultirala većim brojem zabilježenih vrsta, npr. studija libanonske obale (Harmelin-Vivien i sur., 2005.) gdje su zabilježene 62 vrste, uključujući osam lesepsijskih migranata. Međutim, to istraživanje nije provedeno na pojedinačnim, već na različitim staništima (stijena, šljunak, pijesak, makro-alge i morske cvijetnice). Dakle, rezultati istraživanja opisanog u četvrtom radu ukazuju da podvodni strmi stjenoviti i koraligeni grebeni imaju veću biološku raznolikost ribe i gustoću naseljenosti od bilo kojeg drugog stjenovitog staništa na Mediteranu. Budući da se ribolovni napor smatrao glavnim negativnim utjecajem na mediteranske grebenske sustave (Sala i sur., 2012.), možemo pretpostaviti da je jedan od razloga tako velike raznolikosti i gustoće u usporedbi s drugim stjenovitim staništima zbog nižeg ribolovnog napora uzrokovanog specifičnim topografskim značajkama strmog okomitog grebena koje sprječavaju upotrebu većine ribolovnih alata.

Na temelju dobivenih rezultata možemo dalje pretpostaviti da se ograničenje Full HD videozapisa u bilježenju ribljih vrsta, koji je korišten u DVT metodi, odnosi samo na male i kriptobentoske vrste ili u slučajevima kada su podaci o ekologiji poznatih vrsta nesigurni i zbunjujući: primjerice, *G. kolombatovici* smatran je ribom koja obitava na dnu i naseljava lokalitete do 38 m dubine (Kovačić & Miller, 2000), dok je tijekom ovog istraživanja prvi put zabilježen na 50 m. Dakle, ovi rezultati dokazuju značajno poboljšanje DVT metode u odnosu na tradicionalno korištene UVC metode, posebno za veće dubine.

Rezultati ovog istraživanja također pokazuju da, unatoč jakom izravnom i neizravnom utjecaju čovjeka na obalna staništa, još uvijek postoje mikrostaništa koja se ne bi mogla okarakterizirati kao netaknuta (tj. netaknuta od utjecaja čovjeka, s povijesnom strukturom ekosustava i biomasom), ali zapravo su vrlo blizu definiciji netaknutih. Stoga je neophodno identificirati što je moguće više takvih strmih koraligenih grebena i procijeniti njihovo stanje kako bi ih zaštitili kao žarišta morske biološke raznolikosti.

Tijekom posljednjih dvadeset i pet godina, brža alternativa tradicionalnom UVC-u gdje su se podaci zapisivali *in situ*, uvedena je i korištena za procjenu ribljih naselja modificirana metoda gdje su podaci snimani fotografskim aparatom i video kamerom (Greene & Alevison, 1989, Francour i sur., 1999; Willis & Babcock, 2000; Harvey i sur., 2001). Sve veća rezolucija i bolje performanse svake nove generacije fotoaparata te video kamera i njihovih senzora čine podvodni video cenzus sve kvalitetnijom metodom. Jedan od načina kojim se može sigurnije i kvalitetnije istraživati na dubinama većim od 40 m je i korištenjem rebreathera. U petom radu ove disertacije prikazana je metoda u kojoj se koristi podvodni skuter u kombinaciji s tehnikom ronjenja s rebreatherom. Ova metoda ima dvije prednosti u odnosu na standardna UVC i DOV (Diver Operated Video – uzorkovanje korištenjem video kamere) istraživanja ribljih naselja, prva je manje uznemiravanje ribe, a druga je mogućnost veće dubine rada. Bez unaprijed postavljenih transekata, s kratkim vremenom prolaska područjima transekta uz pomoć podvodnog skutera i s tihim disanjem bez ispuštanja mjehurića, uznemiravanje riba trebalo bi biti znatno manje u usporedbi sa standardnim UVC i DOV metodama. Video kamera u kombinaciji s podvodnim skuterom također omogućava da se postigne veća dubina te da se može dulje ostati na toj dubini radi korištenja aparata zatvorenog kruga disanja, rebreathera, koji je elektronički potpuno automatiziran. UVC metode zahtijevaju dugo vrijeme boravka pod vodom radi prikupljanja dovoljne količine podataka, a također trebaju ronioce koji su iskusni ihtiolozi ili bilo koje druge osobe obučene za identificiranje vrsta.

Tijekom posljednja dva desetljeća korištene su i tehnike ronjenja s naprednom ronilačkom opremom i raznim mješavinama plinova kako bi se omogućili sigurni zaroni na dubine od 50-150 m, što premašuje dubine do kojih se može sigurno doći konvencionalnim ronilačkom opremom (Parrish & Pyle 2002). Unatoč tom napretku u ronjenju, općenito je objavljeno samo nekoliko rezultata vizualnog cenzusa ribljih naselja na dubini od 50 m ili dublje (Brokovich i sur., 2008) do 65 m dubine (Bejarano i sur., 2010) do 70 i 85 m dubine (Pinheiro i sur., 2015) do 130 m dubine (Coleman i sur.,2018), vjerojatno zbog povećanih logističkih izazova povezanih s dubinom čak i u današnje vrijeme (Andradi-Brown i sur., 2016). Dvije tehnike ronjenja omogućuju pristup tim dubinama. Prva je korištenje Trimix opreme s otvorenim krugom, koja se još uvijek rijetko koristi za bilo kakva istraživanja riba, s jednim primjerom sakupljanja riba u Jadranu, koja je dio ove disertacije (Glavičić & Kovačić, 2016). Međutim, potrošnja plina kod ronjenja na otvoreni krug ograničava učinkovito vrijeme dna na većim dubinama na najviše 12-15 minuta (Pyle, 1998) pa je stoga njegova upotreba limitirana na kratke zadatke. Druga tehnika je tehnologija aparata zatvorenog kruga (CCR) koja se još rjeđe primjenjuje u istraživanjima riba od Trimix-a otvorenog kruga (Parrish & Pyle, 2002; Bejarano i sur., 2010; Pinheiro i sur., 2015). U usporedbi s Trimix-om otvorenog kruga, CCR ima puno veću autonomiju pod vodom, smanjuje rizik od dekompresijske bolesti, smanjuje ukupne potrebe za plinom, povećava ukupnu sigurnost i bez mjehurića je (Pinheiro i sur., 2015). Zbog ovih karakteristika obećavajuća je metoda za uporabu u vizualnom cenzusu na velikim dubinama. Međutim CCR ima ozbiljna ograničenja u cijeni opreme i cijeni terenskog rada te u potrebi za visokokvalificiranim i obučenim CCR roniocima koji istovremeno moraju biti stručnjaci za identifikaciju ribljih vrsta. Korištenje podvodne video tehnike može prevladati posljednju spomenutu granicu odvajanjem CCR ronilačkih vještina od znanja o identifikaciji vrsta. Tehnike ronjenja rebreatherom u kombinaciji s kraćim zadržavanjem na dnu prilikom vizualnog cenzusa, zahvaljujući većoj brzini podvodnog skutera i visokokvalitetne video kamere, mogle bi učiniti studije vizualnog cenzusa ispod 40 m, čak i do dubine od 100 m, standardnom metodom.

7. ZAKLJUČAK

Obzirom na sve prethodno navedeno, za pravu procjenu ribljih naselja na okomitim stijenama grebena s polušpiljama, špiljama i šupljinama, koji su uobičajena mikro staništa za puno malih vrsta riba, različite metode se moraju kombinirati kako bi se dobili najbolji mogući rezultati. Trenutni problem je u tome što je upotreba quinaldina, kao najbolje metode za procjenu kriptobentonskih i malih epibentoskih vrsta, metoda anestezije koja se kao takva ne primjenjuje u mnogim osjetljivim morskim staništima, osobito u zaštićenim morskim područjima jer pripada destruktivnim metodama. Stoga se buduća istraživanja trebaju usmjeriti na zamjenu anestetika novom nedestruktivnom tehnologijom barem za vidljive epibentoske vrste, kad to već nije moguće za kriptobentoske vrste, tj. one vrste koje su skrivene u dijelovima morskog dna. Kao što je već vidljivo i iz istraživanja opisanih u ovoj disertaciji, UVC metode su značajno poboljšane korištenjem Full HD, odnosno 4K videa, pa se može očekivati da nas napredak tehnologije videozapisa može približiti tom cilju.

Nadalje, za sva ronjenja preko 40 m dubine neophodno je zrak zamijeniti drugim plinskim mješavinama za disanje te koristiti posebne mješavine bogate kisikom za dekompresiju koja je kod takvih ronjenja neizbježna. Zbog tog razloga, a i sukladno konfiguraciji podmorja Hvarskog kanala, u prikazanim istraživanjima nije se prelazilo dubinu od 65 m. Primijenjena metoda pokazala se djelotvornom na mješovitom cirkalitoralu, kako na koraligenu tako i na pijesku, omogućujući sakupljanje kriptobentonskih i malih epibentonskih riba bez pretjeranih poteškoća.

Dubina od 70-75 m je operativno ograničenje za ronjenje s otvorenim krugom disanja Trimixa, uzimajući u obzir veličinu spremnika plina za disanje na dnu (2x12 lit), koji su čimbenik ograničenja vremena na većim dubinama pa se na dnu ne može ostati više od 12-15 minuta (Pyle, 2000). Usljed toga je upotreba trimixa ograničena na kratke ronilačke zadatke često nedovoljne za ovakva biološka istraživanja.

Druga mogućnost je korištenje rebreathera (CCR), aparata zatvorenog kruga disanja. U usporedbi s opremom korištenom u trimix ronjenjima s otvorenim krugom, CCR ima mnogo veću autonomiju pod vodom, smanjuje rizik od dekompresijske bolesti, smanjuje ukupne potrebe za disajnim plinom, povećava opću sigurnost i ne ostavlja mjehuriće. Zbog ovih karakteristika rebreatheri su prikladniji za upotrebu u istraživanjima vizualnog cenzusa na većim dubinama. Međutim, CCR ima ozbiljna ograničenja u cijeni opreme i cijeni terenskog rada, kao i u potrebi za visokokvalificiranim i obučenim roniocima koji bi ujedno trebali biti stručnjaci za identifikaciju ribljih vrsta. Korištenjem podvodne video tehnike može se prevladati posljednje ograničenje odvajanjem vještina ronjenja CCR-a od znanja o identifikaciji ribljih vrsta i kasnijoj post-analizi video zapisa od strane stručnjaka za identifikaciju. Tehnike ronjenja rebreatherom u kombinaciji s vremenski manje zahtjevnom metodom vizualnog cenzusa, zahvaljujući većoj brzini podvodnog skutera i visokokvalitetnoj video kameri, mogle bi učiniti vizualni cenzus ispod 40 m, čak i do dubine od 100 m, standardnom metodom.

Rezultati istraživanja ovog rada će poslužiti boljem razumijevanju ekologije ribljih naselja na područjima vertikalnih podvodnih grebena te njihovom odnosu prema čimbenicima okoliša. Također, rezultati se mogu iskoristiti za prijedlog mjera različitih oblika zaštite pojedinih područja, posebice podvodnih koraligenskih grebena.

Istraživanja prikazana u ovoj disertaciji mogu poslužiti kao polazna točka za daljna istraživanja koja bi dalje unaprijedila postojeća saznanja o koraligenskim ribljim naseljima kako u Jadranu, tako i u Mediteranu.

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A quantitative assessment of the cryptobenthic fish assemblage at deep littoral cliffs in the Mediterranean

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Summary: The present study provides the first quantitative assessment of cryptobenthic fish species diversity and abundance on hard bottoms below 20 m depth by examining Mediterranean underwater reefs with deep vertical cliffs. Quantitative sampling was performed at depths down to about 45 m and yielded 220 cryptobenthic and 61 epibenthic individuals belonging to 21 species, showing that the cryptobenthic fishes highly outnumbered the epibenthic individuals. The study highlights the high diversity and abundance of this unexplored part of the benthic fish community. The cryptobenthic fish assemblage was dominated by the family Gobiidae in terms of both biodiversity (>60% of all species) and abundance (>90% of all specimens). Fish species previously considered rare were present and some of them were even numerous in this assemblage. Three out of fourteen recorded habitat variables (depth, rocky cliff surface area vs. the bottom of the cliff area, and the presence of sand as the bottom substrate) were identified as significant for species occurrence. Species were generalist in their choice of shelters since the size and quantity of semi-caves, caves, cavities and even bio-cover type had no significant influence on the species distribution and abundance.

Keywords: cryptobenthic fishes; multivariate analysis; benthos; littoral zone; underwater cliffs; scuba diving.

Evaluación cuantitativa de la comunidad de peces criptobenónicos en los acantilados profundos del litoral Mediterráneo

Resumen: Este trabajo presenta una primera evaluación cuantitativa de la diversidad y abundancia de los peces criptobentónicos de fondos duros por debajo de 20 m de profundidad, como ejemplo de acantilados litorales sumergidos del Mediterráneo. Se realizó un muestreo a profundidades cercanas a 45 m de profundidad y se obtuvieron 220 individuos criptobentònicos y 61 epibentónicos pertenecientes a 21 especies. El estudio pone de manifiesto la gran diversidad y abundancia de esta fracción inexplorada de la comunidad de peces bentónicos. La familia Gobiidae domina la comunidad de peces criptobentónicos en biodiversidad y abundancia (>60% y >90% del total de especies respectivamente). Se han observado especies de peces de este grupo consideradas raras, incluso algunas son numerosas. Tres de cada 14 variables registradas del hábitat (profundidad, superficie rocosa del acantilado *vs* fondo del acantilado y presencia de arena en el sustrato del fondo) fueron significativas para la presencia de las especies. En la elección de los refugios las especies fueron generalistas ya que el tamaño y la cantidad de semicuevas, cuevas, cavidades e incluso tipos de cubierta biológica no tienen influencia significativa en la distribución y abundancia de las especies.

Palabras clave: peces criptobentónicos; análisis multivariante; bentos; zona litoral; acantilados sumergidos; submarinismo.

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INTRODUCTION

In rocky reefs and coralligenous habitats throughout the world, fish assemblages are important components of coastal biodiversity, contributing crucially to ecosystem functioning and representing important marine resources for humans (Ackerman and Bellwood 2000, Bussotti and Guidetti, 2009). Various underwater visual census (UVC) methods have often been used in ichthyological research, especially on hard littoral bottoms where most widespread methods for the quantitative assessment of fish assemblages, such as capture methods using trawls and other fishing gear, are hard to use (Harmelin-Vivien and Francour 1992, Lipej et al. 2003, Soldo and Glavičić 2013). However, it is confirmed that the standard UVC methods can miss a large number of cryptobenthic fish species (Ackerman and Bellwood 2000, Willis 2001, Smith-Vaniz et al. 2006, Kovačić et al. 2012). A cryptobenthic fish is a species (or a life history stage of a fish species) whose individuals exclusively or predominantly spend their lifetime in cryptobenthic microhabitats, i.e. in the restricted living spaces underneath the bottom surface of the substrate or biocover, with a physical barrier to open spaces (Kovačić et al. 2012). To gain a full understanding of how a fish assemblage is composed and structured on hard littoral bottoms, a study of small cryptobenthic and epibenthic species using destructive techniques is required. Quantitative studies on cryptobenthic fish communities are surprisingly rare worldwide and restricted to coastal reefs with depths of mainly 15 to 20 m (Prochazka 1998, Ackerman and Bellwood 2000, 2002, Willis 2001, Depczynski and Bellwood 2003, 2004, Smith-Vaniz et al. 2006, Beldade et al. 2006, Beldade and Gonçavles 2007, Kovačić et al. 2012). No quantitative research on cryptobenthic fish has been performed at or below 40 m depth (Kovačić et al. 2012), while only recently the methodology for the quantitative research at circalittoral depths was proposed by Glavičić and Kovačić (2016). Other quantitative studies of fish assemblages at deep rocky reefs, i.e. UVC using SCUBA diving with a rebreather, are very rare and limited to tropical seas (Brokovich et al. 2008). Studies that include destructive methods on fish assemblages have been performed at underwater reefs going deeper, i.e. below 40 m in tropical seas, but they used only qualitative methods (Feitoza et al. 2005, Pyle et al. 2008).

Destructive techniques, by definition, remove fishes from a particular area and have a harmful effect upon the fish under investigation (Ackerman and Bellwood 2000). The main destructive methods that have been used for the study of fish assemblages using SCUBA diving are ichthyocides and anaesthetics (Ackerman and Bellwood 2000, Kovačić et al. 2012). Ichthyocides, such as Rotenon, have a slow effect on fishes, e.g. Rotenone takes 10-15 min to asphyxiate them (Ackerman and Bellwood 2000). In the case of repeated samplings during SCUBA dives, sampling with Rotenone requires a lot of time, which is usually limited in deeper dives. Therefore, the use of the anaesthetic Quinaldine, following the procedure of Kovačić et al. (2012), was chosen for the present study as the most appropriate method with high space and time efficiency.

The aim of the present study was: 1) to continue the studies on unexplored Mediterranean littoral benthic habitats potentially harbouring cryptobenthic fishes, following proposals by Kovačić et al. (2012); 2) to provide the first quantitative assessment of cryptobenthic fish species diversity and abundance on hard bottoms below 20 m depth by studying the deep underwater vertical cliffs of the Mediterranean; and 3) to identify the environmental variables that best explain species distribution and abundance patterns in this habitat, and that are therefore expected to shape the cryptobenthic fish community structure in this habitat.

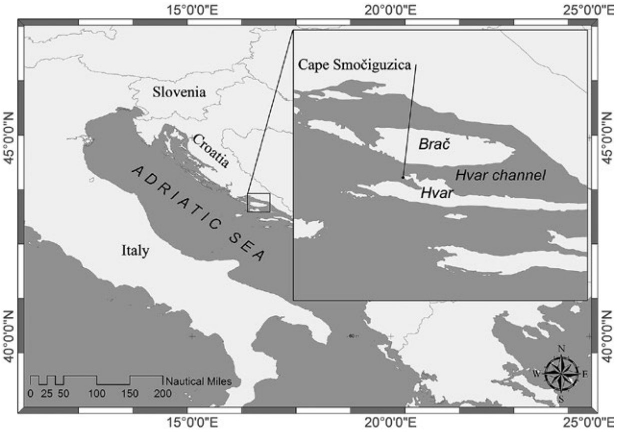

Fig. 1. – Map showing studied locality at Cape Smočiguzica.

MATERIALS AND METHODS

Sampling location and time

The study was carried out in the Hvar Channel, on the north coast of Hvar Island in the central eastern Adriatic (Fig. 1), during the warm season from June to October 2013. All sampling occurred during daytime, between 12 and 3 p.m. The locality studied was a steep underwater reef called Smočiguzica (43°14.124′N, 16°34.393′E), which is a part of the island of Hvar landmass, but more than 200 m away from the coastline. The reef surface structure is made of steep to vertical bedrock, with a few relatively large caves and a lot of smaller ones, and its base is surrounded by a sandy bottom. The reef is shaped as a truncated elliptical and oblique cone. The highest point of the reef is at 9.1 m depth, and the depth of the reef base varies from 43 to 61 m. The most common biocenosis on the reef is the coralligenous community (Ballesteros 2006).

Data collection

The sampling was performed at depths of 15 to about 45 m. To extend the amount of time that the divers could spend at underwater depths, all dives were performed using Nitrox 27. Two divers, both experienced and skilled in diving and in identifying fish and other marine species, were used each time.

A total of 50 one-square-metre bottom quadrants were sampled during 23 SCUBA dives. The number of quadrants performed in one dive varied from 1 to 3 depending on the depth. The protocol on the squares was modified from Kovačić et al. (2012). The anaesthetic used was Quinaldine diluted 1:15 with 96% ethanol and then mixed with sea water 1:5 in 750-mL bottles (modified from Kovačić et al. 2012). Two bottles were usually used for a square, i.e. the total volume of the deployed Quinaldine-ethanol solution was about 300 mL m^{-2}. Specimens observed by visual census were defined as epibenthic specimens. Specimens found only after deploying anaesthetic were defined as cryptobenthic specimens.

Table 1. – Descriptive statistics for all environmental variables. The recorded variables are marked in bold letters (n/a -not available).

Environmental variable	Variable abbreviation	Min	Max	Median	Interquartile range	Median absolute deviation
Depth	depth	15	45	25	20	10
Rock wall base vs. rock wall (wall base=1; rock wall=0)	wallbase	0	1	0		0
Inclination (0-19° not recorded; 20-59°=1; 60-74°=2; 75-90°=3)	inclinat	1	3	3	1	0.72
Sand (%)	sand	0	30	0	10	4.40
Gravel (%)	gravel	n/a	n/a	n/a	n/a	n/a
Cobbles (%)	cobbles	n/a	n/a	n/a	n/a	n/a
Boulders (%)	boulders	n/a	n/a	n/a	n/a	n/a
Bedrock (%)	bedrock	70	100	100	10	4.00
Phanerogams (%)	phanero	n/a	n/a	n/a	n/a	n/a
Short tallus algae (%)	shoalgae	0	90	40	70	26.20
Long tallus algae (%)	lonalgae	n/a	n/a	n/a	n/a	n/a
Calcerous algae (%)	calalgae	0	90	50	58	22.80
Zoo cover (%)	zoocover	0	10	10	0	1.60
No cover (%)	nocover	0	30	0	10	4.60
Number of hidden spaces of entrance size 1: 5×5-10×10 cm	hidd1num	0	4	2	2	1.08
Number of hidden spaces of entrance size 2: 10×10 cm-20×25 cm	hidd2num	0	3	1	1	0.76
Number of hidden spaces of entrance size 3: 20×25 cm-40×50 cm	hidd3num	0	3	0	1	0.58
Number of hidden spaces of entrance size 4: >40×50 cm	hidd4num	0	1	0	0	0.08
Sum of entrance surfaces of hidden spaces	hiddsumP	0.5	32.5	6.5	14	7.32

The following habitat characteristics were included in the recording sheet (modified from Kovačić et al. 2012): “Depth” (15, 25 and 35 m, and the depth at the bottom of the cliff which was about 45 m); “Inclination”, as four estimated classes (gentle [0-19°], medium [20-59°], steep [60-74°]) and very steep/vertical [75-90°]); “Position at the cliff” (rocky cliff surface vs. the base of the cliff); “Bottom substrate” and “Biocover”, as in Kovačić et al. (2012); “Number of semicaves, caves and cavities”, if present, as number of each of four estimated size classes of hidden spaces in each square by entrance size (50-99 cm^2, 100-499 cm^2, 500-1999 cm^2, >2000 cm^2); and “Total surface of the entrances to the hidden spaces”, estimated from the number and size of hidden spaces as percentages of the total surface in each square. The variables were estimated and recorded during dives (see order in protocol above) and most of them were later rechecked in the photos. The position of the collected cryptobenthic specimens in the habitat sensu Kovačić et al. (2012) was not recorded as most of the anaesthetised specimens were collected while floating along the surface of vertical cliffs. Thus, their exact origin and position at the bottom could not be known. Squares were selected to achieve an approximately equal representation of each depth i.e. 15, 25 and 35 m, and the depth of the bottom of the cliff of about 45 m (the depth of the bottom of the cliff in the studied section of the cliff varied from 43 to 50 m, and the square was always fixed at the bottom of the cliff on the border between bedrock and the soft sediment). The number of squares performed for each depth was 14 at 15 m, 12 at 25 m, 12 at 35 m and 12 at 45 m. The position of the square along each isobath was random. The random numbers (ranging 1-50) were generated in advance and employed one by one from the list. Once a particular desired depth was reached going down along the lateral edge of the cliff, the divers moved a number of metres matching the particular random number on the cliff along the isobath. The point on the cliff reached was the position of the square where the 1×1 m frame was fixed on the cliff. All collected specimens were killed, stored, measured and identified as in Kovačić et al. (2012).

Data analysis

Community composition of cryptobenthic and epibenthic fishes was analysed in order to identify environmental variables that best explain species distribution and abundance patterns in the total data set. Among the total number of possible environmental variables included in the field recording sheet, 14 variables were actually recorded during fieldwork (Table 1, the recorded variables marked in bold letters). For the multivariate ordinations the CANOCO 4.5 computer package was used (Ter Braak and Šmilauer 1998). According to Gauch (1982), rare species typically have a minor influence on the results of multivariate statistics and can be perceived in ordination as outliers. However, some of the less abundant species in this research are stenotopic species with a low number of any published records and are, considering the poor knowledge of their influence in communities, interesting for study (Ahnelt and Kovačić 1997, Kovačić and Miller 2000, Kovačić 2008). Consequently, a compromise was chosen and species accounting for less than 1.2% (<4 catches) of the total catch were omitted from the analysis (Table 2). To reveal prevailing patterns of response curves in relation to environment gradients, the data sets were exposed first to detrended correspondence analysis. The first axis gradient length >4 of standard deviation indicates strong nonlinear or unimodal responses, suggesting that canonical correspondence analysis (CCA) is the most appropriate method; values <2 of standard deviation indicate a monotonic response to the environmental gradients and redundancy analysis should be used (Ter Braak and Verdonschot 1995). In order to normalize the highly skewed distribution of species abundance, the data were log(x+1)-transformed. The CCA was run with a focus on inter-species distances and the biplot scaling option. The option down-weight rare species was not selected because some of the abundant species are generally common and eurytopic littoral species, while several less numerous species are very rarely recorded stenotopic species and of unknown influence in communities, so their role should not be underes-

Table 2. – The data from the bottom quadrats of 1 m^2 (n=50) showing recorded species with the number of cryptobenthic (CB) (n=220) and epibenthic (EB) specimens (n=63) and the frequency of species occurrence at the squares. Species in bold letters (≥4 individuals) were included in quantitative data analysis .

Family	Species (abbreviation)	CB specimens	EB specimens	CB frequency of occurrence	EB frequency of occurrence
Gobiidae	***Corcyrogobius liechtensteini*** (*Co li*)	73	0	74.0%	0.0%
Gobiidae	***Odondebuenia balearica*** (*Od ba*)	49	0	52.0%	0.0%
Gobiidae	***Gobius auratus*** (*Go au*)	30	46	36.0%	56.0%
Gobiidae	***Didogobius splechtnai*** (*Di sp*)	13	0	22.0%	0.0%
Blenniiidae	***Parablennnius rouxi*** (*Pa ro*)	13	0	20.0%	0.0%
Gobiidae	***Chromogobius zebratus*** (*Ch ze*)	9	0	16.0%	0.0%
Gobiidae	***Thorogobius macrolepis*** (*Th ma*)	8	1	10.0%	2.0%
Gobiidae	***Zebrus zebrus*** (*Ze ze*)	6	0	8.0%	0.0%
Gobiidae	***Vanneaugobius dollfusi*** (*Va do*)	4	0	6.0%	0.0%
Pomacentridae	*Chromis chromis*	3	0	6.0%	0.0%
Gobiidae	*Gammogobius steinitzi*	3	0	4.0%	0.0%
Gobiidae	***Gobius kolombatovici*** (*Go ko*)	2	2	2.0%	4.0%
Gobiidae	***Gobius vittatus*** (*Go vi*)	1	4	4.0%	8.0%
Scorpaenidae	***Scorpaena notata*** (*Sc no*)	1	3	2.0%	6.0%
Gobiidae	*Speleogobius trigloides*	1	1	2.0%	2.0%
Gobiesocidae	*Apletodon incognitus*	1	0	2.0%	0.0%
Blenniiidae	*Microlipophrys nigriceps*	1	0	2.0%	0.0%
Scorpaenidae	*Scorpaena porcus*	1	0	2.0%	0.0%
Tripterygiidae	*Tripterygion melanurum*	1	0	2.0%	0.0%
Gobiidae	*Thorogobius ephippiatus*	0	3	0.0%	6.0%
Tripterygiidae	*Tripterygion delaisi*	0	1	0.0%	2.0%

timated in advance (Miller 1986, Jardas 1996, Ahnelt and Kovačić 1997, Kovačić and Miller 2000, Kovačić 2008). The first CCA was performed with all environmental variables and the forward selection procedure was then used to extract the variables incorporated in the final model. In order to avoid inflated significance values, redundant predictor variables, i.e. environmental variables showing high multicolinearity (VIF>10), were removed from the model. The significance of the canonical axes and of the environmental variables was determined using Monte Carlo permutation tests (with 499 permutations).

RESULTS

Diversity, abundance and frequency of cryptobenthic and epibenthic fish species

The total number of recorded cryptobenthic and epibenthic fish species in the bottom quadrats was 21 (Table 2). The epibenthic specimens belonged to 8 species and the cryptobenthic specimens belong to 19 species. In other words, 6 species were observed both in cryptobenthic and epibenthic positions and two infrequent species, leopard spotted goby, *Thorogobius ephippiatus* (Lowe, 1839) and *Tripterygion delaisi* Cadenat and Blanche, 1971*,* were found exclusively on the open surface. The family Gobiidae contributed to fish biodiversity with 13 species, more than a half of all the recorded species. Other five families were represented with only one or two species (Table 2). The average total abundance of cryptobenthic and epibenthic fishes in the bottom quadrats was 6.64±0.36 ind. m^{-2} (mean±S.E.) and ranged from 3 to 17 ind. m^{-2}. The cryptobenthic fish, with 220 individuals collected, highly outnumbered the 61 recorded epibenthic individuals. The twelve most abundant species with four or more individuals collected or recorded represented 94.3% of all individuals. The three most numerous species, Liechtenstein's goby *Corcyrogobius liechtensteini* (Kolombatović, 1891), Coralline goby *Odondebuenia balearica* (Pellegrin and Fage, 1907) and Golden goby *Gobius auratus* Risso, 1810, collectively made up 69.1% of all cryptobenthic specimens. The epibenthic individuals of a single species, *G. auratus*, represent 75.4% of all recorded epibenthic fish (Table 2). The most abundant species were exclusively present in cryptic positions, except for *G. auratus*, which was very common in both hidden spaces and open positions (Table 2). The species of the family Gobiidae contributed more than 90% to total fish abundance. The eight most numerous species with cryptobenthic individuals and individuals in general were also the most frequent species in quadrants, ranked from *C. liechtensteini,* recorded in about 3/4 of the quadrants, to the zebra goby, *Zebrus zebrus* (Risso, 1826), which was present in 8% of the quadrants (Table 2).

Important habitat variables and habitat preferences of fish species

The number of the environmental variables that were actually recorded in the field was smaller than expected (Table 1, the recorded variables marked in bold letters). Some bottom substrate and bottom cover variables that usually occur on littoral bottoms were missing in the specific environment of the vertical cliffs.

In the CCA analysis all 14 recorded environmental variables were initially considered. The first exploratory CCAs indicate that, although the global model with 14 environmental variables was statistically significant, some environmental variables were inter-correlated (Fig. 2A). After the removal of redundant variables, the second model was run with only 10 environmental variables (Fig. 2B). The Monte Carlo permutation test indicated that in both analyses the first axis and all other canonical axes were statistically significant (Tables 3 and 4). The first four canonical axes explained 82.2%

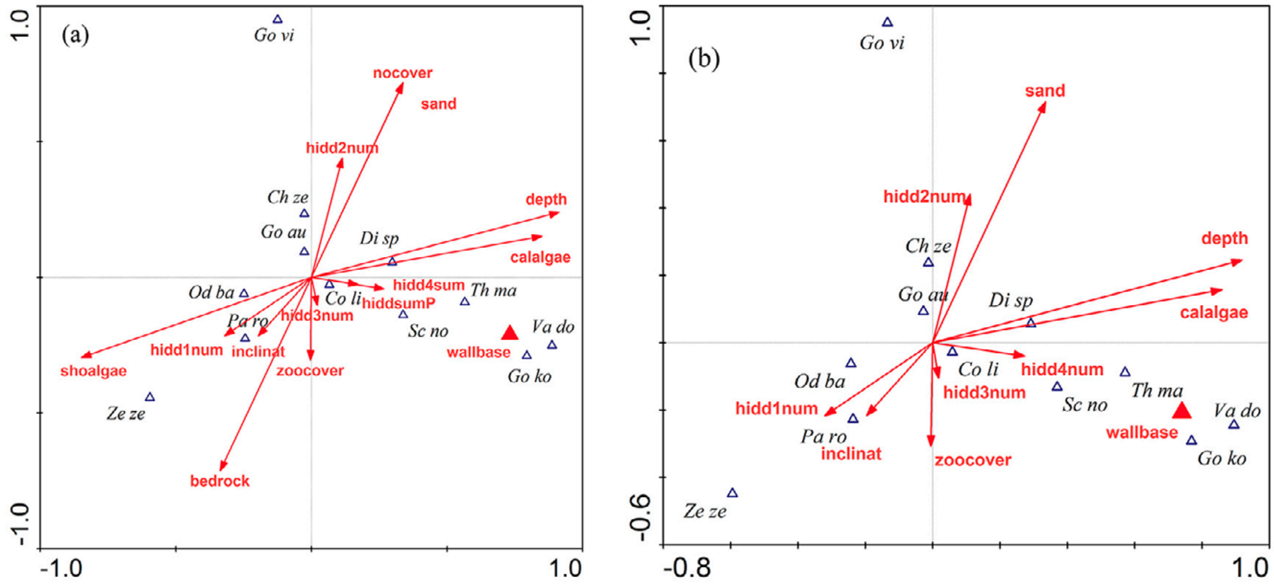

Fig. 2. – CCA species-environment biplot for 14 (A) and 10 fish species (B) (biplot scaling). In the biplot (B), together, the first two axes explain 17.2 % (horizontally λ=0.302; vertically λ=0.179) of the total inertia in the abundance and 58.2 % of variance in the weighted averages and class totals of species with respect to environmental variables. Quantitative environmental variables are represented by arrows and nominal variable is indicated by a triangle, both with abbreviations in regular font. Hollow triangles and abbreviations in italics represent fish species. The abbreviations for variables are explained in Table 1, and for fish species in Table 2.

Table 3. – Outcome of CCA, using 14 environmental variables. Significant environmental variables are reported in Table 5.

Total inertia = 2.796 Sum of canonical eigenvalues = 0.844	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.307	0.179	0.125	0.083
Species-environment (S-E) correlations	0.830	0.757	0.637	0.565
Cumulative % variance of species data	11.0	17.4	21.9	24.8
Cumulative % variance of S-E relation	36.4	57.6	72.4	82.2
Monte Carlo test (P-value, 499 permutations)	0.002		P (all canonical axes) = 0.008	

Table 4. – Outcome of CCA, using 10 environmental variables. Significant environmental variables are reported in Table 5.

Total inertia = 2.796 Sum of canonical eigenvalues = 0.826	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.302	0.179	0.124	0.082
Species-environment (S-E) correlations	0.824	0.756	0.637	0.562
Cumulative % variance of species data	10.8	17.2	21.6	24.6
Cumulative % variance of S-E relation	36.5	58.2	73.3	83.2
Monte Carlo test (P-value, 499 permutations)	0.004		P (all canonical axes) = 0.014	

and 83.2% of species occurrence variation based on 14 and 10 environmental variables, respectively. The datasets with 14 and 10 environmental variables both identified the same three significant variables: depth (P=0.02, forward selection), rocky cliff surface area vs. the bottom of the cliff area (P=0.008, forward selection) and the presence of the sand as bottom substrate (P=0.048, forward selection) (Tables 3, 4 and 5). As can be seen in Table 5, all other variables were not statistically significant. The first axis correlated positively with depth, calcareous algae and wall base, where the amount of calcareous algae is expected to increase with depth, and wall base is exclusively present at the deepest depth. The second axis may correlate with zoocover i.e. animal biocover.

The 12 species included in the model showed different relations to the three significant variables (Table 1, the included species (>4 catches) marked in bold letters; Fig. 2). Habitat preferences of the collected fishes were interpreted by the position of species centroids in the biplot related to significant variables and to other variables closely associated with three significant variables on the graph (Fig. 2). The highest influence among variables was exerted by the position on the cliff, where the group of gobiid species exclusively preferred the cliff base vs. the cliff itself (*Gobius kolombatovici* Kovačić and Miller, 2000, large scaled goby *Thorogobius macrolepis* (Kolombatović, 1891), *Vanneaugobius dollfusi* Brownell, 1978, Table 1, Fig. 2). Those species, as expected, were also more associated with the increasing depth and the increasing amount of calcareous algae, but surprisingly indifferent to another significant variable that was also present at the bottom of the cliff, the sand. In addition to these species found at the bottom of the cliff, the deeper bottom (and the calcareous algae cover) was also preferred by *Didogobius splechtnai* Ahnelt and Patzner, 1995 and by small red scorpionfish, *Scorpaena notata*

Table 5. – Variation explained by 14 or 10 environmental variables. Marginal effect (λ 1) of environmental variable refers to eigenvalues fit if the corresponding variable was the only environmental variable in the model. The conditional effect (λ A) refers to the additional amount of explained variance in species distribution by the correspondent variable when selected by automatic forward selection in Canoco 4.5. The obtained statistics of the Monte Carlo permutation test under the full model with 499 random permutations (P- value and F) are reported for each variable. Significant environmental variables (P≤0.05) are bold. The abbreviations are explained in Table 1.

	Marginal Effects	Conditional Effects after forward selection					
		14 Environmental variables			10 Environmental variables		
Variable	λ 1	λ A	P	F	λ A	P	F
depth	0.27	0.27	**0.02**	5.15	0.27	**0.002**	5.15
shoalgae	0.24	0.03	0.744	0.68	n/a	n/a	n/a
calalgae	0.24	0.02	0.932	0.40	0.03	0.734	0.70
wallbase	0.25	0.13	**0.008**	2.57	0.13	**0.008**	2.57
inclinat	0.10	0.08	0.076	1.66	0.08	0.076	1.66
sand	0.15	0.11	**0.048**	2.20	0.11	**0.048**	2.20
bedrock	0.15	n/a	n/a	n/a	n/a	n/a	n/a
nocover	0.15	n/a	n/a	n/a	n/a	n/a	n/a
hidd1num	0.08	0.06	0.254	1.24	0.06	0.254	1.24
zoocover	0.09	0.05	0.474	0.94	0.05	0.474	0.94
hidd2num	0.08	0.04	0.618	0.81	0.04	0.618	0.81
hidd4num	0.05	0.04	0.598	0.85	0.04	0.598	0.85
hidd3num	0.03	0.01	0.996	0.21	0.02	0.996	0.23
hiddsumP	0.03	n/a	n/a	n/a	n/a	n/a	n/a

Rafinesque, 1810. Contrary to this, a strong association with the shallow depth within the studied range 15 to approx. 45 m depth was shown by *Z. zebrus*, and to some extent by *Parablennnius rouxi* (Cocco, 1833) and *O. balearica*. These three species with a preference for the shallow parts of the cliff were also associated with the steep inclination contrary to most of the species showing indifference to inclination. The remaining four species were more or less indifferent to the depth of the bottom. The bottom substrate was dominated by the bedrock and the only other recorded bottom substrate was sand, which was present in about half (54%) of the quadrants, covering 5%-30% of their surfaces. An association with the presence of sand, i.e. with the mixed bottom of bedrock and sand, was evident for four gobiid species (Kolombatović's goby *Chromogobius zebratus* (Kolombatović, 1819)*, D. splechtnai, G. auratus*, and striped goby, *Gobius vittatus* Vinciguerra, 1883). On the other hand, the three species preferring a steep inclination (*O. balearica, P. rouxi* and *Z. zebrus*) showed an avoidance of sand.

DISCUSSION

The present study is the first quantitative assessment of cryptobenthic fish species diversity and abundance at hard bottoms below 20 m of depth. It was performed on a Mediterranean underwater reef with vertical cliffs going down to about 45 m depth. The recorded fish assemblage is part of the Mediterranean infralittoral and shallow circalittoral ecozones, which are mostly occupied by coralligenous biocenoses (Table 1, quadrants with median of calcareous algae cover of 50%) and to some degree by biocenoses of infralittoral algae (Bakran-Petricioli 2007, 2011). The necessity of a large number of longer dives at greater depths in order to obtain quantitatively useful data could be the reason for the lack of this kind of study in general. These studies need an extended diving time for the use of ichthyocides or anaesthetics, and for the larger number of quadrants necessary for quantitative analysis. The use of the anaesthetic Quinaldine, which takes just 2-3 min to asphyxiate fishes, provided better use of diving time, and the SCUBA diving protocol depending not just on compressed air but on other gas mixtures provided longer diving time. The chosen procedure of Kovačić et al. (2012), with 1m^2 squares, allowed for high space and time efficiency, while the quantity of Quinaldine used and the intensive examination of the substratum should have guaranteed a total census of present fishes.

The only published quantitative study (Kovačić et al. 2012 in the northern Adriatic Sea) that performed methodologically appropriate research on Mediterranean cryptobenthic fish communities was restricted to more shallow depths. Two other quantitative studies focusing on cryptobenthic fishes in the Mediterranean (La Mesa et al. 2004, 2006) used the visual census technique, a method which could not record "real" cryptobenthic fishes, i.e. fishes present in the cryptobenthic microhabitats, where they are never or rarely visible from the outside. Both studies were also depth-limited (to 23 m depth and 15 m depth, respectively). Two studies on cryptobenthic fish communities geographically closest to the Mediterranean were performed on the Atlantic coast of Portugal. Beldade et al. (2006) used only interference visual census with depth limits of 9.5 m, while Beldade and Gonçavles (2007) combined interference visual census with limited use of the anaesthetic Quinaldine with depth limits of 11.2 m. The qualitative studies of cryptobenthic fish communities in the Mediterranean were limited to the infralittoral zone and only one (Patzner 1999) reached 40 m depth, a depth similar to the present study. The qualitative studies of Patzner (1999) were performed in Ibiza, in the western Mediterranean Sea, using SCUBA diving and the anaesthetic Quinaldine, and it showed a high match in species composition with Adriatic researches. All 15 species recorded by Patzner (1999) were also recorded in the Adriatic researches (Kovačić et al. 2012, present research). Among them, 10 species were found in the present research and the 5 remaining shallow water species were recorded by Kovačić et al. (2012). Species biodiversity in Kovačić et al. (2012) was higher than in the present research (27 *vs.* 21 species). However, that study covered a depth down to 20 m, more diverse habitats and a higher total effort (76 quadrants *vs*. 50 quadrants). About half of the species were the same as in the present study. Most of the remaining half of the species diversity in Kovačić et al. (2012) belonged to shallow water species of the families Blenniidae, Gobiidae and Gobiesocidae and, to a lesser degree, species of these families that do not occur on vertical and steep bedrocks (e.g. *Lepadogaster candolii* and *Gobius bucchichi*).

The average density was surprisingly similar between two studies performed on different habitats and depths: the present research had an average total abundance of cryptobenthic and epibenthic fish of 6.64±0.36 ind. m^{-2} (mean±SE), while in Kovačić et al. (2012) the median total abundance of cryptobenthic fish was 6 ind. m^{-2} and of epibenthic fish 1 ind. m^{-2}. The cryptobenthic fish density is even higher on the tropical coral reef where Depczynski and Bellwood (2004) found a mean density of 11 ind. m^{-2}. Both Mediterranean studies showed an abundance of cryptobenthic fish several times higher than that of epibenthic fish (in Kovačić et al. (2012) cryptobenthic fish/epibenthic fish ratio was 7.8; in the present research 3.6). In the present research 13 out of 21 species were found strictly in the cryptobenthic position, compared with 13 out of 27 species found by Kovačić et al. (2012). Therefore, the studies of benthic fish assemblages on infralittoral and circalittoral bottoms rich with shelters should not overlook the influence and role of cryptobenthic fishes in the biological community and in the benthic ecosystem.

The present study used two methods: visual census to study epibenthic fish and anaesthetic to study cryptobenthic fish. The part of the bottom fish assemblage that was not covered by these methods includes hyperbenthic fish and large epibenthic fish, the second one due to the limited size of individual squares, and the relative contribution of cryptobenthic fish to total benthic fish diversity is certainly lower than 62% and 48% from these two studies.

Despite the limited range of ecozones in this study, i.e. the lower infralittoral ecozone and the beginning of the circalittoral ecozone, one of three significant environmental variables that mostly contributed to variation in species occurrence (with an effect of 0.27 explained variance in species distribution) was the bottom depth. The presence of sand was another significant environmental variable that explained species distribution and abundance, with several gobiid species showing preferences for mixed bottoms and a few species showing an avoidance of sand. The third significant variable was the bottom of the cliff area, where some species occurred exclusively or mostly. It seems that the size and quantity of semicaves, caves and cavities, and even the biocover type as the second source of shelters, were without significant influence on species distribution and abundance, i.e. species were generalist in the choice of shelters. In a previous study of Kovačić et al. (2012), the three variables were highly significant for variation in species occurrence: depth, quantity of bedrock and presence of short thallus algae on boulders. Depth was, as in the present study, the variable contributing most strongly to variation in species occurrence in Kovačić et al. (2012). The differences in other significant and highly explanatory variables between the two studies could be the results of habitat differences, because not all the variables were present in both studies (e.g. the bottom of the cliff area in Kovačić et al. 2012). The depth dependence of cryptobenthic species composition for Mediterranean is also clearly visible in descriptive data in Patzner (1999, Fig. 1). The only other research that quantitatively studied, among other variables, the influence of depth on the cryptic fish community composition was also done in temperate waters, in South Africa, at depths of 0-20 m (Prochazka 1998) and showed depth to be the second most important environmental variable explaining cryptic fish community composition.

The indiscriminate use of the term cryptobenthic fishes in the past was criticized by Kovačić et al. (2012). We follow their definition of cryptobenthic fishes (provided here in the Introduction). The terms of cryptobenthic fish species and fish specimen in the cryptobenthic position should be clearly distinguished (see Material and methods). For the general conclusions on species character, even the large number of samples is not enough: the data need to be from various habitats, seasons and parts of day to understand general species relationships to the habitat and behaviour. In the present study, among species included in the quantitative data analysis, specimens of *Z. zebrus, P. rouxi, O. balearica, C. zebratus, D. splechtnai* and *C. liechtensteini* were found exclusively in the cryptobenthic positions. For the four of them the results match those in Kovačić et al. (2012) and represent one more evidence for the cryptobenthic character of these species. The exclusively cryptic behaviour of *P. rouxi* in the present study was surprising compared with previous data and general knowledge on the species (Kovačić et al. 2012), while *D. splechtnai* was not collected by Kovačić et al. (2012). The group of species present at the cliff base (*G. kolombatovici, T. macrolepis, V. dollfusi*) preferred epibenthic positions close to shelters. Another group along the entire cliff surface (*G. auratus, G. vittatus* and *S. notata*) could be termed epicryptobenthic according to Kovačić et al. (2012), considering their ambivalent bottom position records that include open and hidden places. The exclusively cryptic sample of *Chromis chromis* in the present study is too small for any conclusion but intensive use of cryptic spaces by this hyperbenthic species was known (at least for juvenile fish) at night-time (Harmelin 1987) and was recently reported even in daylight (Kovačić et al. 2012).

Some of the species found in the present study are considered to be “rare”, i.e. they have a limited number of total known records (summarized by Francour (2008) for *D. splechtnai*; Kovtun and Manilo (2013) for Steinitz’s goby, *Gammogobius steinitzi* Bath, 1971; Francour and Mangialajo (2007) for *G. kolombatovici*; Fesser (1980) for *Speleogobius trigloides* Zander and Jelinek, 1976; Ahnelt and Kovačić (1997) for *T. macrolepis*; and Kovačić (2008) for *V. dollfusi*). Most of them have been described or rediscovered during the last two decades (*D. splechtnai, G. kolombatovici, T. macrolepis, V. dollfusi*) and two species were described in the 1970s (*G. steinitzi* and *S. trigloides*). In addition, several species recorded in the present study as abundant (*C. liechtensteini, O. balearica*) or common (*C. zebratus, Z. zebrus*) had until recently been considered rare or even very rare species, but the recent study of Kovačić et al. (2012) revealed them to be a common part of the cryptobenthic fish assemblage.

Potential and possible directions of future research on Mediterranean cryptobenthic fish communities, using quantitative sampling by SCUBA diving and anaesthetics were reviewed by Kovačić et al. (2012). They could be summarized as studies of cryptobenthic fish communities at different times of day, seasons or different habitats to those used in Kovačić et al. (2012). The quantitative data on the influence of night and the cold season on the behaviour of Mediterranean benthic fishes and on their utilization of cryptic spaces is still unknown for any habitat. The present study, with a median of cover of calcareous algae in quadrants of 50% (Table 1), mostly studied the coralligenous biocenoses. This habitat was poorly included in the quadrants during the study by Kovačić et al. (2012), so the two studies are quite complementary by depths and bottom types. Other Mediterranean infralittoral benthic habitats well known for their rich cryptobenthic fish fauna, but without any quantitative data on this fauna, are *Posidonia oceanica* meadows and *Cystoseira* forests (Patzner 1999, Hofrichter and Patzner 2000). Future quantitative studies on cryptobenthic fishes should also target these two valuable and endangered Mediterranean habitats that have experienced a dramatic decline over the last decades in the Mediterranean (Boudouresque et al. 2003).

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A QUANTITATIVE SAMPLING METHOD FOR ASSESSMENT OF DEEP CRYPTOBENTHIC ICHTHYOFAUNA USING TRIMIX DIVING

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Abstract. The first quantitative sampling method for deep cryptobenthic and epibenthic ichthyofauna using trimix diving was performed at about 60 m depth at two localities off the island of Hvar (eastern central Adriatic Sea) in October 2014. The safe, efficient, and relatively simple diving technique combined with the quantitative method for collecting fishes on studied depths is described and explained. A total of 16 fish specimens representing four species, i.e., three gobiid and one blenniid species, were collected using anaesthetic quinaldine at six bottom quadrats (squares) of 1 m^2. The cryptobenthic and small epibenthic ichthyofauna on these habitats at circalittoral depths could not be efficiently sampled by any other method. Two of the collected species, *Thorogobius macrolepis* (Kolombatović, 1891) and *Vanneaugobius dollfusi* Brownell, 1978, are considered rare with very few reported records. The deepest findings of species *Parablennius rouxi* (Cocco, 1833), *Gobius auratus* Risso, 1810, and *T. macrolepis* were recorded.

Keywords: circalittoral zone, deep diving, Mediterranean, quadrat sampling, rare fishes, deepest records

During the second half of the 20th century, in situ observation and collection of fishes reached circalittoral bottoms like tropical mesophotic coral ecosystems (Bejarano et al. 2010), most often using research submersibles. However, due to their high cost, submersibles were in general used in the research of deeper habitats, rather than circalittoral depths, and their use to study bottoms at depths of 40–200 m has remained rare (Thresher and Colin 1986, Dennis and Bright 1988). Later, near the end of the 20th century remote operated vehicles (ROVs) became more widely used, but they were greatly limited in their ability to collect specimens (Pyle 1998). The advance of diving techniques at the turn of the century enabled not only observations and visual census of circalittoral ichthyofauna by divers (Brokovich et al. 2008, Bejarano et al. 2010), but also collection of fish specimens. However, studies using SCUBA diving for collecting fishes at marine reefs going deeper than 40 m are rare and restricted to the Western Atlantic and Pacific (Feitoza et al. 2005 to 70 m of depth, Pyle 1999 and his references therein to 150 m of depth, Pyle et al. 2008 to 120 m of depth). The primary goal of these researchers was to get samples for species identification i.e., qualitative data on species presence for results in biodiversity, biogeography and systematics by erratic collecting of fishes (Pyle 1998).

Destructive methods using SCUBA diving and ichthyocides or anaesthetics for quantitative studies on cryptobenthic fish communities are less frequently used and usually restricted to shallow depths, and no published research with quantitative data on cryptobenthic fish has been performed at or below 40 m of depth (Kovačić et al. 2012). One still unpublished slightly deeper quantitative research is the recently submitted work of the present authors from the locality Smočiguzica with a sampling of 50 bottom quadrats (squares) performed at depths of 15–45 m between June and October 2013.

The problem with diving at greater depths is that such diving is associated with a possibility of developing decompression sickness and with problems related to breathing gases causing nitrogen narcosis and oxygen toxicity. Consequently, using compressed air at greater depths for collecting is not just a safety problem, but it influences collecting methods and procedures by a lower level of accuracy and precision of obtained data, primary because of the narcotic effect of nitrogen. Therefore, a breathing gas mixture other than air has to be used. The present sampling was performed at depths between 57 and 62 m (Fig. 1), using trimix 20/30 as a bottom gas and nitrox 50 as a decompression gas (Van Rees Vellinga et al. 2006). Trimix enables safe diving at greater depths and nitrox enables a significant reduction of decompression

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time (a 50% reduction compared to decompression with compressed air) and also safer diving. We used double 12 L diving tanks connected with a manifold, 2 separate first stage diving regulators for trimix and a separate decompression tank of 11 L for decompression with nitrox. We also applied deep decompression stops prior to mandatory shallow stops (Pyle 1997) of 2 min starting from 30 m of depth. Nitrox was used as a tool to accelerate decompression stops and to decrease the risk of decompression sickness (Marinović et al. 2012). The change of breathing gases was performed at a depth of 21 m, corresponding to O_2 partial pressure in nitrox of 1.6 bar. The dives were performed following dive plans prepared in advance using V-Planner (SCUBA diving decompression planning software V-3.89) (Fig. 2), while the Mares Icon HD diving computer was used solely as a bottom timer.

The aim of the presently reported study was to introduce and test the use of trimix diving for quantitative studies of cryptobenthic and epibenthic fish communities of deep littoral bottoms i.e., of the circalittoral zone on the example of the Mediterranean. The method using diving and anaesthetics or ichthyocides is the only method in general that can ensure efficient collection of truly cryptobenthic fishes (Kovačić et al. 2012). It is also the only method that can be used to find and collect small epibenthic fishes on the rugose rocky and mixed bottoms.

The study was carried out during October 2014 in the area of the Hvar Channel, central eastern Adriatic. All sampling occurred during daytime, between 1400 and 1600 hours. The studied localities were two underwater steep reefs, called Smočiguzica (43.2354ºN, 16.573217ºE), and Zala Luka (43.3068ºN, 16.441517ºE), which are a part of the island of Hvar, but more than 200 m away from the coastline. Surfaces of both reefs consist of steep to vertical limestone bedrock surrounded by a sandy bottom. The base of the reefs is at a range of 57–62 m of depth, so that base area was chosen for the study as the deepest reef part.

Two divers, both experienced and skilled in diving and in fish and other marine species identification, performed each collection. The collecting protocol was modified from Kovačić et al. (2012) (Fig. 1):

- Visual census of the epibenthic fishes;
- Fixing a frame (1 × 1 m) onto the cliff;
- Photographing the 1 m² square as reference for the recheck of habitat parameter values assigned in situ;
- Recording habitat characteristics and the temperature in 1 m²;
- Spraying the anaesthetic into the square, catching escaping fishes with a scoop net and waiting for about 2 min;
- Removing biocover and movable parts of the bottom structure and collecting anesthetised fishes.

The anaesthetic used was quinaldine diluted 1 : 15 with 96% ethanol and then mixed 1 : 5 in 750 mL bottles with sea water (modified from Kovačić et al. 2012). Two bottles were usually used for each square i.e., the total volume

Fig. 1. Collecting within an iron bar quadrat with anaesthetics and small scoop net, depth 58 m, locality Smočiguzica; Photo by Dani Laslo

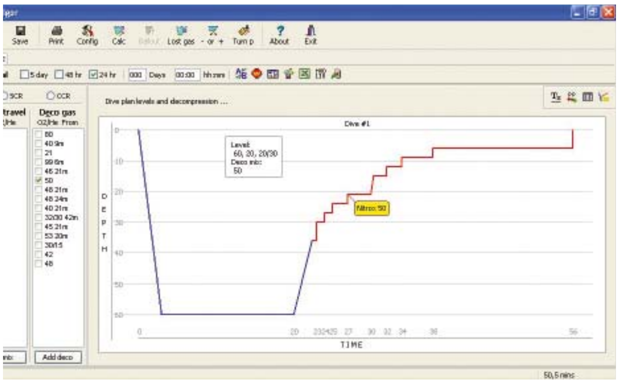

Fig. 2. The graph of the prepared dive plan for performed dives at 60 m of depth and used combination of breathing mixtures; Produced using V-Planner scuba diving decompression planning software and used for all present dives

of deployed quinaldine-ethanol solution was about 300 mL·m^{-2}. The anaesthetic affected only the specimens within the square and only they were collected. No sea currents were present during the collecting, or in one case they were light, so sea currents have not influenced the diving and collecting procedure, including the use of quinaldine. All collected specimens were put to death after reaching the surface by a quinaldine overdose and stored in 65% ethanol solution. The preliminary field species identification of cryptobenthic specimens was later rechecked in the lab. All examined material has been deposited in the Natural History Museum Rijeka (PMR), Croatia.

At six bottom quadrats of 1 m^2 a total of 16 fish specimens representing four species were collected using the anaesthetic quinaldine (4 species at Zala luka and 2 at Smočiguzica). In this number there were three gobiids: *Gobius auratus* Risso, 1810; *Thorogobius macrolepis* (Kolombatović, 1891); *Vanneaugobius dollfusi* Brownell, 1978; and one blenniid species: *Parablennius rouxi* (Cocco, 1833).

The fish assemblage in bottom quadrats was dominated by gobiid specimens, with just one individual belonging to a non-gobiid species, the blenny *Parablennius rouxi*. The most numerous species was *Gobius auratus*. However 7 specimens of this species were all found together as a school at the same quadrat. The more frequent ones were *Thorogobius macrolepis* with 5 specimens present at 2/3 of bottom quadrats and *Vanneaugobius dollfusi* with 3 specimens present at 50% of bottom quadrats. The mean total abundance of fishes in the bottom quadrats was 2.67 ± 1.28 individuals · m^{-2} (mean ± standard error of the mean), but the distribution is heavily right-skewed ranging from 1 to 9 individuals · m^{-2} with a median value of 1.5 and modal value of 1. Some of the *T. macrolepis* and *V. dollfusi* specimens and the majority of the *G. auratus* specimens were observed before the application of the anaesthetic i.e., they were in an epibenthic position.

List of fishes collected: *Gobius auratus*: 2 males, 40.9 + 9.2 and 49.0 + 11.3 mm (standard length + caudal fin length), 5 females, 33.0 + 8.8–40.6 + 9.9 mm (PMR VP3367), Smočiguzica, central eastern Adriatic, 8 October 2014. *Thorogobius macrolepis*: 1 male, 33.8 + 8.0 mm (PMR VP3360), 1 female, 47.7 + 11.1 mm (PMR VP3361), Zala Luka, central eastern Adriatic, 2 October 2014; 2 males, 41.8 + 9.8 and 47.7 + 11.6 mm (PMR VP3363), Zala Luka, central eastern Adriatic, 6 October 2014; 1 female, 43.8 + 10.4 mm (PMR VP3365), Smočiguzica, central eastern Adriatic, 8 October 2014. *Vanneaugobius dollfusi*: 1 female, 25.2 + 6.5 (PMR VP3362), Zala Luka Island, Hvar Channel; central eastern Adriatic, 6 October 2014; 1 female, 36.3 + 7.3 mm (PMR VP3364), 1 male, 36.1 + 7.0 mm (PMR VP3366), Smočiguzica, central eastern Adriatic, 8 October 2014. *Parablennius rouxi*: 1 specimen of unidentified sex, 42.2 + 7.9 mm (PMR VP3368), Smočiguzica, central eastern Adriatic, 8 October 2014. All materials were collected by Igor Glavičić and David Pivalica.

The mixed bottom in studied quadrats was composed of bedrock and of muddy sand and gravel, consisting mostly of shell debris. The recorded biological communities in bottom quadrats belonged to the circalittoral zone. The coralligenous biocoenosis was developed on the bedrock, including the two variations of this biocoenosis: the facies of *Eunicella cavolini* and facies of *Parazoanthus axinellae*. At these depths no trace of biocoenosis of infralittoral algae existed on the bedrock. The coralligenous community consisted of species characteristic of this community: calcareous encrusting algae (*Lithophyllum* sp., *Peyssonnelia rubra*), other sciaphilic algae (*Flabellia petiolata*), and sessile invertebrates (Porifera, Cnidaria, Bryozoa, and Tunicata). The shell debris sediment had rarely scattered surface fauna of biocoenosis of coastal detritical bottoms like *Cerianthus membranaceus*. Hyperbenthic and larger epibenthic fish fauna around bottom quadrats was rare at these depths and it was represented by: *Chromis chromis* (Linnaeus, 1758); *Phycis phycis* (Linnaeus, 1766); *Scorpaena scrofa* Linnaeus, 1758; and *Trigloporus lastoviza* (Bonnaterre, 1788).

Vanneaugobius dollfusi was the only one among four recorded species occurring exclusively in circalittoral bottoms down to 160 m (Ahnelt and Dorda 2004). The presently reported finding at 60 m represents the deepest records of *Parablennius rouxi*, *Gobius auratus*, and *Thorogobius macrolepis* and shows these species for the first time as fishes with width-depth range i.e., infralittoral to circalittoral species. *Thorogobius macrolepis* was hitherto known as an infralittoral species with a depth range of 6–45 m (Ahnelt and Kovačić 1997, Francour et al. 2007). *Gobius auratus* also seemed to be limited to the infralittoral zone with maximum reported depths of 35 m (Herler et al. 2005) and 40 m (Francour et al. 2007). The published lower depth range of *P. rouxi* was 42 m (Zander 1986). *Vanneaugobius* dollfusi is known from only a few records. It was described at the Atlantic coast of Morocco, and two decades passed with only the type record known. It has been found again in the eastern Adriatic Sea (Pallaoro and Kovačić 2000) and in the Aegean Sea (Ahnelt and Dorda 2004). *Thorogobius macrolepis* is known from only a few records from the Adriatic Sea (Ahnelt and Kovačić 1997, Guidetti et al. 2006), from France and the Balearic Islands in the western Mediterranean (Ahnelt and Patzner 1996) and from the eastern Mediterranean at the southern Aegean Sea and eastern Levantine coasts (Francour et al. 2007).

The presently reported study applied bottom quadrats that produced quantitative data on fish bottom abundance and it introduced a safe, relatively simple, and efficient application of using trimix diving for collecting fishes on depths below the reach of compressed-air divers. However, 70–75 m is the operating limit for SCUBA diving with open circuit diving system trimix, considering the carrying limit of the volume of the tanks that can provide the needed diving time to perform tasks in this kind of biological research and collecting. For lower parts of the circalittoral zone i.e., bellow 70–75 m, quantitative research using diving and anaesthetics could be performed only using the complicated and expensive method of closed-circuit rebreather systems with trimix (Pyle 1999).

The applied method showed to be efficient on the mixed circalittoral bottoms, both on coralligenous cover over bedrock and on shell debris, enabling collecting of cryptobenthic fishes and small epibenthic fishes. Only other method for collecting small benthic fishes at circalittoral depths is specially designed beam trawl made to collect samples of epibenthic organisms (Kovačić et al. 2016). It has advantage of covering much larger area of circalittoral bottom than diving. However, the method is limited on flat and soft bottoms. It can also be used on flat maerl beds (Kovačić et al. 2016). In addition, the use of this net has risk of damaging small fragile specimens during dragging. Therefore, the diving method with anaesthetics or ichthyocides remains only method to collect cryptobenthic and small epibenthic fishes at coralligenous communities other than maerl beds and on other steep or rugose rocky and mixed circalittoral bottoms.

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A quantitative assessment of the diel influence on the cryptobenthic fish assemblage of the shallow Mediterranean infralittoral zone

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Summary: Cryptobenthic fishes are an underestimated and probably important component of coastal marine ecosystems that are usually overlooked by standard methods for collecting and studying benthic fishes. Studies focusing on cryptobenthic fishes have been rare and all have been based on samples taken during daytime. The present study tested the difference in epibenthic and cryptobenthic fish composition, diel differences in cryptobenthic fish assemblage and diel shifts of infralittoral fish species between hidden and open bottom spaces. It also looked for the significant habitat variables structuring the cryptobenthic fish assemblage. The daylight, sunset and night samples of epibenthic and cryptobenthic fish assemblages were collected from 78 squares of 1 m^2 shallow water plots (0.5 to 3 m) on Brač island in the eastern Adriatic. The study recorded 27 species, among which the family Gobiidae dominated fish diversity with 14 species. Cryptobenthic specimens highly outnumbered epibenthic specimens, with a ratio of 7.5 to 1. Species composition of cryptobenthic and epibenthic fish assemblages differed significantly. No diel variation in composition, species richness or abundance of the cryptobenthic fish assemblage was detected. Occurrence frequencies in hidden and open bottom spaces of ambivalent species did not change significantly between times of day, so no dial switches between open and hidden places were apparent. In combination, these results suggest that the cryptobenthic fish assemblage has diel stability and is mostly composed of permanent inhabitants of hidden spaces with domination of miniature gobies. Significant habitat variables for species occurrence were the presence of multiple layers, bottom inclination and the presence of cobbles, while depth also had a large but not significant effect.

Keywords: cryptic fishes; diel influence; multivariate analysis; habitat utilization; benthos; littoral zone.

Una evaluación cuantitativa de la influencia diaria sobre la comunidad de peces criptobentónicos en la zona infralitoral mediterránea

Resumen: Los peces criptobentónicos están subestimados y probablemente forman un componente importante de los ecosistemas costeros debido a errores en los métodos de muestreo habituales en peces bentónicos. Los estudios sobre peces criptobentónicos son escasos y generalmente realizados durante el día. El presente trabajo analiza la diferencia en la composición de peces epibentónicos y criptobentónicos, diferencias día-noche entre especies criptobentónicas y cambios diarios de peces infralitorales entre áreas abiertas y ocultas. Asimismo, se analizan las variables del hábitat que estructuran las comunidades criptobentónicas. Se realizaron muestreos durante el día, anochecer y noche de las comunidades epibentónicas y criptobentónicas a partir de 78 cuadrados de 1 m^2, entre 0.5 y 3 m en la isla Brač (Adriático oriental). El estudio registró 27 especies, siendo dominante la familia Gobiidae con 14 especies. Los ejemplares criptobentónicos sobrepasan claramente a los epibentónicos (7.5 a 1). La composición de especies de ambas comunidades son muy diferentes. En la comunidad criptobentónica no se observaron ni variaciones diarias en su composición, ni en la riqueza o abundancia de especies. La frecuencia de especies entre zonas abiertas u ocultas no cambia según la hora del día. En conjunto, estos resultados sugieren que la comunidad criptobentónica tiene una estabilidad diaria y que está formada sobre todo por especies que viven en zonas ocultas y por pequeños góbidos. Las variables del hábitat más significativas son la inclinación del fondo y la presencia de bloques, mientras la profundidad influye, pero sin ser significativa.

Palabras clave: peces crípticos; influencia diaria; análisis multivariante; uso del hábitat; bentos; zona litoral.

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INTRODUCTION

Cryptobenthic fishes most likely play an important role in coastal marine ecosystems, but large gaps of knowledge about their functionality remain (Goatley and Brandl 2017). A cryptobenthic fish is a species (or a life history stage of a fish species) whose individuals exclusively or predominantly spend their lifetime in cryptobenthic microhabitats, i.e. in the restricted living spaces underneath the bottom surface of the substrate or biocover, with a physical barrier to open spaces (according to the habitat or ecological definition of Kovačić et al. 2012). Standard methods for collecting and studying benthic fishes mostly fail to collect or record cryptobenthic fishes (Ackermann and Bellwood 2000, Willis 2001, Smith-Vaniz et al. 2006). According to the comparative study of Smith-Vaniz et al. (2006), the unrecorded biodiversity of the fish assemblage estimated by visual census was very high, as they visually recorded only 36% of 228 fish species they sampled with rotenone. It was estimated that cryptobenthic fishes (known and still undescribed species) could represent more than half of the total coral reef fish biodiversity (Brandl et al. 2018). These fishes by definition have an intimate association with the benthos and provide important food web links between the benthos and nekton communities of coral reefs, with their quantitative significance resulting from their exceptional growth rates and mortality (Goatley et al. 2016). Most quantitative ecological studies that differentially have targeted microhabitats of cryptobenthic fishes have been performed in coral reef and seagrass meadow ecosystems (Depczynski and Bellwood 2003, Ahmadia et al. 2012, Tornabene et al. 2013), a few in south temperate reefs (Prochazka 1998, Willis and Anderson 2003), and only a few in northeastern Atlantic (Beldade and Goncalves 2007) and Mediterranean warm temperate reefs (Kovačić et al. 2012, Glavičić et al. 2016, Thiriet et al. 2016). Mainly on the basis of these studies, cryptobenthic reef fishes are now considered to play a globally significant role in temperate and tropical reefs: they are numerically dominant across different cryptic microhabitats, they may serve as a key trophodynamic component between open water and seafloor habitats, and ecologically highly differentiated species assemblages evolve rapidly in different microhabitats and regions and hence may serve as sensitive environmental indicators (Goatley and Brandl 2017).

Interestingly, all aforementioned studies were based on samples taken during daytime, and virtually nothing is known about diel changes of the cryptobenthic fish assemblage. Except for highly specialized coral or sponge dwelling species, very little is known also about the autecology of most cryptobenthic fish species (Goatley and Brandl 2017).

The studies of diurnal-nocturnal activity of inshore benthic fishes started half a century ago (Hobson 1965), but crepuscular and nocturnal fish assemblages of open benthic habitats remain largely unresearched in shallow coastal environments (Azzurro et al. 2007). In the Mediterranean Sea, only a few published studies on diel variations of littoral assemblage of epibenthic and hyperbenthic fish have been performed on soft sediments down to a few metres depth by beach seine (Dulčić et al. 2004, 2005) or on rocky bottoms by visual census performed by SCUBA dives down to 3 m (Azzurro et al. 2007, 2013). Published studies clearly show diel variations of the littoral assemblage of epibenthic and hyperbenthic fish. Dulčić et al. (2004) found exclusively nocturnal and exclusively diurnal fish species and major diel differences in total fish abundance and biomass. Azzurro et al. (2007) revealed a clear-cut separation between day and night fish assemblages, with significant differences in species composition, species richness and fish abundance.

Considering the lack of any data on diel variations in cryptobenthic fish assemblages and the rarity of studies on diel shifts of other littoral benthic fishes in general, the general goal of the present study was to fill this knowledge gap, which might hide important aspects of the dynamics and relationships of coastal inshore fish assemblages. The quantitative assessment of littoral cryptobenthic fish assemblages of the shallow infralittoral (0.5-3 m depth) also complements earlier studies of littoral cryptobenthic fish assemblages performed deeper by Kovačić et al. (2012) and Glavičić et al. (2016). The present research used the cryptobenthic fish definition of Kovačić et al. (2012) based on the fish position at the bottom, so the aim was to study the fish assemblage in the hidden physical space, "inside bottom" (cryptobenthic in this work), and to compare it with the fish assemblage laying on the open bottom (epibenthic in this work). Therefore, the specimens could be cryptobenthic or epibenthic, according to the place of collection, while the species status as the sum of positions of all specimens could be exclusively or predominantly cryptobenthic, exclusively or predominantly epibenthic or ambivalent (termed epicryptobenthic by Kovačić et al. (2012)) if specimens were considerably recorded in both bottom spaces. In particular, the study was designed (1) to test whether epibenthic and cryptobenthic fish compositions differ; (2) to investigate differences in composition, species richness and abundance of cryptobenthic fish assemblages at day/night/sunset, which could be interpreted as time of day–dependent changes; (3) to test for diel shifts of infralittoral fish species between hidden and open spaces, by testing whether ambivalent species present in both hidden and open spaces exhibit significantly different epibenthic and cryptobenthic frequencies of occurrence at daylight, night and sunset; and (4) to identify environmental variables contributing significantly to the variation of the cryptobenthic fish assemblage in the shallow infralittoral zone (0.5-3 m depth), including also the time of day as an explanatory variable.

MATERIALS AND METHODS

Sampling location

The study was performed in the eastern Adriatic, on the south side of Brač island, about 1.5 km east of the city of Bol, in a small bay (43°15.543′N, 16°38.607′E) located in front of a tourist resort. The sea bottom at the study depths consisted of sand and various mixed rocky bottom types (bedrock, boulders, cobbles and pebbles) covered with sessile biota, including a variety of algae, sponges, sea urchins and sea cucumbers. Areas deeper than 3 m are mostly covered by *Posidonia* meadows. There are no strong currents at the location, underwater visibility is typically 20-25 m at water depths of less than 5 m, the position is daily exposed to the afternoon waves by the maestral wind, and the strongest wave action on this position is produced by an occasional sirocco wind.

Data collection

Data and specimens were collected by two SCUBA divers (IG and MK) during three surveys performed on 9-11 June 2015, 10-14 May 2016 and 10-13 October 2016. The number of squares performed in one dive varied from three to five depending on the time of day and other conditions, with two dives on average per day.

Dives were performed during daylight, sunset or night: 26 daylight, 25 sunset and 27 night bottom squares were sampled, giving the total of 78 sampled bottom squares of 1 m^2. The protocol for selecting squares was adopted and modified from Kovačić et al. (2012): about an equal number of squares were sampled during daylight, sunset and night and about an equal number of squares were studied at each depth in shallow water: 0.5, 1, 2 and 3 m. Underrepresented day time, depth and dominant substrate type categories at a particular moment of the fieldwork were established from the already collected data and targeted for the next dive. Therefore, in addition to daytime and depth, squares were further selected to achieve as equal as possible a representation of each dominant substrate type on each of the isobaths. To achieve this, each isobath was followed until the next not yet sufficiently sampled dominant substrate type was found, at least 10 m from the previous square (Kovačić et al. 2012). The protocol for sampling in squares was also adopted and modified from Kovačić et al., (2012): (1) visual census of the epibenthic fishes, (2) fixing of a reference frame (1×1 m) onto the bottom, (3) photography of the 1 m^2 square taken and stored as a reference for in situ estimations of percentages of habitat parameters “bottom substrate” and “biocover”, and number by size of the habitat parameter “number of semi-caves, caves and cavities”, all previously estimated in situ, (4) recording of habitat characteristics in 1 m^2 and temperature, (5) spraying of the anaesthetic into the square, catching escaping fishes with a handnet and waiting for about 2 min, and (6) removal of biocover and movable parts of the bottom structure and collection of anesthetised fishes. The anaesthetic used was Quinaldine, diluted 1:15 with 96% ethanol and then mixed 1:5 in 750 mL bottles with sea water (modified from Kovačić et al. 2012). Two bottles were usually used for one square with the total volume of deployed quinaldine-ethanol solution per square of about 300 mL/m^2. The habitat variables “bottom substrate”, “number of bottom layers”, “biocover” with categories match Kovačić et al. (2012) as follows: “bottom substrate” as five types of different estimated particle size (sand <2 mm, gravel <60 mm, cobbles <200 mm, boulders >200 mm, bedrock) expressed as estimated percentages (10%, 20%... 100%) of the total surface in each frame (1×1 m); “number of bottom layers”, i.e. no layers, single layer, multiple layers; and “biocover” as six types (no biocover, phanerogams, long thallus algae, short thallus algae, calcareous algae, zoocover). Other variables were modified: “depth” (0.5, 1, 2 and 3 m); “inclination”, as five estimated classes (gentle 0-19°=1, medium 20-59°=2, steep 60-74°=3, very steep to vertical 75-90°=4, overhanging=5); and “number of semi-caves, caves and cavities”, if present, as number of each of four estimated size classes of hidden spaces in each square by entrance size (25-99 cm^2, 100-499 cm^2, 500-1999 cm^2, >2000 cm^2). All collected specimens were killed after SCUBA dives by over-anesthetization with quinaldine and stored in 65% ethanol solution. Standard length of each fish was measured thereafter. Preliminary field species identifications of cryptobenthic specimens were later rechecked in the lab on preserved specimens.

Data analysis

Difference between epibenthic and cryptobenthic samples in species composition in total and at different times of day. Significant differences between epibenthic and cryptobenthic species compositions were tested using analysis of similarities (ANOSIM) with a Bray-Curtis dissimilarity index for the dissimilarity matrix and 999 as the number of permutations.

Differences in cryptobenthic fish assemblage species composition, total fish abundance and species richness between daylight, night and sunset samples. Similarity of species composition among studied squares was graphically represented by two-dimensional non-metric multi-dimensional scaling using the Bray-Curtis similarity matrices (Clarke 1993). Species abundance data were square-root transformed to reduce weighting given to abundant species (Clarke 1993). Significant differences between daylight, night and sunset cryptobenthic species compositions were tested with ANOSIM with a Bray-Curtis dissimilarity index for the dissimilarity matrix and 999 as the number of permutations. One-way analysis of variance (ANOVA) was used to test for significant differences in total fish abundance and species richness between daylight, night and sunset squares. Total abundance and species richness data were square-root transformed for counts per unit data (Underwood 1997). The homogeneity of variance was tested using Cochran's test on transformed data.

Difference in epibenthic and cryptobenthic frequencies of occurrence of ambivalent species between daylight, night and sunset. Significance of differences in epibenthic and cryptobenthic frequencies of occurrence of the ambivalent species between daylight, night and sunset was tested by the Fisher exact test, considering the small size of samples (McDonald 2014). The size of the tested contingency table was limited to 2x2 to fit the Fisher exact test, so three pairs of times of day were compared separately. Since row and column totals are unconditioned, the Fisher exact test is not, strictly speaking, exact. Instead, it is somewhat conservative, having a little less statistical power (McDonald 2014). Since in ambivalent species the total sum of frequencies of two categories of both groups was less than or equal to six, the data from all ambivalent species were pooled together by category and by group, and significance was tested on frequencies summed from all species. *Millerigobius macrocephalus* (Kolombatović, 1891) and *Zebrus zebrus* (Risso, 1827) had numerous specimens found in hidden spaces and single and two specimens, respectively, found on the open bottom (Table 1). Strictly speaking, they are not ambivalent species but they were also included in the test, separately from ambivalent species (Table 1).

Influence of environmental explanatory variables and time of day as an explanatory variable on cryptobenthic fish species as response variables. Community composition of cryptobenthic fishes was analysed in order to detect explanatory variables (including both the habitat variables and the times of day) that best explain species distribution and abundance patterns. Only cryptobenthic specimens from species with four or more cryptobenthic specimens were included in the analyses (modified from Glavičić et al. 2016). Therefore, only the 13 most common species were used for analysis (Table 1). Following the semantic-based approach for transformation (Šmilauer and Lepš 2014), the data were log(x+1)-transformed because we asked for the amount of change of species abundance by unit change of environmental variables. Among the 20 possible environmental variables included in the field recording sheet, 18 variables were actually recorded during fieldwork, of which one (phanerogams) with presence in just two cases was discarded; hence 17 variables were finally included in the analysis (Table 2). To reveal patterns of species response curves in relation to environmental gradients, data sets were first analysed with detrended correspondence analysis. The first axis gradient length measured in turnover (or SD units) was 4.46, indicating strong nonlinear or unimodal responses, so a canonical correspondence analysis was chosen as a constrained ordination method. Because unimodal methods cannot treat empty cases (in the present study squares with none of the 13 dominant species present), only 62 out of 78 cases were analysed. Season and year of fieldwork were introduced into the model as covariables to partial out possible confounding effects varying between fieldwork date using partial constrained analysis. The option "down-weight rare species" was not selected (Glavičić et al. 2016). The first canonical correspondence analysis was performed with all 17 environmental variables, and after forward selection 14 variables were incorporated in the final model. In order to avoid inflated significance values, three redundant predictor variables showing high multicollinearity (VIF>10) were removed from the model (temperature, bedrock and no cover) (Table 2). Significance of canonical axes and of the environmental variables was determined using Monte Carlo permutation tests (999 permutations). Significance of canonical axes was tested on the first axis and also on all axes. Significance of the environmental variables is the significance of the conditional (partial) effect of each variable after the forward selection procedure.

The Fisher exact test, ANOSIM and ANOVA were performed in R software version 3.3.3 using the RStudio 1.0.143 integrated development environment (IDE) for R and packages Vegan version 2.4-4., Outliers version 0.14 and Stats version 3.4.1. For the non-metric multi-dimensional scaling, detrended correspondence analysis and canonical correspondence analysis, the Canoco 5 computer package was used (Ter Braak and Šmilauer 2012).

RESULTS

Diversity, abundance and frequency of cryptobenthic and epibenthic fish

The total number of recorded cryptobenthic and epibenthic fish species in the bottom squares was 27 (Table 1). Epibenthic specimens belonged to 13 species and cryptobenthic specimens to 22 species, with eight shared species occurring in both space layers. Among those eight species, *M. macrocephalus* and *Z. zebrus* had predominantly cryptobenthic specimens (98% and 95.1%). The family Gobiidae dominated fish diversity with 14 species, which is more than a half of all recorded species, followed by the family Blenniidae with six species, while the remaining six families were restricted to one or two species (Table 1). Cryptobenthic fish, with 231 individuals collected, highly outnumbered the 31 recorded epibenthic individuals. The average total abundance of cryptobenthic specimens in the bottom squares was 2.92±0.33 individuals/m^2 (mean±SE), with a maximum of 13 individuals/m^2. The average total abundance of epibenthic specimens in the bottom squares was 0.38±0.8 individuals/m^2 (mean±SE), with a maximum of 3 individuals/m^2. The 13 most abundant cryptobenthic species (species with four or more cryptobenthic individuals collected) represented 92.6% of all cryptobenthic individuals and the 5 most abundant species collectively made up 74.0% of all cryptobenthic individuals. The only species with numerous epibenthic individuals recorded was *Scorpaena porcus* Linnaeus, 1758, with all 11

Table 1. – Species occurrence data from the bottom squares of 1 m^2 (n=78), showing recorded species with the number of cryptobenthic (CB) (n=231), epibenthic (EB) (n=31) and total specimens, the percentage of CB specimens and the frequency of species occurrence in the squares. Species in bold letters (species with cryptobenthic occurrence with ≥4 individuals) are included in detrended correspondence analysis and canonical correspondence analysis.

Family	Species (abbrevation)	CB specimens	EB specimens	Total	CB % of total	CB frequency of occurrence	EB frequency of occurrence
Gobiidae	***Corcyrogobius liechtensteini* (Cor lic)**	51	0	51	100.0%	20.5%	0.0%
Gobiidae	***Millerigobius macrocephalus* (Mil mac)**	50	1	51	98.0%	37.2%	1.3%
Gobiidae	***Zebrus zebrus* (Zeb zeb)**	39	2	41	95.1%	32.1%	2.6%
Pomacentridae	***Chromis chromis* (Chr chr)**	17	0	17	100.0%	12.8%	0.0%
Gobiidae	***Chromogobius zebratus* (Chr zeb)**	14	0	14	100.0%	16.7%	0.0%
Scorpaenidae	*Scorpaena porcus* (Sco por)	0	11	11	0.0%	0.0%	12.8%
Tripterygiidae	***Tripterygion melanurum* (Tri mel)**	6	3	9	66.7%	7.7%	3.8%
Gobiidae	***Gobius bucchichi* (Gob buc)**	4	3	7	57.1%	5.1%	2.6%
Gobiidae	***Gobius incognitus* (Gob inc)**	6	1	7	85.7%	7.7%	1.3%
Blenniidae	***Parablennnius zvonimiri* (Par zvo)**	7	0	7	100.0%	9.0%	0.0%
Gobiidae	***Gobius fallax* (Gob fal)**	6	0	6	100.0%	3.8%	0.0%
Blenniidae	***Parablennnius incognitus* (Par inc)**	5	1	6	83.3%	6.4%	1.3%
Gobiesocidae	***Lepadogaster lepadogaster* (Lep lep)**	5	0	5	100.0%	5.1%	0.0%
Gobiidae	***Chromogobius quadrivittatus* (Chr qua)**	4	0	4	100.0%	5.1%	0.0%
Blenniidae	*Parablennnius rouxi* (Par rou)	3	1	4	75.0%	2.6%	1.3%
Gobiidae	*Pomatoschistus bathi* (Pom bat)	0	4	4	0.0%	0.0%	2.6%
Gobiidae	*Odondebuenia balearica* (Odo bal)	3	0	3	100.0%	3.8%	0.0%
Gobiidae	*Didogobius splechtnai* (Did spl)	2	0	2	100.0%	2.6%	0.0%
Lottidae	*Gaidropsarus mediterraneus* (Gai med)	2	0	2	100.0%	2.6%	0.0%
Gobiidae	*Gobius paganellus* (Gob pag)	2	0	2	100.0%	2.6%	0.0%
Blenniidae	*Parablennnius gattorugine* (Par gat)	2	0	2	100.0%	2.6%	0.0%
Tripterygiidae	*Tripterygion tripteronotum* (Tri tri)	1	1	2	50.0%	1.3%	1.3%
Gobiidae	*Gobius cruentatus* (Gob cru)	0	1	1	0.0%	0.0%	1.3%
Gobiidae	*Gobius geniporus* (Gob gen)	0	1	1	0.0%	0.0%	1.3%
Blenniidae	*Microlipophrys adriaticus* (Mic adr)	1	0	1	100.0%	1.3%	0.0%
Blenniidae	*Parablennnius sanguinolentus* (Par san)	0	1	1	0.0%	0.0%	1.3%
Sciaenidae	*Sciaena umbra* (Sci umb)	1	0	1	100.0%	1.3%	0.0%

Table 2. – Environmental variables including all possible variables, with the unrecorded variables in italics letters. The simple (marginal) effect is the amount of variability in the response data that is explained by a constrained ordination model using that variable as the only explanatory variable. The conditional (partial) effect is the additional amount of explained variance in species distribution of the corresponding variable when 14 environmental variables are selected by "blind" forward selection in Canoco 5. The variation in species distribution explained (Explains%) by 14 environmental variables included in the final model by the forward selection procedure are represented with pseudo-F value and P for conditional effect. The categorial variables part of day and number of layers were decomposed in Canoco 5 into daylight, sunset and night and into no layers, one layer and multiple layers, respectively. Environmental variables with a significant conditional effect (p≤0.05) are in bold.

Environmental variable	Simple effects	Conditional effects		
	Explains%	Explains%	pseudo-F	P
Time of day: daylight	2.2	1.6	1.1	0.351
Time of day: sunset	1.2	1.6	1.1	0.32
Time of day: night	2.1	1.9	1.3	0.211
Depth	6.5	2.5	1.7	0.091
Temperature	n/a	n/a	n/a	n/a
Inclination (as five estimated classes)	**5.4**	**4.5**	**12.9**	**0.002**
Sand (%)	1.2	0.3	0.2	0.993
Gravel (%)	1.4	1.0	0.7	0.725
Cobbles (%)	**6.3**	**3.6**	**2.3**	**0.008**
Boulders (%)	3.6	1.0	0.7	0.762
Bedrock (%)	n/a	n/a	n/a	n/a
Number of layers: no layers	3.6	2.3	1.6	0.083
Number of layers: one layer	2.8	2.3	1.6	0.115
Number of layers: multiple layers	**6.7**	**6.7**	**4.2**	**0.001**
Phanerogams (%)	n/a	n/a	n/a	n/a
Short tallus algae (%)	1.8	1.1	0.7	0.693
Long tallus algae (%)	n/a	n/a	n/a	n/a
Calcerous algae (%)	3.2	0.5	0.3	0.971
Zoo cover (%)	2.5	1.6	1.1	0.352
No cover (%)	n/a	n/a	n/a	n/a
Number of hidden spaces of entrance size 25-99 cm^2	1.2	2.3	1.6	0.118
Number of hidden spaces of entrance size 100-499 cm^2	3.4	1.5	1.0	0.404
Number of hidden spaces of entrance size 500-1999 cm^2	1.8	1.7	1.2	0.26
Number of hidden spaces of entrance size >2000 cm^2	n/a	n/a	n/a	n/a

individuals recorded on open bottoms, and it is also the only species with just epibenthic specimens recorded. Other species with epibenthic specimens had, in addition to collected cryptobenthic individuals, one to three epibenthic specimens recorded per species. Species of the family Gobiidae contributed to fish abundance with 78.4% of all cryptobenthic individuals sampled and the second most abundant family, Blennidae, with 7.8%. The five most numerous species with cryptobenthic individuals were also the most frequent species in

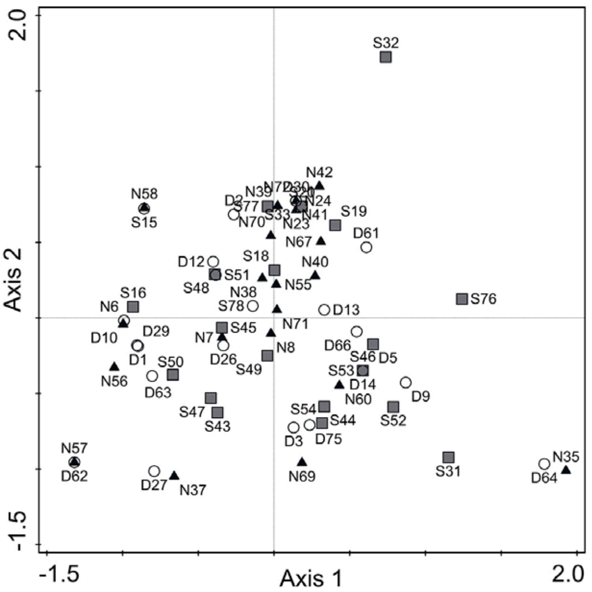

Fig. 1. – Non-metric multi-dimensional scaling ordination plot of individual squares comparing diurnal (hollow circles), sunset (grey quadrats) and nocturnal (black triangles) cryptobenthic fish assemblage species composition.

squares, ranked in presence from about 1/8 to about 2/5 of all squares (Table 1).

Comparison of cryptobenthic and epibenthic fish assemblages

Cryptobenthic and epibenthic fish assemblages showed a significantly different species composition for all data (ANOSIM R=0.2949; p=0.001) and also for each time of day: at daylight (ANOSIM R=0.22; p=0.006), at sunset (ANOSIM R=0.3978; p=0.003) and at night (ANOSIM R=0.3221; p=0.001). Even the qualitative data alone showed the clear difference between the two assemblages, with a limited number of shared or ambivalent species (Table 1). Among species recorded in the cryptobenthic space, 63.6% were exclusively cryptobenthic, and among species recorded in the epibenthic space, 38.5% were exclusively epibenthic.

Diel influence on the cryptobenthic fish assemblage

There was no separation on the non-metric multi-dimensional scaling ordination plot between the diurnal, sunset and nocturnal cryptobenthic fish assemblage species composition (stress=0.063) (Fig. 1). The cryptobenthic fish assemblage showed no significantly different species composition between daylight, night and sunset samples in total (ANOSIM R=0.01; p=0.274) and in pairwise comparison of daylight vs. night (ANOSIM R=0.04432; p=0.114), daylight vs. sunset (ANOSIM R=–0.01622; p=0.674) and night vs. sunset (ANOSIM R=0.00715; p=0.334). In addition, R values were close to 0, meaning that there was no separation between levels of the time of the day, so this factor had not just no significance but also no detectable effect at all on cryptobenthic fish assemblage species composition.

Further, one-way ANOVA did not detect differences between different times of day either for species richness of cryptobenthic fish assemblage (d.f.=2, MS=0.3323, F=1.568, p=0.215) or for total fish abundance of the cryptobenthic fish assemblage (d.f.=2, MS=0.7072, F=1.507, p=0.228).

Diel variation of ambivalent species presence in open and hidden spaces

The data for all ambivalent species were combined, and significance was tested on combined frequencies with the Fisher exact test. No significantly different epibenthic and cryptobenthic frequencies of occurrence were found in any data subset between daylight and night, between night and sunset or between daylight and sunset (Table 3), meaning that with the available data it was not possible to identify any significant switch from open to hidden places or vice versa for ambivalent species between times of day. *M. macrocephalus* and *Z. zebrus*, two species with very few epibenthic records, had sufficiently large frequencies at different time of day to be tested separately with the Fisher exact test and also showed no significance difference, i.e. no evidence of utilization of hidden and open spaces at different times of day was established (Table 3).

Important habitat variables structuring the cryptobenthic fish assemblage

The first partial canonical correspondence analysis was performed with 17 environmental variables on 13 species as response variables (Fig. 2A). The Monte Carlo permutation test was statistically significant on the first axis and all other axes (Table 4A). The cumulative percentage of variance in the response

Table 3. – The frequencies of cryptobenthic and epibenthic specimens at the different times of day, summed together for ambivalent species and separately for the predominantly cryptic *Millerigobius microcephalus* and *Zebrus zebrus,* with the results of the Fisher exact test on these frequencies at the different times of day.

	All ambivalent species summed together		*M. macrocephalus*		*Z. zebrus*	
Frequencies	cryptobenthic	epibenthic	cryptobenthic	epibenthic	cryptobenthic	epibenthic
Daylight	9	6	7	0	12	0
Night	8	3	23	1	8	2
Sunset	8	1	20	0	19	0
Fisher exact test	p-value		p-value		p-value	
Daylight vs. night	0.6828		1		0.1948	
Night vs. sunset	0.5913		1		0.1108	
Daylight vs. sunset	0.1907		1		1	

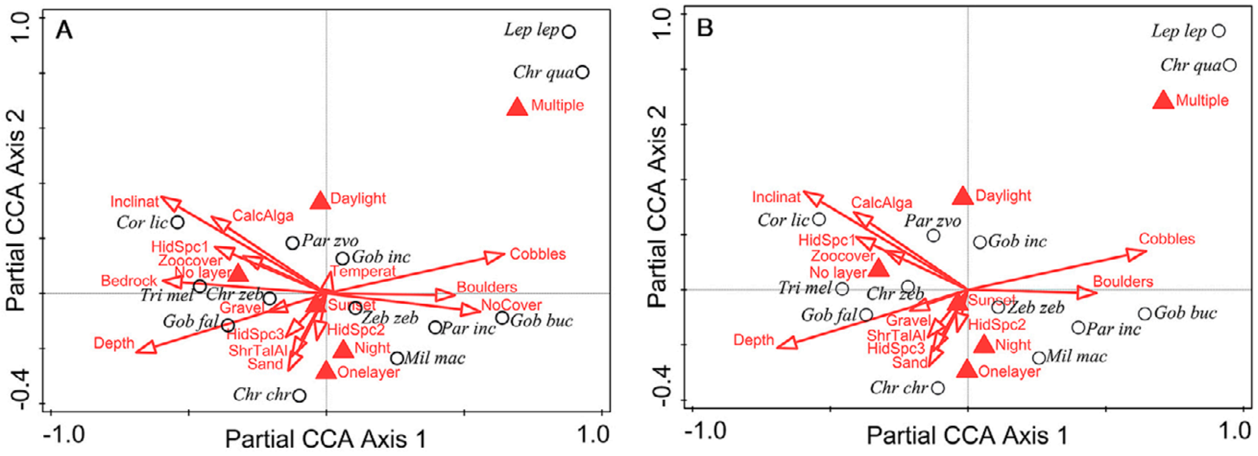

Fig. 2. – Canonical correspondence analysis species–environment biplot for 17 (A) and 14 (B) predictors (with a focus on response variable distances and biplot scaling) based on analysis of 62 cases with data on abundance of 13 fish species. Summary statistics of the biplot are presented in Table 4. Quantitative environmental variables are represented by red arrows, and each category of factors is indicated by a full red triangle, both with abbreviations in regular font. The abbreviations for explanatory variables: Temperat, temperature; Inclinat, inclination; Onelayer, one layer; Multiple, multiple layers; ShrTalAl, short tallus algae; CalcAlga, calcerous algae; Zoocover, zoo cover; NoCover, no cover; HidSpc1, number of hidden spaces of entrance size 25-99 cm^2; HidSpc2, number of hidden spaces of entrance size 100-499 cm^2; HidSpc3, number of hidden spaces of entrance size 500-1999 cm^2. Hollow circles and abbreviations in italics represent fish species. The abbreviations for fish species are explained in Table 1.

data explained by the first four constrained axes was 25.12%, and the first four axes explained 72.83% of variation in the response data explained by explanatory variables, i.e. of the variation of fitted response values (Table 4A). Total variation and partial variation of all eigenvalues are presented in Table 4. The model with 14 variables (after the forward selection procedure excluded redundant variables of temperature, bedrock and no cover) showed slightly lower cumulative explained variation and slightly higher cumulative fitted explained variation than the model with 17 environmental variables (Fig. 2B, Table 4B). Canonical eigenvalues were also slightly lower from the forward selection procedure (Table 4), resulting in explanatory variables accounting for 34.0% of partial variation vs. 34.5% in the model with 17 environmental variables. The conditional effect of daylight, night and sunset in the forward selection procedure was not significant in explaining species response variables (Table 2). Even simple effects of each time of day were not significant (daylight P=0.229, night P=0.257 and sunset P=0.77). Therefore, the time of day did not reveal a significant correlation with cryptobenthic fish composition and abundance. The forward selection identified three highly significant variables: multiple layers, inclination and cobbles (Table 2), all others being non-significant. After forward selection, the three highly significant variables explained 14.8% of total variation excluding the effects of the covariates, contributing 42.6% to the variation explained by the complete set of explanatory variables. Among highly significant variables, the first axis was positively correlated with inclination and negatively correlated with cobbles, while a further positive correlation was detectable with another quantitative predictor of a large but not significant effect, depth (Fig. 2, Table 2). The projection of the signifi-

Table 4. – Summary statistics of partial canonical correspondence analysis on the distribution of the 13 most common cryptobenthic fishes over 62 sample sites in relation to (A) 17 and (B) 14 environmental variables.

A Total variation=4.76 All eigenvalues=4.51092 Canonical eigenvalues=1.55566	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.5083	0.2830	0.1879	0.1538
Explained variation (cumulative)	11.27	17.54	21.71	25.12
Pseudo-canonical correlation	0.9045	0.6871	0.7137	0.6175
Explained fitted variation (cumulative)	32.67	50.87	62.95	72.83
Monte Carlo test (P-value, 999 permutations)	0.007		All axes=0.023	
B Total variation=4.76 All eigenvalues=4.51092 Canonical eigenvalues=1.5333	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.5064	0.2789	0.1856	0.1537
Explained variation (cumulative)	11.23	17.41	21.52	24.93
Pseudo-canonical correlation	0.9018	0.6822	0.7125	0.6156
Explained fitted variation (cumulative)	33.03	51.22	63.32	73.34
Monte Carlo test (p-value, 999 permutations)	The significance of conditional (partial) effect of each variables are reported in Table 2			

cant explanatory factor variable multiple layers onto the arrow lines of cobbles and boulders shows high average value of these quantitative explanatory variables in cases of the multiple layers class (Fig. 2). The most notable small distance between species point and the centroid of the explanatory factor variable predicting high relative species abundance in the class is that of *Lepadogaster lepadogaster* (Bonnaterre, 1788) and *Chromogobius quadrivittatus* (Steindachner, 1863) to multiple layers. The projections of species points of *Corcyrogobius liechtensteini* (Kolombatović, 1891) to inclination and of *Gobius bucchichi* Steindachner, 1870 to boulders show species optima at high values of these quantitative explanatory variables. On the other hand, *L. lepadogaster* and *C. quadrivittatus* species optima are at very small values of depth. The species optima estimated by the calibration variable arrow tool was inclination between steep and very steep to vertical for *C. liechtensteini*, more than 30% of boulders cover for *G. bucchichi* and less than 1 m depth for *L. lepadogaster* and *C. quadrivittatus*.

DISCUSSION

Overall species composition of cryptobenthic and epibenthic fish assemblages differed significantly. This overall differentiation remained stable over the course of the day. In the explicit testing for diel changes in species composition, abundance and richness of the cryptobenthic fish assemblage and for diel changes in habitat choice of ambivalent species, in our study we detected no significant differences. These results suggest that the cryptobenthic fish assemblage has diel stability and that it is mostly composed of permanent inhabitants of hidden spaces with a numerical dominance of miniature gobies. We can hypothesize that, having temporal stability, in the bottom hidden spaces there is a permanent resident cryptobenthic fish community distinct from the epibenthic fish community, i.e. that which is on the bottom surface above them. If species composition in cryptobenthic habitats had drastically changed over different times of day, that would indicate that hidden spaces are mostly daytime shelters inhabited in circadian rhythm mostly by non-strictly cryptobenthic and specialized fishes. This cryptobenthic fish community inhabits various hidden bottom microhabitats and is shaped, according to the results of the present study, mainly by the number of layers present in their respective habitats (multiple layers), bottom inclination, type of layer particles (cobbles) and depth.

Cryptobenthic and epibenthic fish communities in the present study were dominated by gobiid species (14 species) and blennies (Blenniidae, six species), while other families were represented each by only one or two species (Table 1). Cryptobenthic and epibenthic fish communities exhibited a significantly different species compositions across all samples. About two thirds of the species recorded in cryptobenthic position were exclusively cryptobenthic, and more than one third of the species recorded in epibenthic position were exclusively epibenthic. The four most frequent gobiid species, i.e. *C. liechtensteini*, *M. macrocephalus, Z. zebrus* and *C. zebratus*, had exclusively or predominantly cryptobenthic specimens, with no evidence for regular or significant habitat switches between cryptobenthic and epibenthic layers (Table 1). Similar to the findings of comparable studies, more than half the specimens sampled in our study belonged to the three most frequent species, all three gobies (Gobiidae) of minute size (Kovačić et al. 2012, Glavičić et al. 2016, Goatley et al. 2016). Among those five most frequent species, *C. liechtensteini*, *Z. zebrus* and *Chromogobius zebratus* were confirmed to be cryptobenthic and abundant, as found by Kovačić et al. (2012) and Glavičić et al. (2016), whereas *M. macrocephalus* was absent or poorly represented in earlier studies due to the deeper depth ranges targeted by Kovačić et al. (2012) and Glavičić et al. (2016). The fourth most frequent species, *C. chromis*, was a damselfish (Pomacentridae), whose adults are known to be hyperbenthic to benthopelagic (Abel 1961). The exclusively cryptic sample of *C. chromis* in the present study confirms previous findings on use of cryptic spaces of this species, which is otherwise present in the water column. The use of cryptic spaces of this species was first found for juvenile fish at night-time (Harmelin 1987), and it was recently also reported in cryptic spaces in daylight (Kovačić et al. 2012, Glavičić et al. 2016). The species is obviously a switcher between hidden spaces and the water column. Since the present method did not sample the water column, the study revealed the exclusively cryptic sample of *C. chromis*. Specimens of all five dominant fish species accounted for approximately 75% of all sampled individuals, whereas the remaining approximately 25% of specimens belonged to 22 species. Their low frequency precluded statistical testing for positive species-specific assignations to hidden or open bottom spaces, with the notable exception of *S. porcus* (Scorpaenidae), whose 11 specimens were exclusively found in epibenthic positions. Cryptobenthic individuals highly outnumbered epibenthic individuals, with a ratio of 7.5, matching results of earlier studies in the Mediterranean Sea (Kovačić et al. 2012: 7.8, Glavičić et al. 2016: 3.6). This further supports the significance of cryptobenthic fishes for the benthos in particular and for the complete ecosystem of infralittoral and circalittoral bottoms if they are rich in cryptic spaces.

Our results highlight the overall importance of diel day/night analyses in fish community studies. To confirm the general temporal stability of the cryptobenthic fish assemblage and complement present day/night analyses, we suggest additional studies on the seasonal changes in cryptobenthic fish assemblages. Future researches should also have alternative approaches to study the cryptobenthic fish community and the trophodynamic functionality of small hidden spaces on sea bottoms. There are virtually not data on the ecological significance of small cryptic spaces on Mediterranean bottoms. The composition, abundance and functioning of sessile and mobile cryptobenthic invertebrates and fishes in these habitats are unknown. Though tropical studies of cryptic spaces are very rare and hard to perform, Scheffers et al. (2003) estimated that the cryptic space volume could account for 30%-75% of the total coral reef volume; and coelobites, i.e. cryptobenthic organisms living in these spaces, have been calculated to be responsible for phytoplankton removal equivalent to 22% of the gross community metabolism of the entire reef (Richter et al. 2001). To shed light on the ecology of small benthic cryptic spaces on Mediterranean bottoms and the role of cryptobenthic fishes inhabiting them, a comparative stable nitrogen and carbon isotopic study within and across open and hidden bottom spaces in the Adriatic Sea is now being undertaken by a team of researchers, including one of the present authors (M.K.). The stomach content analysis of night and day in situ samples of dominant cryptobenthic fish species is also one of the possible directions of research into the problem of where and how these species are included in the trophic web. Targeted in situ or aquarium observations of these species would further help to understand their autecology and foraging behaviour, and thus help to confirm indirect observations such as those presented here. Our quantitative day/night analyses of cryptobenthic fish community composition patterns is just the first step in research aimed at understanding the processes occurring in the small hidden spaces of Mediterranean littoral bottoms and the role of cryptobenthic fish species in these processes.

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Underwater Visual Census of Deeper Vertical Rocky Reefs

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Abstract

Diver-based underwater visual census (UVC), particularly transect-based survey, is a widely used method for the study of tropical and temperate fish assemblages. However, due to logistical constraints associated with conventional SCUBA diving, deeper habitats, such as vertical rocky reefs, are rarely studied and poorly known. This paper describes the Deep Vertical Transect (DVT) method as a safe and effective method for assessing fish in waters up to 50 m of depth. It is based on sampling of vertical transects browsing within it (S-type transect) by divers using Full HD video cameras. The diving profile includes the use of deep decompression stops and Nitrox 50 as a decompression gas. Hence, the study yields information on fish assemblages associated with deeper vertical coralligenous reefs. The results of 51 recorded species, yielded 41 considered as reef-associated and 10 as occasional. This suggests that underwater steep coralligenous reefs are marine biodiversity hotspots. They may be considered to represent a distinctive marine subecosystem, possessing its own food chain, with the depth, in relation to temperature, as the most important factor responsible for the diversity of fish assemblages within this habitat.

Introduction

The most common observational method for studying fish assemblages and habitats is underwater visual census (UVC). At first, UVC has been developed for use in tropical coral reef fish studies (Brock, 1954). Nowadays, the application of UVC is widespread in temperate waters such as the Mediterranean Sea, and in many Marine Protected Areas (MPAs), as well as in other non-protected but important marine habitats, e.g. rocky reefs, submarine caves and seagrass meadows (Falcon, Bortone, Brito, & Bundrick, 1996; Tunesi, Molinari, Salvati, & Mori, 2006; Bussotti & Guidetti, 2009). A variety of UVC methods have been developed but those focusing on reef fishes are mainly based on use of SCUBA divers conducting a survey, due to their better ability to search within such complex habitat compared to a stationary camera used by different remote underwater video methods (Colton & Swearer, 2010). The main disadvantage of SCUBA diving is depth restriction because of safe diving considerations. The build-up of residual nitrogen in the divers blood dictates short maximum dive times. Furthermore, as the dive depth increases the allowable dive time decreases and requires a sufficient no-dive recovery period between dives. Thus, most of the studies have been constrained to depths of 3-25 m, rarely exceeding 30 m (Fasola, Canova, Foschi, Novelli, & Bressan, 1997; Gül *et al*, 2011, Quimpo *et al,* 2018). Consequently, traditional UVC methods are not applicable to the deeper, steep and vertical slopes of Mediterranean rocky reefs (Warnock, Harvey, & Newman, 2016) that are characterized by habitat complexity and heterogeneity (Bussotti & Guidetti, 2009). Therefore, there is an ongoing need for a fish assemblages sampling method at deeper depths that is safe and efficient for divers using SCUBA equipment.

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Underwater steep rocky reefs are crucial contributors to ecosystem functioning as physical complexity of such reef habitats (i.e. rugosity, slope, number and size of boulders) is positively related to species richness and abundance of fish (Garcia-Charton & Perez-Ruzafa, 2001). In the Adriatic, as well as across the Mediterranean Sea, there are long stretches of calcarenitic rocky coast characterized by the presence of many steep and vertical slopes covered by coralligenous formations, which are complex of biocoenoses rich in biodiversity. They are defined as a hard substrate of biogenic origin mainly produced by the accumulation of calcareous encrusting algae growing in dim light conditions (Ballesteros, 2006). Previous studies confirm the role of rocky habitat for the diversity of coastal fish assemblages (Piazzi, Cecchi, & Serena, 2012) which have a key role in coastal habitats due to their economic value and ecological importance. Thus, the knowledge of their diversity patterns is fundamental for the management of coastal ecosystems (Smith, Ajani, & Roberts, 1999). Moreover, depth, together with morphological habitat characteristics, is considered an important factor to determine the diversity of fish assemblages (Lorance, Souissi, & Uiblein, 2002; Piazzi *et al*. 2012). Steep and vertical rocky reefs usually extend to depths deeper than 30 m. Field transects are the most widely used survey method of UVC (Lam *et al,* 2006) and are based on sampling of horizontal areas, instead of vertical. Consequently, currently there are no descriptive or comparative studies on fish assemblages associated with deeper vertical reefs in the Mediterranean.

The reason for depth restrictions in conventional SCUBA diving derives from limitations in commonly used existing technology. Most divers worldwide are using open circuit diving gear with compressed air as breathing gas. Over the past years, an increasing number of divers have been experimenting with mixed-gas diving technology, to extend the depth limits of conventional SCUBA. Mixed-gas and closed-circuit rebreather gear have been used previously in sampling coral-reef-associated fishes and habitats at deeper depths (Pyle, Earle, & Greene, 2008; Glavičić & Kovačić 2016; Glavičić, Paliska, Soldo, & Kovačić, 2016). However, such a technique can be classified more as a random method than widely used and comparable transect method, as, to date, most of the research coming out of these deep reef (mesophotic) surveys have focused on biodiversity surveys including the collection of species rather than targeted surveys to describe communities using UVC methods. Additionally, disadvantages of using mixed-gas and closed-circuit rebreather is the equipment high cost and requirement for extensive advanced training. This restricts the application to a small group of highly trained scientific divers.

Thus, the aim of this study was: 1) to describe new transect method of UVC designed for deeper and vertical areas, named Deep Vertical Transect (DVT) method, that has to be low cost and usable by a wide diving population; 2) to describe the abundance and diversity of fish assemblages across different depth isobaths in the Adriatic Sea associated with deeper vertical coralligenous reefs.

Materials and Methods

In recent years there is an increasing trend of using technical breathing gases such as nitrox (a mixture of oxygen and nitrogen) instead of air for all kinds of dives to extend diving time and accelerate decompression (Brebeck *et al*. 2018). However, despite the positive effects of nitrox on decreased nitrogen loading during compression that acts to decrease venous gas bubbling, diving with nitrox at deeper depths is associated with exposure to higher oxygen partial pressures which causes oxygen toxicity syndrome. Thus, diving with solely nitrox is actually decreasing the depth that can be achieved by air diving.

Both authors have been enrolled actively in studies performed by the University of Split School of Medicine aimed to compare in detail various effects of diving on human physiology, especially to compare the effects of air and nitrox dives using the same diving profiles. Results from these studies (Marinović *et al*, 2012), showed significant benefits of nitrox dives compared to air dives. Thus, the authors decided to use a combination of compressed air, as the main breathing gas, and Nitrox50 (a mixture of 50% oxygen and 50% nitrogen) as a decompression gas. Hence, authors also decided to incorporate deep decompression stops of two minutes into the method as such a procedure is recommended for decreasing bubbling (Bennett *et al*, 2007).

The DVT method was tested at the underwater rocky reef in the area of the island of Hvar channel, central eastern Adriatic (43^{O}14’02” N, 16^{O}34’29” E). The study was carried out during sunny days from mid-June until the end of August 2017. Each dive started around 1 p.m., in order to have the best possible light conditions. The reef is 300 m from the coastline, is elliptically shaped with a truncated and oblique cone with the highest reef point of 9.1 m and deepest at 60.7 m. Reef cover is coralligenous and it is surrounded by a sandy bottom. A large part of the reef has a high slope. For the targeted study area, a section was chosen in the SE-NW direction with a slope of a 90 degrees angle and the deepest point at 50 m of depth. After measuring the reef by transect tape and depth gauge the study area from surface to 50 m was divided into 10 vertical transects. Each transect was 50 m in length and 5 m in width (depth/height), which correspond to commonly used transect surfaces (Garcia-Charton & Perez-Ruzafa, 2001).

The DVT method was performed by two divers, both with more than 10 years of experience in the identification of marine fish during diving. The experience has been shown to significantly improve the results (Bernard, Gotz, Kerwath, & Wilke, 2013; Quimpo *et al,* 2018). Both divers used the same diving computers with the possibility of storing depth and temperature data. A total of 24 dives were conducted. First 3 initial dives were used for measuring the reef geometry. After the study area was mapped, 18 dives from the surface to 50 m were performed and focused on sampling fish in each of 10 transects. Divers went from the surface immediately to 50 m and started sampling from the deepest transect toward the surface (Figure 1). Since the study area was divided into 10 vertical transects, each transect was sampled 18 times using a Full HD video recording. Additionally, 3 more dives were performed using still cameras for high resolution photos of small fish species in order to have accurate identification at the species level. A common problem of different UVC methods is that direct comparison of the data collected by the two observers conducting a transect method relied on the assumptions of 1) independence of data, 2) that only fish within the survey area were counted, 3) that all these fish were equally visible to both observers, and 4) that the different species were correctly identified (Bernard *et al*, 2013). To overcome such biases, the first diver used a high definition (Full HD) video camera and instead of using conventional I-type transects (swimming in a straight line at a constant speed) swam through the transect by browsing within it (S-type transect) and varying in depth, angle and zooming when needed or notified by other diver to a small or hidden fish individuals which are usually missed if I-type transects are used (Pelletier, Leleu, Mou-Tham, Guillemot, & Chabanet, 2011). The DVT method combines 1) a use of Full HD video, as a new video standard of higher resolution and quality than standard-definition, and 2) a S-type transect. Both procedures provide more accurate and precise data. Due to the low-light level at the dive depths custom made LED lights 2x25 W, producing light temperature of 6400 °K (sunlight color temperature) were used during video recording for easier post-identification of the species. Although the speed was not constant throughout the transect, each transect divers maintained an overall conventional speed (Watson, Harvey, Anderson, & Kendrick, 2005) and taking approximately 5 min to complete one.

As the sampling period for each depth transect was 5 min, the use of 2 min deep decompression stops were performed at an average of 18 m. This did not interfere with the sampling protocol as it was incorporated within the overall 5 min transect period. Hence, each diver changed the breathing gas at the 18 m depth, corresponding to the partial pressure of oxygen in Nitrox50 mixture of 1.4 bar. Based on the gained experience, it is recommended to use at least a 18 liter diving tank for compressed air and a 9 liter tank for nitrox.

Post-analysis of the recorded video was carried out on a 55"screen, using standard software that enables pausing, slow motion viewing and zooming of the images. Video analysis was conducted within 24 hours after the dives (to use information still fresh in the memory of divers if necessary) with all fish identified and counted per species. For each species observed, the frequency of occurrence *f* (the number of dives when species has been recorded) was analyzed by classifying them as 1) very frequent ($f \geq 70\%$), 2) frequent ($40\% \leq f \leq 70\%$), 3) common ($15\% \leq f \leq 40\%$) and 4) occasional species ($f \leq 15\%$) (Garcia-Charton *et al*, 2004). Depth and temperature were constantly stored by diving computer within the dive profile data with a precision of 10 cm per depth and 0.1°C per temperature.

Standardized numbers of individuals of all the species recorded at each depth were employed to construct a Bray-Curtis similarity matrix using the PRIMER multivariate statistical package (Clarke &

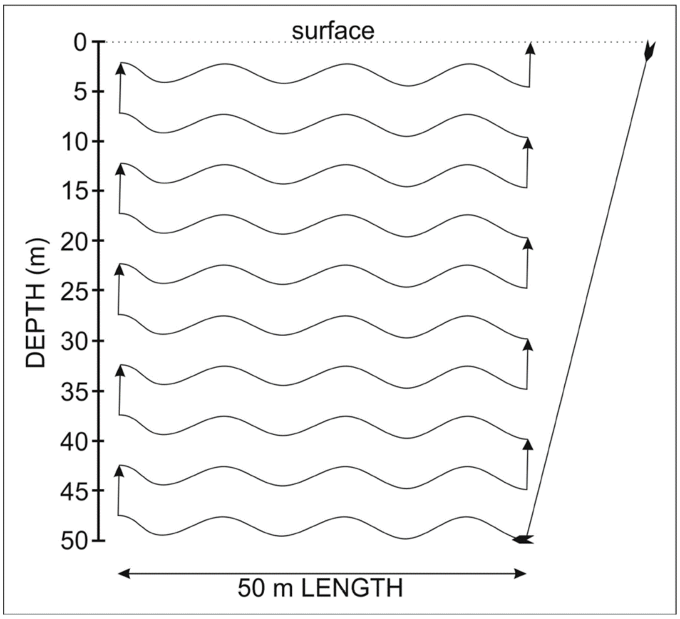

Figure 1. Sampling/diving protocol.

Gorley, 2006). The resulting rank similarity matrix was subjected to non-metric multidimensional scaling (MDS) ordination. Subsequent one-way analysis of similarity (ANOSIM) was used to test whether the species composition differed between different depths. The R-statistic value from ANOSIM for significant comparisons was used to ascertain the degree to which a priori groups of samples were dissimilar.

Similarity percentage (SIMPER) analysis was used to determine which species contributed most to any significant dissimilarities between different temperature zones. The cut-off percentage for low contributions was set at 50%.

Results

Sessile species covering each transect varied by depth and included both algae and animals, but the dominant were green algae (Chlorophyta) *Codium bursa* (Olivi) C.Agardh 1817 (from 9 to 15 m) and *Flabellia petiolata* (Turra) Nizamuddin 1987 (from 15 to 40 m), bryozoan (Bryozoa) *Pentapora fascialis* (Pallas, 1766) (from 15 to 25 m), ascidian (Ascidiacea) *Halocynthia papillosa* (Linnaeus, 1767) (from 20 to 45 m), and corals (Anthozoa) *Eunicella cavolini* (Koch, 1887) (from 20 to 35 m) and *Parazoanthus axinellae* (Schmidt, 1862) (from 30 to 50 m).

The total number of recorded fish species was 51 from 21 families (Table 1). According to the frequency of occurrence, 41 species from 15 families were categorized as reef-associated, while 10 species from 6 families were considered as occasional (Table 1). 49 fish species were identified at the species level by Full HD video, while 2 species at the genus level. However, both species were subsequently identified as *Tripterygion delaisi* Cadenat and Blache, 1970 and *Gobius kolombatovici*, Kovačić and Miller, 2000, by images taken with the high resolution photo camera. Within reef-associated species, the assemblage was taxonomically dominated by Sparidae (9 species), Labridae (6 species), Gobidae (6), Serrenidae (5), Scorpaenidae (3), Centracanthidae (3), while other 9 families were represented by only one species. The average fish abundance (number of recorded fish) per dive was 1144.3 fish. The most common species were *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817), followed by *Chromis chromis* (Linnaeus, 1758) and *Boops boops* (Linnaeus, 1758)*, Gobius auratus* Risso, 1810 and *Spicara flexuosa* Rafinesque, 1810 (Table 2).

B. boops, C. chromis, and *S. flexuosa* were the only species observed in all transects from surface to 50 m, followed by *Coris julis* (Linnaeus, 1758), *Diplodus annularis* (Linnaeus, 1758) and *D. vulgaris* which were absent from surface to 5 m depth and *G. auratus* that was observed in all transects, except in water column.

Sea temperature ranged from 17.3°C at the bottom to 27.1°C near the surface. Thermocline usually occurred from 13 to 16 m. In transects beneath the thermocline, the lowest number of species was observed. This points to the temperature as one of the most important parameters influencing the depth distribution of the species during the summer period (Figure 2). Hence, thermocline is functioning as a sort of physical barrier as 6 species were observed exclusively above it and 18 species underneath. Multivariate analysis based on the number of individuals recorded, showed statistically significant differences in species composition between all depths, except between 20-25 and 25-30 depth strata (Figure 3.). Furthermore, a statistically significant difference was also observed between different temperature zones (above and below thermocline) (ANOSIM, R= 0.667, p= 0.001) (Figure 4). The similarity percentage (SIMPER) analysis showed that *G. auratus* and *D. vulgaris* contributed most to the differences between different temperature zones (Table 3).

Discussion

Fasola *et al*. (1997) conducted one of the rare studies in the Mediterranean investigating deeper areas. They recorded 39 species from 13 families of fish during the census along 32 horizontal transect at the rocky slopes of southwestern Adriatic, from surface to 42 m of depth. Most of the species that have been observed during that study were also recorded during our study, with the exceptions of some labrids and combtooth blennies that are missing. The difference in species observed was likely due to the homogeneity of our investigated area (entirely coralligenous) compared to different investigated habitats from Fasola *et al*. (1997) study, as well as lack of substrate transects from surface to 9,1 m in our study, where these species usually occur. Regardless of habitat homogeneity and absence of shallow substrate, the total number of species recorded in our study of 10 vertical transects using DVT method is considerably higher than the number of species recorded along 32 horizontal transects by conventional visual census. There are UVC studies in the Mediterranean that resulted with higher number of recorded species, e.g. the study on the littoral fish community of the Lebanese rocky coast (Harmelin-Vivien, Bitar, Harmelin, Harmelin, & Monestiez, 2005) where 62 species were recorded, including 8 Lessepsian migrants. However, that study was performed not over single but different habitats (rock, gravel, sand, macro-algae and *Cymodocea nodosa* seagrass). Thus, our findings suggest that underwater steep rocky reefs have the highest fish biodiversity and population density than any other rocky habitats in the Mediterranean. As fishing pressure has been considered as a major stressor on Mediterranean reef systems (Sala *et al*, 2012), we can presume that one of the reasons for such high diversity and density compared to other rocky habitats is due to lower fishing pressure caused by specific topographic characteristics of the steep vertical reef preventing use of the most fishing gear. Hence, the composition of reef-associated fish species, comprised

Table 1. Fish assemblage structure according to depth transects and frequency of occurrence (FREQ. OCC.) classification (O-occasional; C-common; F-frequent; VF-very frequent).

REEF ASSOCIATED SPECIES		FREQ. OCC.	DEPTH (m)									
			0-5	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45	45-50
Pomacentridae	*Chromis chromis*	VF	+	+	+	+	+	+	+	+	+	+
Labridae	*Coris julis*	VF		+	+	+	+	+	+	+	+	+
	Symphodus melanocercus	VF		+	+	+		+			+	+
	Symphodus mediterraneaus	VF			+	+			+		+	+
	Symphodus tinca	VF		+	+							
	Labrus bimaculatus	VF								+	+	
	Thalosoma pavo	C		+	+							
Phycidae	*Phycis phycis*	F										+
Gadidae	*Merlangius merlangus*	C								+	+	+
Congridae	*Conger conger*	F										+
Blennidae	*Parablennius rouxi*	VF			+				+	+	+	+
Gobiidae	*Gobius auratus*	VF			+	+	+	+	+	+	+	+
	Gobius geniporus	VF							+			+
	Gobius cruentatus	VF							+		+	+
	Gobius bucchichi	VF									+	+
	Gobius kolombatovici	VF									+	+
	Thorogobius ephipiatus	F								+	+	+
Tripterygiidae	*Triperygion delaisi*	VF		+	+							
Serranidae	*Serranus cabrilla*	VF						+		+	+	+
	Serranus scriba	VF			+	+						
	Serranus hepatus	VF										+
	Epinephelus marginatus	F		+	+	+						
	Epinephelus costae	F		+	+	+						
Scorpaenidae	*Scorpaena porcus*	VF							+	+	+	+
	Scorpaena scrofa	VF									+	
	Scorpaena notata	VF						+	+			+
Sparidae	*Diplodus vulgaris*	VF		+	+			+	+	+	+	+
	Diplodus puntazzo	VF		+	+							+
	Diplodus annularis	VF		+	+	+	+		+	+	+	
	Oblada melanura	F		+	+							
	Sparus aurata	F		+								
	Sarpa salpa	F		+	+							
	Spondyliosoma cantharus	F								+	+	
	Lithognathus mormyrus	F									+	+
	Boops boops	VF	+	+					+	+	+	+
Mullidae	*Mullus surmuletus*	F			+							+
Centracanthidae	*Spicara flexuosa*	VF		+	+	+	+		+	+		+
	Spicara maena	F		+	+							
	Spicara smaris	VF								+	+	+
Muraenidae	*Muraena helena*	F					+	+				
Apogonidae	*Apogon imberbis*	F			+	+						
OCCASIONAL SPECIES												
Mugilidae	*Liza aurata*	O										
	Oedalachilus labeo	O										
Triglidae	*Trigloporus lastoviza*	O										
Coryphaenidae	*Coryphaena hippurus*	O										
Carangidae	*Lichia amia*	O										
	Seriola dumerili	O										
	Trachurus trachurus	O										
Scombridae	*Sarda sarda*	O										
	Thunnus thynnus	O										
Myliobatidae	*Myliobatis aquilla*	O										

Table 2. Number of fish and SD (standard deviation) per each species and each transect.

	Depth transects (m)									
Species	0-5	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45	45-50
Apogon imberbis	-	-	2.15 (0.27)	1.96 (0.31)	-	-	-	-	-	-
Boops boops	12.45 (2.89)	46.2 (12.8)	33 (6.82)	11.23 (3.02)	5.69 (1.38)	4.58 (1.27)	4.08 (0.87)	4.07 (0.74)	5.31 (1)	4.23 (0.81)
Chromis chromis	33.38 (9.29)	94.73 (22.5)	57.87 (7.16)	16.47 (2.78)	7.33 (1.21)	6.27 (1.03)	9.4 (1.87)	11.8 (2.72)	8.87 (1.52)	6.2 (0.94)
Conger conger	-	-	-	-	-	-	-	-	-	1 (-)
Coris julis	-	9.6 (0.98)	16.73 (0.9)	7.2 (0.82)	4.13 (0.64)	3.6 (0.41)	4.71 (0.7)	5.5 (1.14)	4.47 (0.61)	6.13 (1.05)
Diplodus annularis	-	7.27 (0.83)	16.33 (0.96)	7.67 (0.81)	5.07 (0.61)	2.91 (0.49)	2.5 (0.33)	2.27 (0.3)	2.43 (0.29)	2.07 (0.25)
Diplodus puntazzo	-	6.4 (0.82)	6.2 (0.55)	-	-	-	-	-	-	2 (-)
Diplodus vulgaris	-	134.93 (17.05)	87.13 (16.08)	11.79 (2.93)	4 (0.56)	3.36 (0.62)	2.71 (0.32)	3.36 (0.45)	3.93 (0.37)	4.27 (0.36)
Epinephelus marginatus	-	1.14 (0.1)	1 (-)	1 (-)	-	-	-	-	-	-
Epinephelus costae	-	1 (-)	1 (-)	-	-	-	-	-	-	-
Gobius auratus	-	-	7.6 (0.67)	13.33 (1.03)	23.4 (1.3)	25 (1.2)	24 (1.17)	12.07 (1.41)	6.6 (0.31)	6 (0.57)
Gobius bucchichi	-	-	-	-	-	-	-	-	5.93 (0.46)	9.13 (0.49)
Gobius cruentatus	-	-	-	-	-	-	1.67 (0.21)	1.93 (0.2)	1.64 (0.13)	1.38 (0.26)
Gobius geniporus	-	-	-	-	-	-	1.25 (0.16)	1.4 (0.16)	1.93 (0.25)	2.6 (0.21)
Gobius kolombatovici	-	-	-	-	-	-	-	-	1.53 (0.17)	2.33 (0.13)
Labrus bimaculatus	-	-	-	-	-	-	-	-	2.07 (0.07)	1.73 (0.12)
Lithognathus mormyrus	-	-	-	-	-	-	-	-	1.77 (0.28)	1.5 (0.5)
Merlangius merlangus	-	-	-	-	-	-	-	-	1.37 (0.14)	1 (-)
Mullus surmuletus	-	-	-	-	-	-	-	-	-	4.47 (0.31)
Muraena helena	-	-	-	-	1 (-)	1 (-)	-	-	-	-
Oblada melanura	-	9.13 (1.66)	6.1 (0.8)	-	-	-	-	-	-	-
Parablennius rouxi	-	-	3 (0.91)	-	-	-	2.8 (0.53)	3.36 (0.53)	3.79 (0.42)	4.2 (0.2)
Phycis phycis	-	-	-	-	-	-	-	-	-	1.07 (0.07)
Sarpa salpa	-	7.47 (0.29)	5 (0.5)	-	-	-	-	-	-	-
Scorpaena notata	-	-	-	-	-	1.3 (0.21)	1.53 (0.13)	-	-	2.27 (0.21)
Scorpaena porcus	-	-	-	-	-	-	1.36 (0.13)	1.14 (0.1)	1.6 (0.16)	2.2 (0.17)
Scorpaena scrofa	-	-	-	-	-	-	1 (-)	1 (-)	1 (-)	-
Serranus cabrilla	-	-	-	-	-	1.67 (0.33)	1.83 (0.4)	1.89 (0.2)	2.13 (0.27)	2.87 (0.29)
Serranus hepatus	-	-	-	-	-	-	-	-	-	2.73 (0.18)
Serranus scriba	-	5.27 (0.76)	7.8 (0.47)	4.14 (0.91)	-	-	-	-	-	-
Sparus aurata	-	2.33 (0.3)	1 (-)	-	-	-	-	-	-	-
Spicara flexuosa	13.57 (2.09)	21.33 (3.3)	17.47 (1.85)	4.31 (0.78)	4.4 (1.11)	3 (0.94)	2.85 (0.54)	7.31 (2.47)	5 (1.47)	4.69 (0.96)
Spicara maena	-	5.33 (0.61)	4.18 (0.62)	-	-	-	-	-	-	-
Spicara smaris	-	-	-	-	-	-	3.33 (1.45)	4.44 (0.73)	8.2 (1.19)	13.4 (1.41)
Spondyliosoma cantharus	-	-	-	-	-	-	-	1.5 (0.34)	1.71 (0.27)	-
Symphodus mediterraneaus	-	-	2.33 (0.27)	1.93 (0.3)	-	-	1.6 (0.27)	1.4 (0.22)	1.6 (0.24)	2.2 (0.47)
Symphodus melanocercus	-	1.33 (0.33)	1.27 (0.21)	1.53 (0.22)	-	-	1.38 (0.18)	1.11 (0.11)	1.07 (0.07)	-
Symphodus tinca	-	9.13 (0.6)	8.73 (0.72)	-	-	-	-	-	-	-
Thalosoma pavo	-	2.05 (0.3)	2.07 (0.20)	-	-	-	-	-	-	-
Thorogobius ephipiatus	-	-	-	-	-	-	1 (-)	1.27 (0.12)	1.27 (0.12)	1.07 (0.07)
Triperygion delaisi	-	3 (0.17)	2.4 (0.34)	-	-	-	-	-	-	-

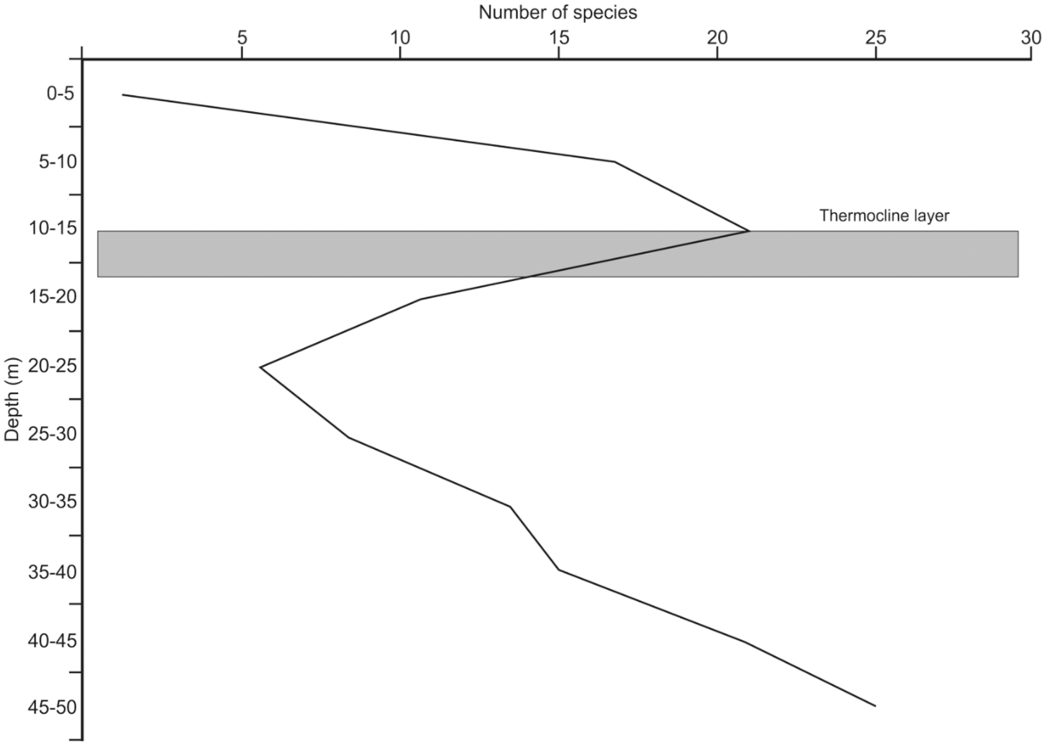

Figure 2. Number of species per each depth transect.

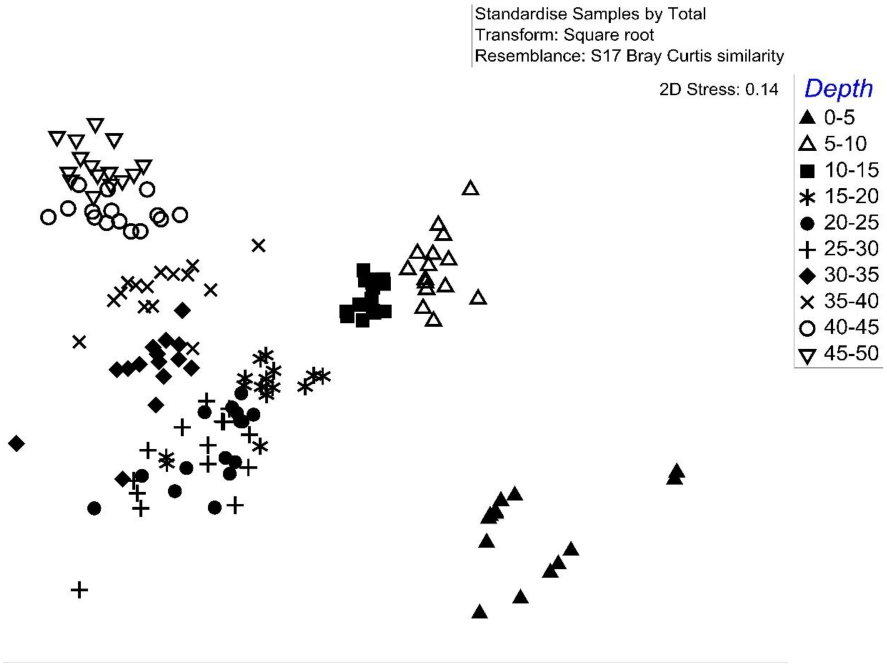

Figure 3. Multivariate analysis based on differences in species composition between all depths.

from small and cryptic species to predator species, but less mobile, such as groupers, moray eel and conger, points to a conclusion that underwater steep coralligenous reefs are not just day time shelter extensions of open water habitats. The results from this study suggest that steep coralligenous reefs represent a distinctive marine subecosystem with its own food web and associated microhabitat diversification with the depth, in relation with temperature, as the most important factor responsible for the diversity of fish assemblages within this habitat.

Composition of occasional species, mainly active and more mobile predators, points that such underwater reefs are also important feeding areas for

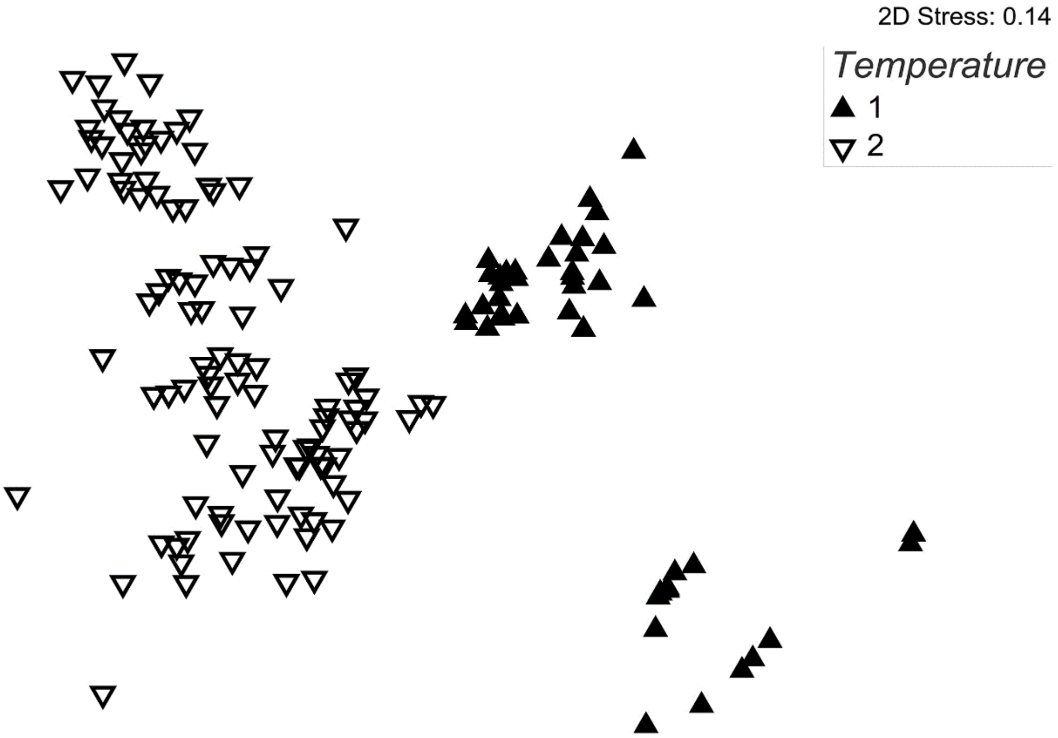

Figure 4. Multivariate analysis based on species composition between different temperature zones (1-above and 2-below thermocline).

Table 3. Results of SIMPER analysis showing species that mostly contributes to determine differences between different temperature zones.

Species	Above thermocline	Below thermocline	Average dissimilarity= 60.62
	Average abundance	Average abundance	Contribution (%)
Gobius auratus	0.63	5.02	13.81
Diplodus vulgaris	3.7	2.26	8.76
Chromis chromis	5.21	3.61	7.09
Spicara flexuosa	3.39	1.91	6.5
Boops boops	3.3	2.28	5.8
Coris julis	1.4	2.66	4.89
Diplodus annularis	1.3	2.11	4.34

many fast moving predators. The results from this study show that despite the heavy direct and indirect human impact on coastal habitats there are still microhabitats that could not be characterized as pristine (i.e. intact by human impact, with historical ecosystem structure and biomass) but as pristine as it can be. Therefore, it is essential to identify as much as possible such steep coralligenous reefs and asses their conservation state in order to protect them as marine biodiversity hotspots.

With regard to the efficiency of the DVT method, it has to be noted that UVC surveys can be biased by problems associated with behavioral responses of fish to divers. However, during the survey, such behavior was not observed, probably as the investigated area is a commercial diving area where fish are accustomed to contact with divers. Effects of observer bias associated with misidentification of fish were also not observed as recorded high resolution video was sufficient for easy identification of most of the species. Based on obtained results we can presume that a certain restriction of Full HD video could be related only to identification of small species with similar body characteristics, such as *Tripterygion sp.*, or in cases when known species ecology data are uncertain and confusing: *G. kolombatovici* was considered as bottom-dweling fish inhabiting localities to 38 m of depth (Kovačić & Miller, 2000), while during this study it was recorded for a first time at 50 m. Thus, these results prove a significant improvement of DVT method over traditionally used UVC methods, especially for deeper depths.

Hence, the use of nitrox mixture and deep decompression stops for safety reasons did not interfere with commonly used sampling methodology, thus allowing obtained results to be compared between different areas. Moreover, as nitrox gas and nitrox diving training courses are, nowadays, relatively cheap and globally available, the field part of the DVT method can be carried out by a large diving population with less scientific expertise, e.g. MPAs staff divers. To conclude, DVT method can be used as a cost-effective and powerful tool for studies of deeper areas, especially vertical reefs that, so far, have been rarely investigated.

Although this study covered a limited geographical area, the study by Piazzi, Balata, Cecchi, Gennaro and Serena (2014) suggested that coralligenous assemblages show a homogeneous structure if subjected to similar environmental conditions. Thus, results from our study could provide information that can be compared to data collected in other Mediterranean areas to validate the usefulness of the DVT method and its applicability for vertical coralligenous habitats across the Mediterranean basin.

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ORIGINAL SCIENTIFIC PAPER

The influence of depth on the Mediterranean reef fish assemblage revealed by the new video on underwater scooter method

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The new video on underwater scooter method in combination with rebreather diving techniques was introduced and applied for visual census of fish assemblages. The presently applied method facilitates deeper visual census studies below 40 m, where they have rarely been done before due to the increasing research challenges with depth. The video on underwater scooter method is also expected to be less disturbing and faster compared to swimming divers. The method was applied in visual census study of fish assemblages from 8 to 50 m depth conducted at two locations in the east Adriatic using 102 video transects. The environmental variables contributing to the variation of the reef fish community in the depth range of the infralittoral and upper circalittoral bottoms were also recorded. Thirty-one fish species were recorded on transects and 10.3% of all individuals were not identified to the species level. The average density on transects was 0.8 individuals/ m^2, with C. chromis, C. julis and G. auratus *being both the most abundant and the most frequent species. Three environmental variables, depth, bottom with zoocover and eastern orientation of the coast, were identified as highly significant for species occurrence. Abundance and species richness of fish assemblages showed no significant variation with depths, inclinations, orientations and sites. A significant difference in the fish assemblage structure was found among orientations, depths and inclinations with the gradient change of species composition with increasing depth and with increasing inclination.*

Key words: Underwater visual census, fish community, video transect, depth, Adriatic Sea, closed circuit rebreather

INTRODUCTION

The most common observation technique used to study reef fish assemblages is underwater visual census (UVC) with observing and recording the data *in situ* by SCUBA divers (WATSON *et al.*, 2005). The UVC method was developed in 1950' but was not applied in the Mediterranean until two decades later (BROCK, 1954; HARMELIN-VIVIEN & HARMELIN, 1975; HARMELIN-VIVIEN *et al.,* 1985). Today, UVC recorded by SCUBA divers is a standard technique used to estimate fish abundance in the field (WARD-PAIGE *et al.,* 2010). The alternative method of UVC, diver-operated video (DOV), has been increasingly used and tested in recent years for observation of infralittoral fish assemblage thanks to improvements and increased quality of video techniques (WATSON *et al.,* 2005; TESSIER *et al.*, 2005; 2013; LAM *et al.* 2006; PELLETIER *et al.,* 2011; JIND, 2012; ASSIS *et al.,* 2013; HOLMES *et al.*, 2013; WARTENBERG & BOOTH, 2015; ANDRADI-BROWN *et al.,* 2016a). The DOV is supposed to be able to reach deeper depths, removes diver effects, be cost effective and provide permanent records (CALDWELL *et al*., 2016).

Underwater visual census methods, in general and in the Mediterranean Sea in particular, have been limited and mostly performed at shallow depth (<20 m), with the published UVC results on fish assemblages down to 30 m depth in Mediterranean not common (COLL *et al.,* 1998; GUIDETTI *et al.,* 1998; GUIDETTI *et al.,* 2002; LETOURNEUR *et al.,* 2003; PAIS *et al.,* 2004) and down to 40 m-50 m depth even more rare (FASOLA *et al.,* 1997; RENONES *et al.,* 1997; KOVAČIĆ, 2002; SOLDO & GLAVIČIĆ, 2013). The long time spent underwater to collect sufficient amount of data and need for qualified and experienced divers who are also skilled enough for identification of fish species, prevent the broad use of UVC at greater depths. In studies, using UVC on the fish assemblage composition in the Mediterranean the depth as variable was rarely assessed. A few published works assessing particularly the influence of depth were limited to 30 m depth (LETOURNEUR *et al.,* 2003; PAIS *et al.,* 2004), except RENONES *et al.* (1997) where the deepest transect reached 41 m depth.

Video-CountN transection was recommended by WARTENBERG & BOOTH (2015) as the most appropriate method for conducting DOV research and was therefore presently applied. The aims of the paper are:

1) to apply a new video on the underwater scooter method in combination with the rebreather diving techniques for visual census of fishes which is expected to be less disturbing and faster compared to swimming divers, and also facilitate visual census studies of deeper reef fish communities,

2) to identify environmental variables significantly contributing to the variation of the reef fish community at the depth range of the infralittoral and upper circalittoral bottoms by ordination statistics methods, and

3) to particularly statistically test the effects of physical and geographical factors (depth, bottom inclination, coastline orientation, locations) on the total fish abundance, species richness, and structure of the reef fish assemblage.

MATERIAL AND METHODS

Study area and sampling design

The study was carried out at two locations in the eastern Adriatic Sea on the islands of Hvar: Cape Smočiguzica and Cape Glavna (Fig. 1). Both sites are reefs with a limited width of about 200-300 m and with steep to vertical limestone bottom at depths ranging between 8 and 55 m. Both locations are similarly exposed to the prevailing weather with waves coming from the NNE, SE and SW.

The visual census was performed on 102 video transects in total from both localities at depth from 8 to 50 m depth (Table 1). The visual census was carried out by two divers, the main observer (DL) and the supporting diver (IG), using video on underwater scooter (Fig. 2A). The divers used the rebreather equipment JJ- CCR (Closed circuit rebreather) and the scooter Suex Model: XJOY7. Camera was Canon EOS 5D MK II with lens Canon EF 17-40/4L USM, 17mm with a diagonal angle

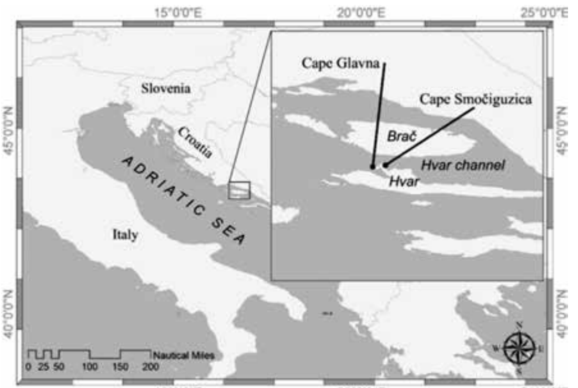

Fig. 1. Map showing studied locations at Cape Smočiguzica and Cape Glavna

Table 1. Number of transects

Depth (m)	Number of transects
8 to 10	29
15 to 20	27
30 to 35	23
45 to 50	23
Total	102

of view 104 degrees. The pretesting for finding the optimal equipment and the optimal values and conditions of shooting, and the training of the divers for the procedure took two weeks in September 2015. To avoid seasonal variability the data sampling was carried out in about one week, 2-9 June 2016. All video recordings were taken at approximately the same part of day i.e. between 11 and 16 hours. The visual census belt transect size was 25x2m. The Video-CountN transect method was adapted from WARTENBERG & BOOTH (2015).

In the pretesting process the optimal equipment, values and recording condition were investigated, including the choice of camera model, camera lenses, lighting, the best camera angle to record both epibenthic and hyperbenthic fishes, the maximum speed of the underwater scooter at which the fish are still identifiable and countable, and the distance of camera from the ground needed to adjust 2 m transect width. During the two-week pre-test phase, in addition to the finding of the optimal equipment and values and condition of shooting, the divers were trained for procedure and to maintain the distance to the bottom, to keep the correct camera angle, and to follow isobaths of the appropriate depths.

The width of the present digital transects was determined by the field of view of the camera lens (WARTENBERG & BOOTH, 2015). The conclusion from the pretesting was that the cameraman should drive an underwater scooter 1.5 m above the bottom and hold the video camera steady in front of him at an angle of 45 degrees to the bottom to ensure width of the transects of 2 m (Fig. 2B and 2C). However, due to the steep to vertical bottom, camera had to be angled 45 degrees to left-right and not up-down direction (Fig. 2A and 2B).

In order to avoid deploying of the transect line needed to define the transect length, thereby to reduce the diver disturbance of the bottom and to optimize the dive time (WARTENBERG & BOOTH, 2015), the transects were terminated after a fixed time, based on relationship of distance and time from the constant speed of the underwater scooter. Regarding the speed of the underwater scooter at which fishes are still identifiable, the pretesting showed that the slowest among tested underwater speeds was the best for the recognizing of fishes. The speed of a scooter with an equipment configuration (Fig. 2A) was 0.7 m/s, resulting in 36 seconds of time needed to pass 25 m transect. The visual census was performed in the conditions of absent sea current so the length of transects could be determined on the time passed. The continuing recording of transects along the same depth should be separated by intervals (ANDRADI-BROWN *et al.,* 2016a; 2016b). Therefore, the divers conducted continuous filming of several transects and the intervals between them at once in a line approximately following isobaths.

Sampling was conducted by divers in buddy team. During transecting, the primary observer (DL) in a buddy pair set the speed of underwater scooter and drove it, recording digital footage and control time for the termination of transect series. The role of supporting diver (IG) at the beginning of each transect series was to check if the horizontal visibility is good enough and if the sea current is absent not to influence the visual census and distance estimation; to establish the

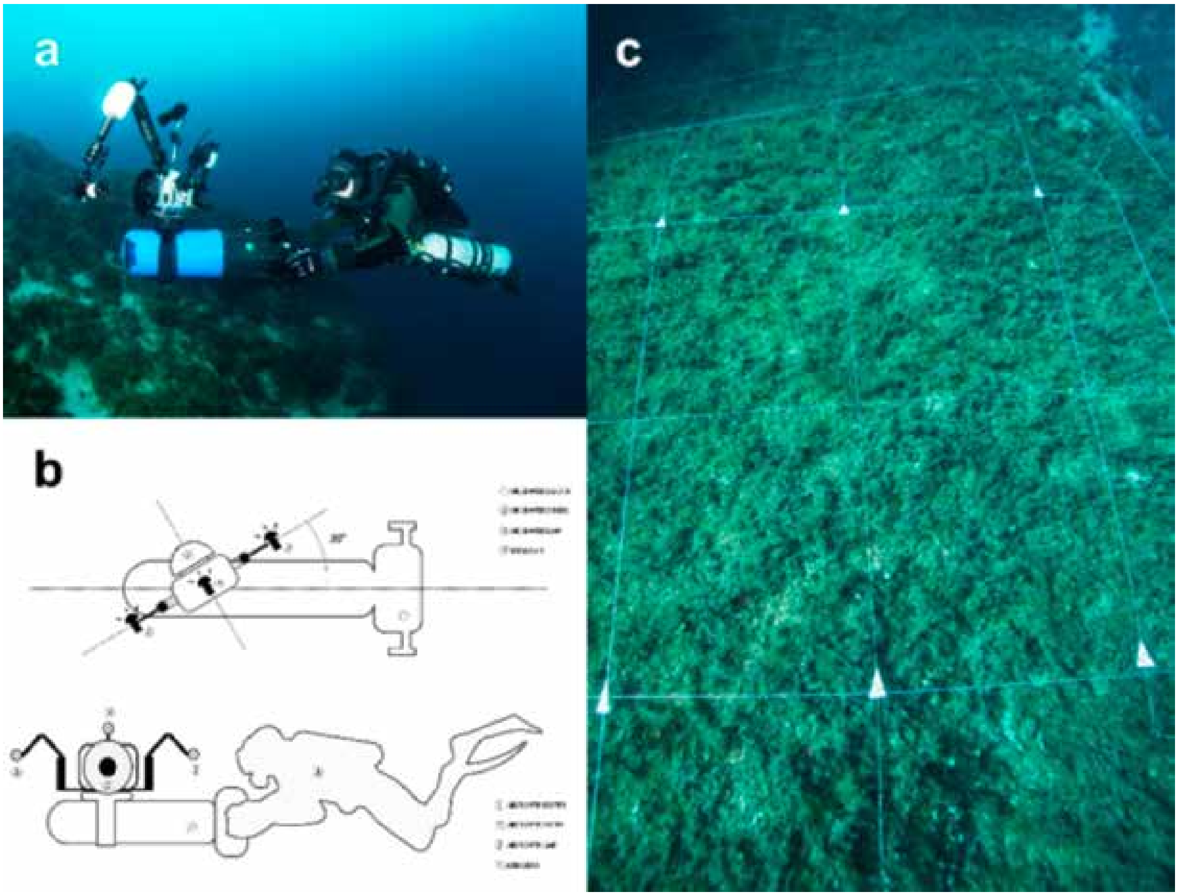

Fig. 2. **A**) The primary observer with full equipment, **B**) sketch of the mounted equipment and camera angle, **C**) camera view when positioned 1.5 m from the bottom and with an angle of 45 degree, grid 1 by 1 m, the view width at the closest point 2 m, widening forward out of 2 m wide marks, the view length about 5 m, visible from grid. The photos and drawing by Dani Laslo

starting point of transect series and to check at shooting beginning the distance of camera from the bottom by calibrated stick. The occurrence of a sea current was tested with a very fine bottom sediment, fine sand was dropped by second diver about 50 cm above the bottom before each survey depth, in case the sediment descends vertically to the bottom, no current is present and sampling can start. The supporting diver also recorded in situ water temperature, depth, date, time and orientation of each transect. The role of supporting diver who followed the first one during transects on the second underwater scooter was to control the procedure i.e. to control if the camera distance from the bottom and the depth i.e. following of isobaths were kept.

Media techniques and analysis

The video clips of 36 seconds from each continuous filming, which is equal to 25 m long transects (see in Study area and sampling design above), were extracted from original shooting, each with additional 14 seconds excluded as interval between transects. The initial part of video with preparation and calibration on the beginning of each shooting was also excluded. Each transect images were analyzed on a 27’ screen by the fish expert (MK), using standard viewing software that enables slow view and zooming (MPC-HC 1.7.10), HPC-HC team, http://hpc-hc.org).

Video clips were analysed on the half of the normal speed, but also using multiple pauses, additional slowing down, rewinds and repeated playbacks whenever necessary (WARTENBERG & BOOTH, 2015). Video-CountN transecting is recommended as the most appropriate method for conducting DOVs providing highest estimates of richness and abundance, the lowest variability, the most representative diversity being also the most efficient considering time consuming (WARTENBERG & BOOTH, 2015). The CountN analysis simply identifies and enumerates all individuals observed in digital transects, effectively imitating in situ slate-transect enumeration. Individuals that could not be accurately identified to the species level because they were too far away, moved through the census area too rapidly, or were obscured due to poor lighting, camera angle, individual orientation or other factors, were categorized as unidentifiable, even if it was possible to identify them to higher taxon (WARTENBERG & BOOTH, 2015). In digital CountN analyses of all transects, first check of video clip was performed to record all present species; the second check focused on search for possible presence of fish at bottom, staying still; on the third check number of individuals of species occurring at lower densities, i.e. <10 individuals per transect, were counted simultaneously. The species that occurred at higher abundances, i.e. >10 individuals per transect were counted in separate checks/counts for each species. Counts from digital transects were corroborated by reanalyzing the media. It was assumed that if two counts for a particular transect were sufficiently similar, then those counts would have been acceptably close to the true count generated by the technique. Re-analysis was conducted 2 weeks after the original counts were completed (WARTENBERG & BOOTH, 2015). Percentage agreement was applied to the species observed (presence/absence) and to species-specific abundance for each digital transect-type analysis technique combination (WARTENBERG & BOOTH, 2015). Minimum agreement in species presence/absence was set to 100% because if a species is observed during initial analysis it should be observed in all subsequent reviews. Minimum species-specific abundance agreement was set to 100% for species observed with <10 individuals per transect, and to 90% for species observed with >10 individuals per transect. A 10% difference in counts was considered acceptable due to the challenges of counting abundant or shoaling species in motion. When single species abundances were between 90% and 100% agreement, the mean of the two abundance estimates from the two stages of analysis was accepted as the final value. When more than one reanalysis was necessary, the percentage agreement rule was applied to the two last rounds of analysis where the minimum 90% agreement was reached. The same percentage rules were also applied to fishes categorized as unidentifiable thereby corroborating counts of all observed individuals. In addition to abundance of each recorded fish species on each transects, the supplementary habitat variables were also estimated from each digital transects in the separate check of video for each habitat variable after the fish counts: bottom inclination, bottom substrate and biocover. The variable categories were modified from KOVAČIĆ *et al.* (2012). The samples were taken at four distinct, non-contacted depth ranges and "Depth" variable was accordingly divided: 8-10 m, 15-20 m, 30-35 m and 45-50 m depth. "Inclination" as three estimated classes: gentle to moderate (<30º), steep (30º – 60º) and very steep to vertical (>60º). "Bottom substrate" as four types of different estimated particle size and three kinds of bedrock surfaces (sand, gravel <60 mm, cobbles <200 mm, boulders >200 mm, bedrock with more or less smooth surface, bedrock with crevices and cavities, high relief bedrock composed of ridges and trenches). "Biocover" as six types (no biocover, phanerogams, long thallus algae, short thallus algae, calcaerous algae, zoocover). Each of the categories of "Bottom substrate" and "Biocover" present in particular sample was expressed as estimated percentage (10%, 20% …100%) of the total surface in that sample. "Orientation" of transect was general orientation parallel to the coast as North, East and West, missing South orientation due to the coast orientation at localities. "Depth", "Inclination", "Orientation" therefore can be used as factors with levels, contrary to the recorded estimated

Table 2. The total number of recorded individuals for species and the frequency of species occurrence at the transects (n = 102). The number of fish is a sum on all transects. Some transects values were with decimal fractions, being the average of two counts (see Media techniques and analysis in Material and methods), with the rounding done only on the final numbers presented here

Species	Abbreviation	Total number of recorded individuals	Species frequency of occurrence
Boops boops (Linnaeus, 1758)	Bo bo	2	2.0%
Chromis chromis (Linnaeus, 1758)	Ch ch	2667	85.3%
Coris julis (Linnaeus, 1758)	Co ju	318	72.5%
Diplodus annularis (Linnaeus, 1758)	Di an	17	9.8%
Diplodus puntazzo (Cetti, 1777)	Di pu	1	1.0%
Diplodus sargus (Linnaeus, 1758)	Di sa	6	2.0%
Diplodus vulgaris (Geoffroy St. Hilaire, 1817)	Di vu	113	35.3%
Gobius auratus (Risso, 1810)	Go au	321	55.9%
Gobius geniporus (Valenciennes, 1837)	Go ge	1	1.0%
Gobius vittatus (Vinciguerra, 1883)	Go vi	9	2.9%
Labrus mixtus (Linnaeus, 1758)	La mi	2	2.0%
Mullus surmuletus (Linnaeus, 1758)	Mu su	2	1.0%
Oblada melanura (Linnaeus, 1758)	Ob me	2	1.0%
Parablennius rouxi (Cocco, 1833)	Pa ro	13	9.8%
Phycis phycis (Linnaeus, 1766)	Ph ph	4	2.0%
Sarpa salpa (Linnaeus, 1758)	Sa sa	1	1.0%
Scorpaena porcus Linnaeus, 1758	Sc po	3	2.9%
Serranus hepatus (Linnaeus, 1758)	Se he	1	1.0%
Serranus scriba (Linnaeus, 1758)	Se sc	43	23.5%
Serranus cabrilla (Linnaeus, 1758)	Se ca	13	12.7%
Spicara smaris (Linnaeus, 1758)	Sp sm	74	4.9%
Spicara flexuosa Rafinesque, 1810	Sp fl	3	2.0%
Spondyliosoma cantharus (Linnaeus, 1758)	Sp ca	5	3.9%
Symphodus doderleini (Jordan, 1890)	Sy do	2	2.0%
Symphodus mediterraneus (Linnaeus, 1758)	Sy med	13	9.9%
Symphodus melanocercus (Risso, 1810)	Sy mel	3	2.9%
Symphodus ocellatus (Linnaeus, 1758)	Sy oc	3	2.0%
Symphodus tinca (Linnaeus, 1758)	Sy ti	26	16.7%
Thorogobius ephippiatus (Lowe, 1839)	Th ep	5	3.9%
Tripterygion delaisi Cadenat & Blanche, 1971	Tr de	3	2.9%
Tripterygion tripteronotum (Risso, 1810)	Tr tr	2	2.0%

percentages of bottom substrate and of the percentages of biocover types.

Data analysis

In order to determine the relationship between environmental variables and the fish assemblage Canonical correspondence analysis (CCA) (TER BRAAK, 1986; 1994) was carried out using CANOCO software (TER BRAAK, 1992). All fish species recorded on the transects were used for analysis (Table 2). The species abundance were log(x+1) transformed to normalize the distribution. To reveal patterns of species response curves in relation to environmental gradients, data sets were first analyzed with Detrended Correspondence Analysis (DCA). The first axis gradient length measured in turnover (or SD units) indicated nonlinear or unimodal responses, therefore a Canonical Correspondence Analysis (CCA) was chosen as a constrained ordination method. Among the 19 possible environmental variables included in the field recording sheet (in CCA factors with levels being just qualitative categories were decomposed and each level treated as separate environmental variables), 14 variables were actually recorded during fieldwork (Table 3).

Locality was introduced into the model as co-variable to partial out possible confounding effects varying between locations using partial constrained analysis. The first CCA was performed with all actually recorded environmental variables and the forward selection procedure was then used to extract the variables incorporated in the final model. In order to avoid

Table 3. Variation explained by 14 and by 11 environmental variables. Marginal effect (λ 1) of environmental variable refers to eigenvalues fit if the corresponding variable was the only environmental variable in the model. The conditional effect (λ A) refers to the additional amount of explained variance in species distribution by the correspondent variable when selected by automatic forward selection. The obtained statistics of the Monte Carlo permutation test under the full model with 999 random permutations (P- value and F) are reported for each variable. Significant conditional effect in models with 14 and with 11 environmental variables (P≤0.05) in bold

	Marginal Effects	Conditional Effects after forward selection					
		14 Environmental variables			11 Environmental variables		
Variable	λ 1	λ A	P	F	λ A	P	F
Depth	0.15	0.15	**0.002**	**4.21**	0.15	**0.002**	**4.21**
Zoocover	0.13	0.1	**0.002**	**2.91**	0.11	**0.002**	**2.86**
Calcareous algae	0.12	0.07	**0.016**	**1.96**	0.07	**0.016**	**1.93**
High relief bedrock composed of ridges and trenches	0.1	0.11	**0.014**	**2.95**	n/a	n/a	n/a
Short thallus algae	0.1	0.03	0.554	0.77	0.02	0.598	0.69
Inclination	0.1	0.06	**0.008**	**1.82**	0.05	**0.046**	**1.59**
East	0.09	0.09	**0.002**	**2.56**	0.09	**0.002**	**2.5**
Sand	0.08	0.05	0.224	1.31	0.06	0.07	1.69
Long thallus algae	0.08	0.07	**0.018**	**2.08**	0.07	**0.022**	**2.08**
West	0.07	n/a	n/a	n/a	n/a	n/a	
No biocover	0.07	0.02	0.9	0.62	0.02	0.94	0.57
Bedrock with crevices and cavities	0.05	0.03	0.392	0.9	0.07	**0.012**	**2.06**
Bedrock with more or less smooth surface	0.05	0.02	0.844	0.69	0.05	**0.046**	**1.52**
North	0.03	0.04	0.22	1.18	n/a	n/a	n/a

inflated significance values, redundant predictor variables, i.e. environmental variables showing high multicolinearity (VIF>10), were removed from the model resulting in the final model with 11 environmental variables. The significance of the canonical axes and of the environmental variables was determined using Monte Carlo permutation tests (with 999 random permutations). The CCA was run with a focus on interspecies distances and the biplot scaling option.

The ANOVA assumptions were not met in the data for the comparisons of abundance (the total number of all individuals) and species richness (total number of recorded species) at different depths, inclinations, orientations and sites. Therefore the changes in abundance and richness among depths (8-10 m, 15-20 m, 30-35 m, 45-50 m), inclinations (<30º, 30º – 60º, >60º), orientations (N, E, W) and between two sampling sites (Cape Glavna, Cape Smočiguzica) were each examined with one-way Permutational multivariate Analysis of variance (PERMANOVA) (ANDERSON, 2001) with 999 permutation on untransformed data using Euclidian distances.

To address the possible differences in fish assemblage structures among depths, inclinations, orientations and between sampling sites we used one-way PERMANOVA, for each factor separately, with 999 permutation on log(x+1) transformed data using Bray-Curtis similarity matrix. Relevant factors were additionally tested for interaction effect with two-way PERMANOVA. All factors were treated as fixed. To graphically visualize the difference in fish assemblage structures, nonparametric multidimensional scaling (nMDS) plots (FIELD *et al.,* 1982) were performed. In cases when one-way PERMANOVA was significant ($P<0.05$), pairwise comparison was conducted using *pairwiseAdonis* (ARBIZU, 2019) in R. The nMDS ordination plots were run using Bray-Curtis similarity matrix. We used *R* statistical package and *vegan* library to run all analyses.

RESULTS

Diversity, abundance and frequency of fishes

Thirty-one fish species were recorded on the 102 transects with the total number of 3677 identified individuals (Table 2) and with additional 396 individuals (10.3% of all individuals) unidentified to the species level for the reasons listed in Material and methods. The present method was able to detect and identify benthic fish down to the size of about 8-10 cm (*Gobius geniporus* (Valenciennes, 1837), *Thorogobius ephippiatus* (Lowe, 1839)). Below that size only strikingly colored benthic species were noticed and identified due to the limits of resolution and illumination (*Gobius auratus* Risso, *Gobius vittatus* (Vinciguerra, 1883), males of *Tripterygion delaisi* Cadenat & Blanche, 1971 and *Tripterygion tripteronotum* (Risso, 1810)). The average number of all individuals per transect was 39.9, resulting in average density of 0.8 individuals/m^2. *Chromis chromis* (Linnaeus, 1758) was the most abundant species, with 72.5% of all identified individuals belonging to this species, followed by *Gobius auratus* Risso, 1810 (8.7%), *Coris julis* (Linnaeus, 1758) (8.6%) and *Diplodus vulgaris* (Geoffroy St. Hilaire, 1817) (3.1%). None of the other species was recorded with more than hundred recorded individuals, and 20 species were recorded with less than ten individuals, which all together represented just 1.6% of all identified individuals (Table 2). The most frequent species were again the same three most abundant species: *C. chromis* (frequency of occurrence on transects 85.3%), *C. julis* (72.5%) and *G. auratus* (55.9%), four more species were more frequent than 10%, but 8 species were recorded on just two transects and 6 species on only one transect (Table 2). The most diverse family was Sparidae (9 species) followed by Labridae (7 species).

Important habitat variables structuring the fish assemblage at the infralittoral and upper circalittoral rocky and mixed bottoms

The first partial CCA was performed with 14 environmental variables on all 31 species as response variables (Fig. 3A). The Monte Carlo permutation test was statistically significant on the first axis and also on all canonical axes (Table 3). The cumulative percentage of variance in the response data explained by the first four constrained axes was 14.2%, and the first four axes explained 64.1% of variation in the response data explained by explanatory variables *i.e.* of the variation of fitted response values (Table 3). The model with 11 environmental variables, after the forward selection procedure excluded redundant variables (North, West, High relief bedrock composed of ridges and trenches), showed slightly lower cumulative explained variation (13.7%) and slightly higher cumulative fitted explained variation (68.0%) than the model with 14 environmental variables (Fig. 3B, Tables 4 and 5).

Canonical eigenvalues are also slightly lower in the model with 11 environmental variables (Tables 4 and 5). The forward selection identified three highly significant environmental variables: Depth, Zoocover and East (P=0.002%), and, in the model with 11 environmental variables, the five other significant variables: Inclination, Bedrock with crevices and cavities, Bedrock with more or less smooth surface, Long thallus and Calcareous algae (Table 3). Among highly significant variables the first axis was positively correlated with Depth and Zoocover and negatively correlated with East, while a further positive correlation is detectable also with other three significant variables (Inclination, Bedrock with crevices and cavities, Calcareous algae) (Fig. 3, Table 3). The second axis correlate, among significant environmental variables, with Long thallus algae. The projections of species points of *Phycis phycis* (Linnaeus, 1766), *Boops boops* (Linnaeus, 1758), *Labrus mixtus* Linnaeus, 1758 and *T. ephippiatus* onto highly significant environmental variables Depth and Zoocover, but also onto significant Inclination, Bedrock with crevices and cavities and Calcareous algae, show optima of these species at high values of these environmental variables i.e. at deep, steep bedrock with crevices and cavities covered with sedentary animals and calcareous algae, contrary to species negatively correlated to these variables: *G. geniporus*, *Mullus surmuletus* (Linnaeus, 1758), *Serranus scriba* (Linnaeus, 1758), *T. tripteronotum*. The projections of species points of several wrasses (*Coris julis* (Linnaeus, 1758), *Symphodus doderleini* Jor-

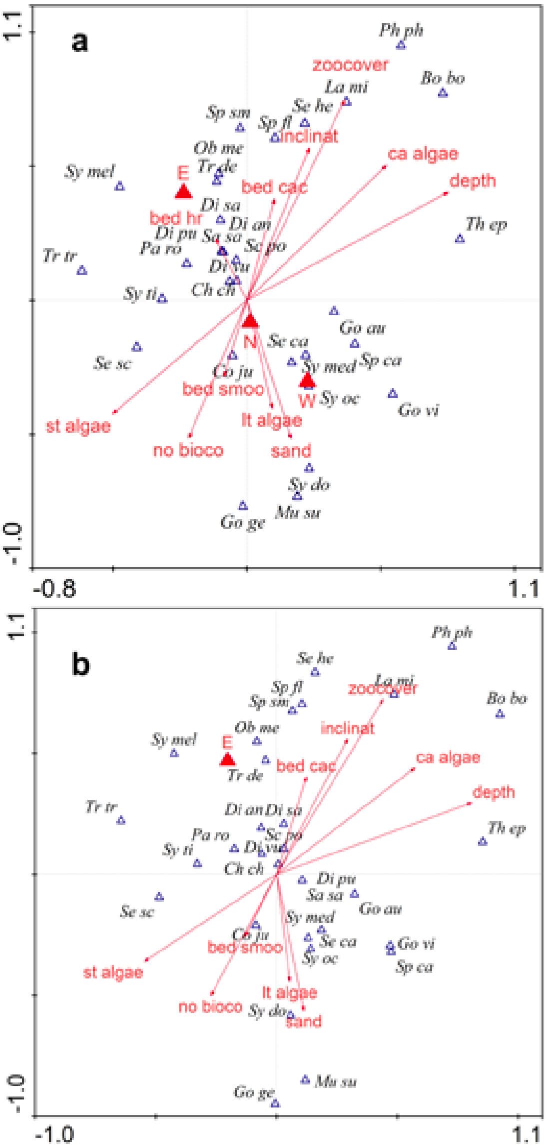

Fig. 3. CCA species-environment biplot for all 14 environmental variables (A) and final model with 11 environmental variables (B) (biplot scaling). Quantitative environmental variables are represented by arrows and nominal variable is indicated by a triangle, both red and with abbreviations in regular font. The abbreviations for explanatory variables: depth- Depth, E – East, W – West, N – North, inclinat – Inclination, sand - Sand, bed_smoo – Bedrock with more or less smooth surface, bed_cac – Bedrock with crevices and cavities, bed hr –High relief bedrock composed of ridges and trenches, no_bioco – No biocover, lt_alage – Long thallus algae, st algae – Short thallus algae, ca algae – Calcareous algae, zoocover - Zoocover. Hollow triangles and abbreviations in italics represent fish species. The abbreviations for fish species are explained in Table 2.

Table 4. Outcome of CCA, using 14 environmental variables. Significance of environmental variables are reported in Table 3

Total inertia = 3.855					
Sum of canonical eigenvalues =	0.837	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues		0.182	0.151	0.123	0.081
Species–environment (S–E) correlations		0.782	0.741	0.715	0.629
Cumulative % variance of species data		4.8	8.8	12.0	14.2
Cumulative % variance of S–E relation		21.8	39.8	54.4	64.1
Monte Carlo test (P-value, 999 permutations)		0.002	P (All canonical axes) = 0.016		

Table 5. Outcome of CCA, using 11 environmental variables. Significance of environmental variables are reported in Table 3

Total inertia = 3.855					
Sum of canonical eigenvalues =	0.764	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues		0.177	0.146	0.118	0.078
Species–environment (S–E) correlations		0.779	0.740	0.700	0.600
Cumulative % variance of species data		4.7	8.5	11.6	13.7
Cumulative % variance of S–E relation		23.2	42.3	57.8	68.0
Monte Carlo test (P-value, 999 permutations)		0.004	P (All canonical axes) = 0.014		

dan, 1890, *S. mediterraneus* (Linnaeus, 1758)*,* *S. ocellatus* (Linnaeus, 1758)), and also of *Spondyliosoma cantharus* (Linnaeus, 1758) and *Serranus cabrilla* (Linnaeus, 1758) on significant environmental variable Long thallus algae show positive correlation of these species to the habitat with long thallus algae, while the similar biplot positions of *G. geniporus*, *M. surmuletus* and also *G. vittatus* is probably more related to another quantitative predictor, Sand, which is highly overlapped with Long thallus algae, and had large but not significant effect. The deep bottom species *P. phycis* and *L. mixtus,* benthopelagic species *Spicara flexuosa* Rafinesque, 1810, *S. smaris* (Linnaeus, 1758) and *Oblada melanura* (Linnaeus, 1758), and also *Serranus hepatus* (Linnaeus, 1758) avoided of Long thallus algae habitat.

The variation of the fish abundance, species richness and fish assemblage structure with depths, inclinations, orientations and sites

PERMANOVA for the differences in total fish abundance and the species richness showed no significant differences with depths, inclinations, orientations and sites (Table 6).

However, the PERMANOVA test on the fish assemblage structure indicates the significant difference in fish assemblage structures among different orientations, depths and inclinations, while sites didn't have significant influence on the fish assemblage composition (Table 7). The post hoc test reveals that the fish assemblage at shallow depth, 8-10 m, is different from the fish assemblages at all other depths, but also that the fish assemblage composition at the depth 15-20 m is again significantly different from the largest depth, 45-50 m (Table 7). The pairwise PERMANOVA also indicated the significant difference in fish assemblage between extreme

*Table 6. Results of one-way PERMANOVA tests comparing total fish abundance and species richness of fish assemblages according to four factors: Depth (8-10 m, 15-20 m, 30-35 m, 45-50 m), Inclination (<30º, 30º – 60º, >60º), Orientation (North, East, West) and Sites (Cape Glavna, Cape Smočigizica). *P<0.05; **P<0.001; n.s.=not significant. Analyses were conducted on untransformed data using Euclidian distances*

		ABUNDANCE			SPECIES RICHNESS		
Source of variation	df	Pseudo F	P (perm)		Pseudo F	P (perm)	
Site	1	0.014	0.923	n.s.	0.625	0.426	n.s.
Orientation	2	1.425	0.259	n.s.	0.642	0.544	n.s.
Depth	3	0.956	0.407	n.s.	0.465	0.725	n.s.
Inclination	2	1.758	0.182	n.s.	2.394	0.084	n.s.

inclinations i.e. <30º and >60º (Table 7). The results of the pairwise comparison for depths and for inclinations therefore imply the gradient changes in species composition with the increasing depth and with the increasing inclination. In addition, it looks like the intensity of changes in species composition was larger in shallow water since 8-10 m and 15-20 m were the only neighboring depth ranges with significantly different fish assemblage composition. The nMDS plots illustrated the differentiation of fish assemblage species composition among depths (Fig. 4A), inclinations (Fig. 4B) and orientations (Fig. 4C) found by the PERMANOVA test, while there was no separation on the nMDS ordination plot between fish assemblage species composition at different sites, matching again the PERMANOVA results (Fig. 4D). Even the significant differences established from the pairwise comparison are visible on the nMDS plots as the larger distances between centroids of those levels on the plots (Table 7, Fig. 4A and 4B). There was also the significant interaction between site and depth, site and orientation and orientation and depth found by the two-way PERMANOVA (Table 8), meaning that the effect of depth varied between sites and among different orientations and the effect of orientation varied between sites.

*Table 7. Results of PERMANOVA tests comparing fish assemblage structure according to four factors: Depth (8-10 m, 15-20 m, 30-35 m, 45-50 m), Inclination (<30º, 30º – 60º, >60º), Orientation (North, East, West) and Sites (Cape Glavna, Cape Smočigizica). *P<0.05; **P<0.001; n.s.=not significant. Results of pairwise comparison are reported only for factors with more than two levels having significant (P<0.05) difference. All data were log(x+1) transformed*

Source of variation	df	Pseudo F	P (perm)	
Site	1	0.812	0.537	n.s.
Orientation	2	3.369	0.03	*
Depth	3	15.589	<0.001	**
Inclination	2	9.574	<0.001	**
Pairwise comparison				
Orientation				
E vs N		2.58	0.06	
E vs W		4.10	0.006	*
N vs W		3.36	0.022	*
Depth (m)				
8-10 vs 15-20		12.05	<0.001	**
8-10 vs 30-35		15.952	<0.001	**
8-10 vs 45-50		19.464	<0.001	**
15-20 vs 45-50		3.421	0.025	*
Inclination				
<30º vs >60º		9.31	<0.001	**

*Table 8. Results of two-way PERMANOVA tests comparing fish assemblage structure. *P<0.05; **P<0.001; n.s.=not significant*

Source of variation	df	Pseudo F	P (perm)	
Site x Depth	3	2.376	0.01	*.
Site x Orientation	2	3.660	0.02	*
Site x Inclination	5	0.221	0.956	n.s
Depth x Inclination	5	0.925	0.522	n.s
Depth x Orientation	5	2.020	0.015	*

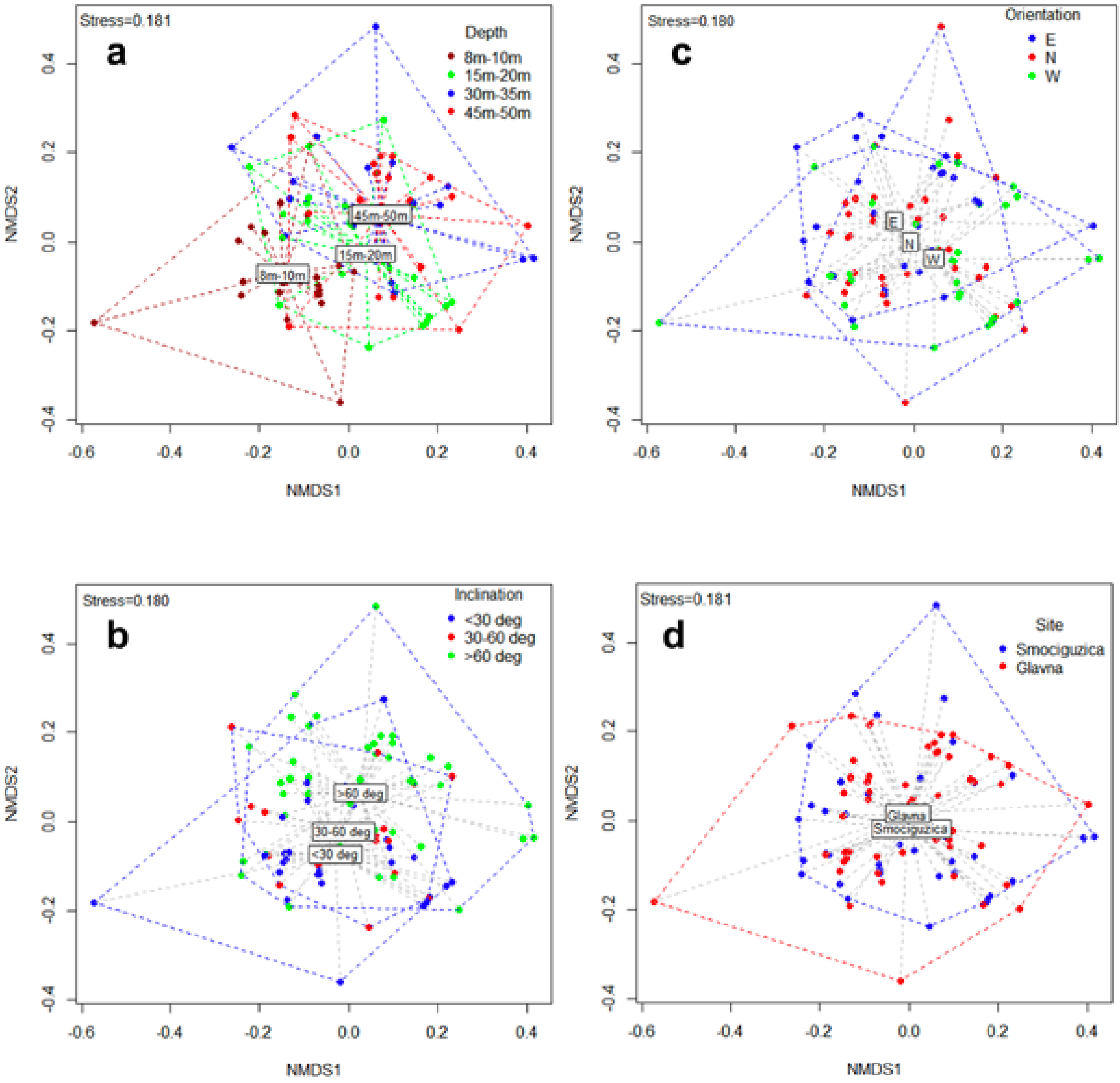

Fig. 4. Non-metric multi-dimensional scaling (nMDS) ordination plot of individual transects comparing (A) depth: 8-10 m (brown circles), 15-20 m (green circles), 30-35 m (blue circles), 45-50 m (red circles); (B) inclination: <30° (blue circles), 30° – 60° (red circles), >60° (green circles); (C) Orientation: North (blue circles), East (red circles), West (green circles); (D) Site: Smočiguzica (blue circles), Glavna (red circles). Stress values presented on each graph.

DISCUSSION

The present method showed to be limited to detect and identify benthic fish below a size of about 8-10 cm, except for the conspicuously colored fish, due to the limits of resolution and illumination. Similar to other DOV studies and to UVC methods in general, in the present study we were also not able to detect cryptobenthic fishes (KOVAČIĆ *et al.*, 2012), which are by definition, hidden in bottom space and out of reach by the visual methods. Therefore, the presently described fish assemblages are incomplete lacking these parts of the fish assemblage. This general limitation of visual methods prevents the visual census from being able to describe the entire fish assemblage of the bottom habitats.

In comparison with other visual censuses carried out, which also examined the influence of depth on the fish assemblage composition in the Mediterranean, the present data showed lover total diversity (31 species recorded over

48 species in RENONES *et al.* (1997); 62 species in LETOURNEUR *et al.* (2003); and 47 species in PAIS *et al.* (2004)). However, those studies were conducted on a more variable range of habitats compared to this study which was limited to underwater cliffs. Interestingly, the most abundant fish species in all studies was *C. chromis*, becoming candidate for the most abundant fish species of the non-sandy infralittoral bottoms of the northern Mediterranean coasts. Furthermore, part of the *C. chromis* population is known to hide in cryptic bottom spaces even during daylight (KOVAČIĆ *et al*., 2012), so the total abundance of *C. chromis* is probably even larger than the abundance of the exposed visible individuals established by the present visual census. Unfortunately, in RENONES *et al.* (1997), LETOURNEUR *et al.* (2003) and PAIS *et al.* (2004) no data on average density of the whole sample was available for comparison.

In contrast to the other significant environmental variables that influence species composition, e.g. depth and inclination, bottom structure (bedrock with crevices and cavities or with more or less smooth surface) and biocover (zoocover, calcareous algae or long thallus algae), the high significance of eastern position for the fish assemblage structure is the only puzzling result among the explanatory variables. It is positively correlated with the second axis, and although the eastern position could be expected to have better sunlight conditions, its centroid position on the biplot (Fig. 3) is opposite to environmental variables that depend on sunlight *e.g.* long thallus algae. During the fieldwork, no prevailing influence of any particular depth, inclination, bottom structure or biocover was observed on the eastern transects, which is confirmed from the biplot where almost no other explanatory variables are directed near the eastern position centroid (Fig. 3). Another significant environmental variables in this study have clearly structured the fish assemblage and the results showed that the fish assemblages of deep, steep bedrock with crevices and cavities covered with sedentary animals and calcareous algae (*P. phycis, L. mixtus*, *T. ephippiatus*) or of the long thallus algae bottoms (Labridae, *S. cantharus*) are composed of species which presence there match our general knowledge of the species habitat preferences (JARDAS, 1996).

The abundance and species richness of the fish assemblages did not show significant variation with depths, inclinations, orientations and sites. However, in contrast to the results of previous studies (RENONES *et al.,* 1997; LETOURNEUR *et al.,* 2003; PAIS *et al.,* 2004), we were able to find, by Permutational multivariate analysis of variance, the significant difference in fish assemblage among different depths, with the shallowest depth, 8-10 m, having the most unique composition and with the gradient change of species composition with increasing depth down to the deepest transects at 45-50 m depth. This is consistent with the results of our Canonical correspondence analysis, which reveals depth as a highly significant environmental variable that influences species composition. LETOURNEUR *et al.* (2003), from the Canonical correspondence analysis, showed that depth in itself was not a significant factor explaining fish species patterns from 0 to 30 m depth and that the most significant factors were bottom substrate (sand) and biocover (macroalgae and *Posidonica oceanica*). The research carried out by PAIS *et al.* (2004) at 0 to 30 m depth was limited by the method to reveal significant differences in fish assemblage at different depths, as the depth as a factor was only tested by ANOVA for density of each fish species, with only two species, *C. chromis* and *S. maena*, showing significantly different density with depth. Finally, RENONES *et al.* (1997) showed by cluster analysis the greatest difference in similarity between their deeper stations at 41 m depth and all other stations and by correspondence analysis they found difference among block stations, vertical cliff stations and again for two deep stations at 41 m depth. The presently found depth importance for the fish assemblage structure was probably not established in previous three works probably for several reasons:

1) they did not focus any of their analyses primarily on depth as an environmental variable with appropriate hypothesis testing tool,

2) they mostly limited their analysis to the

infralittoral zone while the present research was extended to the upper circalittoral,

3) in contrast to the more homogenous bottoms along the depth range in the present research, the variability of the involved bottom types in earlier researches could obscure the differences by depth in previous studies.

The present study did not compare the results of the video on underwater scooter method with the UVC and or with the DOV data. The simultaneous comparison, like in comparisons between UVC and DOV results (e.g. TESSIER *et al*., 2013), would be hard to achieve considering the speed of the underwater scooter against the diver swim. Slate recorded UVC requires a long underwater time to collect sufficient data and also requires divers who are experienced ichthyologists or others trained in identifying species and in some cases estimating individual fish sizes. However, it is well known that the slate recorded UVC gather better information as method since the video census is less accurate than standard UVC for determining species richness and video census also underestimates abundance, especially for low values (TESSIER *et al*., 2005). Anyway, the present video on underwater scooter method in combination with rebreather diving techniques could have two advantages, both over standard UVC and over DOV studies of fish assemblages, the decreased disturbance of fish assemblages and the greater depth that could be reached. Without pre-constructed transects, with the short time of smooth passage of the transect areas with the underwater scooter and with the noiseless breathing of the mixed-gas closed-circuit rebreather the disturbance of fish assemblages should be greatly reduced compared to standard UVC and DOV methods. LINDFIELD *et al*. (2014) already showed that the use of the mixed-gas closed-circuit rebreather for fish surveys clearly minimizes behavioral biases associated with fish avoiding open-circuit SCUBA divers. The video on underwater scooter method in combination with the rebreather diving techniques also allows to reach greater depth, being faster with the underwater scooter and staying deeper and longer, thanks to the mixed gas closed circuit rebreather system, so the deeper underwater visual census could be the niche for the application of the new presented method.

Over the last thirty years, the faster alternative to the UVC, using underwater video techniques, has been introduced and used for assessing fish assemblages (GREENE & ALEVISON, 1989, FRANCOUR *et al.,* 1999; WILLIS & BABCOCK, 2000; HARVEY *et al.,* 2001). The ever increasing resolution and performance in low light conditions of each new generation of cameras and their sensors make underwater video census increasingly promising method. Over the last two decades, diving techniques with advanced dive equipment and different gas mixtures have been used to allow safe excursions to depths of 50–150 m, which exceeds the depths that can be safely reached by conventional SCUBA (PARRISH & PYLE, 2002). Despite these advances in SCUBA diving, few results have generally been published on the visual census of fish assemblages at depths of 50 m or deeper (BROKOVICH *et al.,* 2008 to 65 m depth; BEJARANO *et al.,* 2010; 2014 to 70 and 85 m depth; PINHEIRO *et al.,* 2015 to 130 m depth), probably due to the increased logistical challenges associated with depth even today (ANDRADI-BROWN *et al.,* 2016a; 2016b). Two diving techniques make it possible to approach to these depths. The first one is open-circuit trimix SCUBA equipment, which has rarely been used for studies of fishes, with the single example of collecting fishes in Mediterranean (GLAVIČIĆ & KOVAČIĆ, 2016). However, gas spending of this open-circuit rig limited the effective bottom times at greater depths to no more than 12-15 minutes (PYLE, 1999) so its use is limited to short tasks. The second technique is mixed gas closed circuit rebreather (CCR) technology which is used even more rarely applied in studies of fishes than open-circuit trimix SCUBA technology (PARRISH & PYLE, 2002; BEJARANO *et al.,* 2014; PINHEIRO *et al.,* 2015). Compared to open-circuit trimix SCUBA equipment, CCR has much higher autonomy underwater, decreases the risk of decompression sickness, reduces overall gas requirements, increases overall safety, and is bubble-free (PINHEIRO *et al.,* 2015). These properties make it more promising for use in the visual census of deep bottom fish assemblages. However, CCR has serious limitations in terms of the price of equipment and the cost of fieldwork, as well as the need for highly skilled and trained CCR divers who must also be at the same time experts in fish species identification. The use of underwater video technique can overcome the later limitation by separating the CCR diving skills from the fish species identification knowledge. Rebreather diving techniques combined with the less time consuming visual census method thanks to the higher speed of the underwater scooter and the high quality video camera could make visual census studies below 40 m, even down to 100 m depths, a standard method.

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Utjecaj dubine na zajednicu riba stjenovitog dna u Mediteranu istraženu novom metodom videa sa podvodnog skutera

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SAŽETAK

Nova metoda videa sa podvodnog skutera uz rebreather ronilačku tehniku uvedena je i primjenjena za vizualni census zajednica riba. Ova metoda omogućuje istraživanja vizualnim cenzusom na dubinama većim od 40 m, na kojima se, zbog teškoća u ronjenju na većim dubinama, vizualni cenzus rijetko izvodi. Metoda videa sa podvodnog skutera uz rebreather ronilačku tehniku također bi trebala biti manje uznemirujuća i brža u odnosu na plivajuće ronioce. Metoda je primjenjena u istraživanju vizualnim cenzusom na dubinama 8 do 50 m na dvije postaje na istočnom Jadranu sa 102 video transekta. Zabilježene su također varijable okoliša koje utječu na varijaciju u sastavu zajednice riba stjenovitog dna infralitorala i cirkalitorala.

Trideset i jedna vrsta riba je zabilježena, uz 10,3% primjerka koji nisu određeni do razine vrste. Prosječna gustoća riba po transektu bila je 0,8 primjeraka/m^2, a najčešće i najbrojnije vrste bile su *C. chromis*, *C. julis* i *G. auratus*. Tri varijable okoliša: dubina, dno sa životinjskim obraštajem i istočna orjentacija dna, bile su izrazito značajne za prisutnost vrsta. Brojnost primjeraka i bogatstvo vrsta nisu pokazale značajnu promjenjivost sa promjenama dubine, nagiba, orjentacije i postaja. Utvrđena je značajna promjena u sastavu zajednice riba sa promjenom orjentacije obale, dubine i nagiba dna na transektima. Sastav vrsta postepeno se mijenjao sa porastom dubine dna i s povećanjem nagiba dna.

Ključne riječi: Podvodni vizualni census, zajednica riba, video transekt, dubina, Jadransko more, rebreather zatvorenog kruga