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Performance of a baited underwater video system vs. the underwater visual census technique in assessing the structure of fish assemblages in a Mediterranean marine protected area

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Abstract

Accurate, rapid, and cost-effective fish assemblage monitoring is fundamental for marine protected area (MPA) management as a pivotal tool to verify whether and to what extent MPA conservation objectives have been achieved and to redefine these objectives in the framework of an adaptive management. Recently, there has been a sharp increase in the number of video-based methods to study fish fauna, such as baited remote underwater video (BRUV) systems, that, depending on the objectives of the monitoring, can provide complementary or additional data to the more commonly used underwater visual census (UVC). Even though BRUV systems have been widely used in a wide range of geographic contexts and habitats, their use in the Mediterranean basin is still sporadic and the evaluation of the efficiency of BRUV systems and whether they can be used to complement other techniques needs investigation. Thus, the objective of this study was to assess the performance of a BRUV system in a Mediterranean MPA and to evaluate its effectiveness in assessing the structure of fish assemblages (abundance and species richness) by comparing estimates with those obtained by the UVC technique. The fish fauna was monitored by BRUV and UVC in the Capo Caccia – Isola Piana Marine Protected Area (Sardinia, Italy), in July and October-November 2020, at four sampling sites and two areas, hundreds of meters apart, for each site. Overall, 46 taxa and a total of 3620 individuals were observed by BRUV, while 36 taxa and a total of 2995 individuals were observed by UVC. The species first observed in front of the camera's field of view and able to reach the maximum abundance were the planktivores (*Chromis chromis* and *Oblada melanura)* followed by several carnivorous species belonging to the families Labridae, Serranidae and Sparidae, and lastly two carnivores (*Mullus surmuletus* and Mugilidae spp.) and some high-level predators (*Dentex dentex, Seriola dumerili, Sphyraena viridensis, Dicentrarchus labrax*). The maximum species richness and abundance were reached between 39 and 50 min. The cumulative species richness increased until around 30 min. Species richness was higher during the BRUV compared to the UVC monitoring. The consistency in findings between BRUV and UVC and a better performance of BRUV in detecting some species (mainly high-level predators), supports BRUV as an additional technique for describing and quantifying species richness and abundance also in the Mediterranean Sea. Based on the results of this study, the advantages/disadvantages, shortcomings, suggestions and resources needed for the two techniques are outlined.

Keywords: Fish fauna monitoring; video-based system; BRUV; Mediterranean Sea.

Introduction

Conservation of marine biodiversity and the associated ecosystem services are the main goals of marine protected areas (MPAs; Costanza *et al*., 1997; Fletcher *et al*., 2011; Leenhardt *et al*., 2015). Due to its complexity, managing an MPA requires tools and strategies to balance conservation and economic interests to foster the achievement of the objectives for which the protected area has been established (Rigby *et al.,* 2019). Commonly, the ecological effectiveness of MPAs is estimated based on the recovery of fish assemblages, probably as the re-establishment of depleted species is the most obvious response to restrictions on fishing pressures (Molloy *et al.,* 2009). Besides being one of the main components of biodiversity, fish fauna also play an important role in both the ecological processes of marine ecosystems (Guidetti, 2006) and the economic activities of coastal communities, such as fisheries and sea-related tourism (e.g., diving, snorkeling and excursions; Harasti *et al.*, 2015). Therefore, accurate, rapid, and cost-effective fish assemblage monitoring is a pivotal tool for MPA management (Baker *et al*., 2016), to verify whether and to what extent the MPA conservation objectives have been achieved, thus evaluating the reserve effectiveness, and redefining these objectives in the framework of the adaptive management (Rojo *et al*., 2021). In fact, an inadequate assessment of the state of fish assemblages can lead to an increase in exploitation quotas, in the case of excessive estimates of their abundance, or to an unnecessary tightening of restrictions on human activities, in the event of underestimation (Ward-Paige *et al*., 2020).

Estimating fish assemblages may require complex sampling techniques. Several methodologies have been developed to investigate fish abundance and diversity (Kingsford & Battershill, 1998), however, the most frequently used in temperate reefs is based on the direct observation by scientist-divers, known as "underwater visual census" (UVC; Harmelin-Vivien *et al*., 1985). If not properly planned, UVC results can be affected by some potential sources of error: i) environmental conditions, such as differences in water clarity and habitat characteristics among surveys, may affect the detectability of fishes (Thresher & Gunn, 1986; Pais *et al*., 2014; Figueroa-Pico *et al*., 2019); ii) the difference in the type and number of target species being counted simultaneously (Lincoln Smith, 1989); iii) the divers' swimming speed (De Girolamo & Mazzoldi, 2001) and the divers' effects on fish behavior (Dickens *et al*., 2011); iv) the survey area dimensions (Sale & Sharp, 1983; Cheal & Thompson, 1997; Jones *at al*., 2015) and the survey methodology utilized (e.g. transect length and width or stationary vs. point count method; Cheal & Thompson, 1997; Colvocoresses & Acosta, 2007; Prato *et al*., 2017; Pais & Cabral, 2018); v) characteristics of target fishes (large, non-schooling fishes tend to be more likely counted than small, schooling, highly mobile, or cryptic fishes that can be missed (Lincoln Smith, 1989; Samoilys & Carlos, 2000; Stewart & Beukers, 2000; Willis, 2001; Kulbicki *et al*., 2010; Bozec *et al*., 2011); vi) fish behavior (Pais & Cabral, 2018); vii) inter-observer variability (differences among observers), and intra-observer variability (inexperienced divers who gain experience during the surveys) (Harvey *et al*., 2001; Williams *et al*., 2006; Andradi-Brownet *et al.,* 2016 and references therein). The latter aspect deserves particular attention in the planning of long-term MPA monitoring required by management since a turnover of observers over time is likely to occur.

Recently, there has been a sharp increase in the number of video-based methods to study fish fauna due to the spread of relatively cheap digital devices and the availability of software for image processing (Stobart *et al.,* 2007). Baited remote underwater video (BRUV) systems (video systems equipped with bait to attract fauna close to the cameras) may provide a convenient complementary technique with respect to the most used UVC (Cappo *et al*., 2006), especially in the case of monitoring that simultaneously sample different types of species (Willis, 2000). BRUV systems are non-destructive methods, that do not involve the withdrawal of fish resources and are well suited to the needs of MPA. The advantages of such systems are a reduction in observer errors linked to the lack of identification or incorrect recognition of the species observed (Harvey *et al*., 2004; Cappo *et al*., 2006), the possibility of being used at night and at depths greater than 40 m (Cappo *et al*., 2004). They can also provide complementary data, such as those related to fish behaviour (Cappo *et al*., 2006; Lowry *et al*., 2012), which can be stored indefinitely for further analysis and used to extract videos and images suitable to disseminate scientific content to the public (Cappo *et al*., 2006). However, even BRUV systems are subjected to potential biases such as: i) the surface of the area under investigation remains unknown, since the attraction exerted by the bait varies according to many factors (e.g. currents, seabed topography, type of bait, appetite of fish and fish behavior) ii) species richness and abundance may be influenced by the competition between different species attracted by the bait (Willis & Babcock, 2000; Cappo *et al*., 2003), leading to an overestimation of some trophic groups, such as carnivores (Andradi-Brown *et al*., 2016). To reduce the sources of bias related to both UVC and BRUV systems, the simultaneous use of multiple methods to obtain more precise estimates in the abundance and richness of fish fauna has been suggested (Willis *et al.,* 2000; Cappo *et al.,* 2004; Aglieri *et al*., 2020).

BRUV systems have been frequently used in a wide range of geographic contexts (Malcolm *et al.,* 2007; McLean *et al.,* 2011; Rees *et al.,* 2014) and habitats (Yeh & Drazen, 2009; Malcolm *et al.,* 2011; Harvey *et al.,* 2012; Lindfield *et al.,* 2014), but their use in the Mediterranean basin is still sporadic (Stobart *et al.,* 2007; Stobart *et al.*, 2015; Whitmarsh *et al.,* 2017; Aglieri *et al*., 2020; Torres *et al*., 2020; Cattaneo *et al*., 2021). Even when BRUV was used in the same geographic contexts (e.g., temperate area), the optimal scenario (related to the number of replicates, soak time, bait type) was not clearly identified, showing that the most appropriate sampling strategy and method to use should be planned on a case-by-case basis (Whitmarsh *et al*., 2017). The evaluation of the efficiency of BRUV systems and whether they can be used to complement other techniques is necessary to validate their applicability, especially when they are used in MPAs, where surveys are needed to address conservation and management decisions (Baker *et al*., 2016). Thus, the objectives of this study were to: i) assess the performance of a BRUV system in a Mediterranean MPA, in terms of species richness and diversity; ii) determine the effective BRUV soak time; iii) evaluate BRUV effectiveness by comparing estimates with those obtained by a contemporary monitoring by UVC technique; iv) calculate a cost/benefit ratio to estimate cost and precision of both techniques.

Materials and Methods

Study area and experimental design

The fish fauna was investigated using two techniques, BRUV and UVC, during July and October- November 2020 in the Capo Caccia – Isola Piana Marine Protected Area (hereafter MPA), in the western Mediterranean Sea (Sardinia, Italy). The MPA was established in 2002 and comprises three zones with different levels of protection: i) A zone, integral protection (only authorized scientific research is permitted); ii) B zone, partial protection, and C zone, general protection (fishing activity, anchoring and mooring are permitted but regulated and boat speed limits in B zone are 5 knots lower than in C zone).

BRUV system and deployment

Four sampling sites within the MPA were selected: one in the A zone (Sant'Antonio, SA), one in the B zone (Punta Giglio, PG), and two in the C zone (Bramassa, BR and Mugoni, MU, Fig. 1). The BRUVs were deployed on three rocky sites with small patches of the seagrass *Posidonia oceanica* at a depth between 7 and 15 m (BR, PG and SA), and one sandy site with *P. oceanica* dead matte or *Cymodocea nodosa* within 5 m of depth. These sites were chosen to satisfy the MPA's management needs of monitoring both rocky and sandy habitats, representing all the protection levels of the MPA. Within the boundaries of the MPA, only one site in the C zone is characterized by sandy bottom. At each site, two areas hundreds of meters apart were chosen. In each area, three BRUV units were randomly deployed in July and October and between 9 am and 3 pm (to avoid changes in fish behaviour during crepuscular time; Bond *et al.*, 2018), obtaining a total of 48 recordings/replicates (24 in summer and 24 in autumn). BRUV units were never deployed simultaneously in the same area, in order to minimise the overlap of bait odour and obtain independent data (Willis & Babcock, 2000; Harvey *et al.*, 2007). However, two BRUV units were deployed on the same day and site, at least 2 hours apart from each other (Andradi-Brown *et al.*, 2016). The time necessary to deploy the BRUV unit and leave the site by boat was approximately 1 hour, while the soak time was set for 50 min: a trade-off between the minimum time suggested for BRUV monitoring of a rocky reef (30 min; Harasti *et al.*, 2015) and the camera's battery capacity (55-60 min).

Fish richness and abundance were measured by means of a customised BRUV system equipped with a high-resolution camera (GOPRO Hero 6 or 7) inside waterproof housing with horizontal orientation. The camera was mounted on the lowest part of a PVC frame at a forward-facing angle, 30 cm above the seafloor, and set to record a wide field of view (horizontal 130°, vertical 94°) with 2.7k resolution (2704x1520) and 30 FPS (frames per second). A 100 cm long bait pole was located 50 cm above the camera and equipped with a PVC mesh bait bag, containing 400 g of chopped, locally sourced pilchards (*Sardina pilchardus)*. Pilchards were chosen as bait because they are considered among the most common (Whitmarsh *et al.*, 2017) and effective (Dorman *et al.*, 2012; Harasti *et al.*, 2015), and the quantity to be used was evaluated after a series of deployment tests (quantities larger than 400 g were excessive due to the low bait predation rate). The

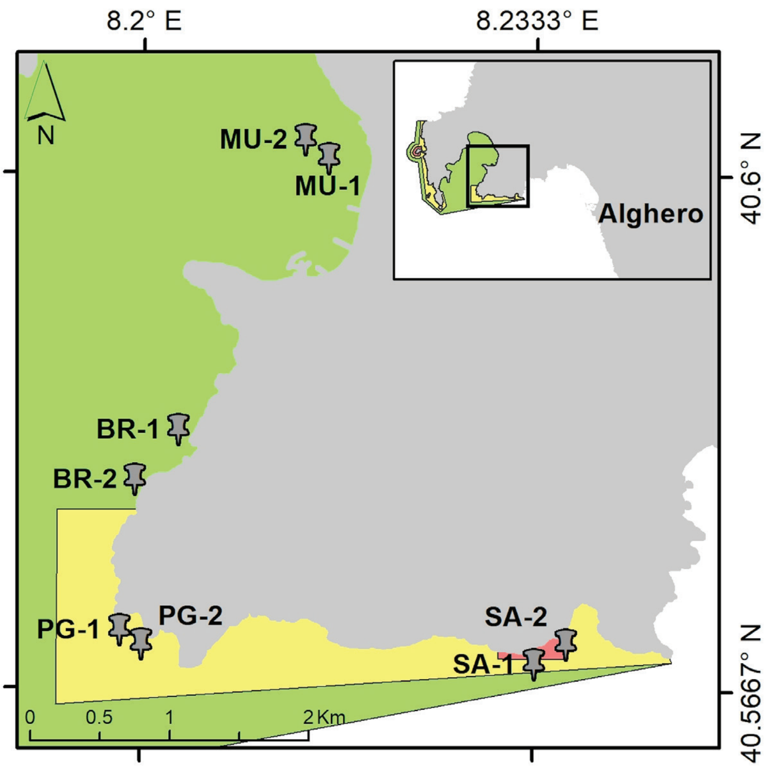

Fig. 1: Study area and sampling sites. Mu: Mugoni; BR: Bramassa; PG: Punta Giglio; SA: Sant'Antonio. 1 and 2: areas. A zone in red; B zone in yellow; C zone in green.

elevated position of the bait with respect to the bottom was aimed at increasing odour plume dispersion (Bosh *et al.*, 2017). The pole to which the bait was attached was 2.5 cm wide, thus, its presence did not limit the camera's field of view. A 2 kg ballast was attached to each base of the frame to improve BRUV stability, while a floating label was attached to the upper site of the frame to help in recovery. BRUV systems were deployed from the boat and a free diver checked for the correct position on the seafloor, moving the BRUV and searching for a better location in a few cases. After deployment, the research boat left the area within 4 minutes. To avoid boat disturbances to fish behaviour, the first 4 min of video were not considered in the analysis.

UVC technique

Following the same experimental design, UVC was conducted at three of the four sites monitored by BRUV (SA, PG, MU), in the same periods and areas. BR was not included due to logistical limits. To avoid any dependence of one technique's data on the other, BRUV and UVC were not performed simultaneously in the same site and day.

UVC involved a fish count along three transects of 25 m in length and 5 m in width (Harmelin-Vivien *et al.*, 1985) in each area. In order to limit the effect of the operator on fish behaviour, data collection was performed simultaneously with the deposition of a metric string on the seabed to precisely define the length of the transects (Kulbicki, 1998; Edgar *et al.*, 2004; Dickens *et al.*, 2011; Franzitta *et al.*, 2019), while its width was visually estimated. The speed along the transect was kept to about 3-4 m per minute. Samplings were performed in calm sea conditions with at least 25 m of visibility between 9:00 am and 3:00 pm to minimise the temporal variability in fish assemblages throughout the day (Willis *et al.*, 2006). In each transect, all the species and number of individuals for each were recorded. Fish species were identified to the lowest possible taxonomic level (species or family level) and the abundance of schooling fish (e.g., *Chromis chromis*) was grouped into abundance classes.

Video and data analysis

Video files were analysed via the SeaGIS Event Measure software (www.seagis.com.au). A trained observer analysed all the videos, while a senior supervisor randomly checked for quality and consistency of fish identification (Langlois *et al.*, 2020). Footage was analysed for the 50 min soak time, recording the MaxN value of each species (the maximum number of individuals observed in one single frame, Priede *et al.*, 1994) every 30 seconds (Willis & Babcock, 2000). MaxN is considered a conservative measure of relative abundance of a species, since it reduces the effect of double counting the same individuals (Willis *et al.*, 2000; Coppo *et al.*, 2003). This is the most widely used metric for BRUV (Whitmarsh *et al.*, 2017) and allows for the comparison of data collected in different studies. Taxa were identified to the lowest possible taxonomic level (species or family level; Langlois *et al.*, 2020). Individuals were not counted nor included in the analysis in case of lack of confidence in the identification. For each species, the time of the first seen was also recorded.

The frequency of occurrence of each species was calculated as the percentage of samples in which the species was observed (Colton & Swearer, 2010). For each deployment, the following variables were calculated: i) species richness, ii) total abundance (TotMaxN) as the sum of the MaxN of each species present in a deployment, iii) the Shannon-Wiener diversity index and iv) evenness, using MaxN as an abundance proxy. The differences in the values of these variables as a function of site (random factor, four levels: SA, PG, BR, MU), period (fixed factor, two levels: summer and autumn) and area (random factor, two levels: 1 and 2; nested in site) were explored with four three-way ANOVAs. After testing for outliers, the normality of the data distribution with the Shapiro-Wilk test and heterogeneity with the Bertlett test, the TotMaxN and the Shannon-Wiener diversity index variables were square root transformed.

A non-metric multidimensional ordination (nMDS) was produced from the sample similarity matrix to visually represent the similarity of fish assemblage structure between sites and periods. The MaxN values for each species were fourth root transformed before calculating the Bray-Curtis similarity in R (similarity matrix), to reduce the influence of schooling fish species present in large numbers and patchily distributed (Clarke & Gorley, 2006). A one-way non-parametric similarity analysis (ANOSIM) was applied on the same matrix to test the null hypothesis that there was no difference in species composition between the levels of each factor (site and period).

BRUV effectiveness and comparison with UVC

The effectiveness of the BRUV technique was tested in various ways. To verify if the soak time (50 min) was suitable to identify all the species present, the following variables were calculated: i) the mean time needed to reach the MaxN for all species together and for each species observed in at least three samples and ii) the average time of first seen for each species observed in at least three samples. Further, the cumulative number of species was plotted (for each single site and for all sites combined) against the soak time, in blocks of 5 min.

The comparison between the BRUV and UVC techniques was carried out on the three common sites: Sant'Antonio (SA: A zone, rocky bottom), Punta Giglio (PG: B zone, rocky bottom) and Mugoni (MU: C zone, sandy bottom), for a total of 36 replicates per technique. Since the UVC recorded all the individual fish seen along the transect, while the BRUV recorded the MaxN of each species from a fixed position, we could not compare the raw fish abundance values between the two techniques, thus only species richness was compared quantitatively. Species richness was tested with a two-way ANOVA using the factor technique (fixed factor, two levels: BRUV and UVC) and site (random factor, three levels: PG, SA, MU). Additionally, a non-metric multidimensional ordination (nMDS) was produced from the sample similarity matrix to visually represent the similarity of the species composition between techniques and sites. To identify the trophic groups influenced by bait or SCUBA diver presence, all species were grouped into a trophic level: herbivore, detritivore, carnivore, high-level predator, or planktivore (Sala *et al*., 2012; Roberson *et al.,* 2015). The trophic levels were qualitatively compared between the two techniques.

To verify if the number of samples was sufficient, a species accumulation curve was calculated with the "speccacc" function of the Vegan package in R. Generally, the curve increases rapidly with some units of sampling. Then, as the number of samples increases, the slope curve increases slower until the asymptote. To compare the two techniques, BRUV and UVC, the same curve was constructed using the number of BRUV deployments and the number of transects by UVC. All statistical analyses were performed in R (R Core Team, 2015).

At the end, a cost/benefit ratio was calculated for both techniques using the following formula (Souza and Barros, 2014): $CB = (C_t / (1-p))/1000$, where C_t is the total cost and p is the "precision". Thus, a small value generated by lower cost and higher precision produces a better cost-benefit ratio. The total cost was calculated considering the expenses for field work, data entry, analysis and reporting. Personnel costs were estimated based on the collective labor agreement in force in Italy for the Education and Research sector. The costs of the equipment necessary for both techniques were not included because they are durable goods subject to depreciation, and the economic impact of both options on the total costs (for the period of use considered) can be considered negligible. Precision was calculated as SE/X (Souza and Barros, 2014), where SE is the standard error and X is the mean species richness. The value obtained from the ratio is inversely related to precision. Namely, when the SE is small compared to the mean, the ratio is lower, and the precision is higher (Andrew & Mapstone, 1987). The SE was calculated considering mean species richness and standard deviation and a sample size of 36, corresponding to the number of UVC transects and BRUV deployments.

Results

Fish richness and abundance

BRUV monitoring has overall recorded 46 taxa, belonging to 18 families. In particular, 32 species were observed at BR (C zone, rocky bottom), 37 species at PG (B zone, rocky bottom), 33 species at SA (A zone, rocky bottom) and 18 species at MU (C zone, sandy bottom). Nine species were present in 92% to 60% of the deployments (*Diplodus sargus, Coris julis, Diplodus vulgaris, Chromis chromis, Symphodus tinca, Oblada melanura, Serranus cabrilla, Diplodus annularis, Sarpa salpa*), seven taxa between 46% and 20% (*Thalassoma pavo, Symphodus melanocercus, Serranus scriba, Sphyraena viridensis, Sparus aurata,* Mugilidae*, Diplodus puntazzo*), six species between 19% and 10% (*Mullus surmuletus, Seriola dumerili, Symphodus mediterraneus, Symphodus doderleini, Symphodus ocellatus, Symphodus roissali*), and the remaining 24 taxa were present in less than 10%. A significant effect of the site on species richness and fish abundance was found (Table 1 and 2; Fig. 2). The Shannon-Wiener and the evenness index did not change with site, area and between periods (Table 2). Consistently, the nMDS indicated a separation between sites and no separation between periods (Fig. 3). These results were confirmed by the one-way non-parametric similarity analysis (ANOSIM), which identified a significant difference in the species composition by site (ANOSIM, $p = 0.025$, $R = 0.30$) and no difference by period (ANOSIM, $p = 0.1265$, $R = 0.025$).

Table 1. Descriptive statistics (mean, standard error, maximum and minimum) of MaxN, species richness, Shannon-Wiener index, and evenness, for all sites and for each site.

	MaxN	Richness	Shannon	Evenness
All sites				
Mean	95.19	11.04	1.52	0.66
SE	11.61	0.68	0.08	0.03
Max	339	21	2.32	0.98
Min	1	1.00	0.00	0.00
BR				
Mean	79	12.00	1.79	0.72
SE	62	3.30	0.45	0.16
Max	261	19.00	2.32	0.92
Min	19	8.00	0.73	0.35
PG				
Mean	159	13.50	1.53	0.59
SE	87	3.45	0.57	0.22
Max	339	18.00	2.28	0.94
Min	24	8.00	0.53	0.23
SA				
Mean	126	13.50	1.49	0.58
SE	60	3.94	0.43	0.17
Max	209	21.00	2.07	0.93
Min	18	7.00	0.64	0.33
MU				
Mean	17	5.17	1.29	0.73
SE	14	2.29	0.64	0.35
Max	42	8.00	1.89	0.98
Min	1	1.00	0.00	0.00

Table 2. Output of the ANOVAs run on species richness, abundance (TotMaxN), Shannon-Wiener index and evenness monitored by BRUVs, as a function of the factors period, site, area and their interactions (":"). Significant values in bold.

	df	SumSq	Mean Sq	F value	P value
SPECIES RICHNESS					
Period	1	1.33	1.33	0.1091	0.76290
Site	3	570.25	190.08	12.8146	**0.01612**
Period: Site	3	36.67	12.22	1.1508	0.34188
Site:Area	4	59.33	14.83	1.3967	0.25471
Residuals	36	382.33	10.62		
ABUNDANCE (TotMaxN)					
Period	1	21.28	21.28	6.8366	0.07936
Site	3	495.11	165.04	8.7147	**0.03152**
Period: Site	3	9.34	3.113	0.4168	0.74197
Site:Area	4	75.75	18.938	2.5361	0.05683
Residuals	36	268.83	7.467		
SHANNON WIENER					
Period	1	0.006	0.0062	0.1337	0.73889
Site	3	0.516	0.17197	5.1233	0.07422
Period: Site	3	0.139	0.04644	0.4341	0.72990
Site:Area	4	0.1343	0.03356	0.3137	0.86691
Residuals	36	3.8518	0.10699		
EVENNESS					
Period	1	0.01659	0.01659	0.8683	0.4202
Site	3	0.24623	0.082078	2.2651	0.2230
Period: Site	3	0.05732	0.019108	0.3049	0.8216
Site:Area	4	0.14495	0.036236	0.5783	0.6803
Residuals	36	2.25596	0.062665		

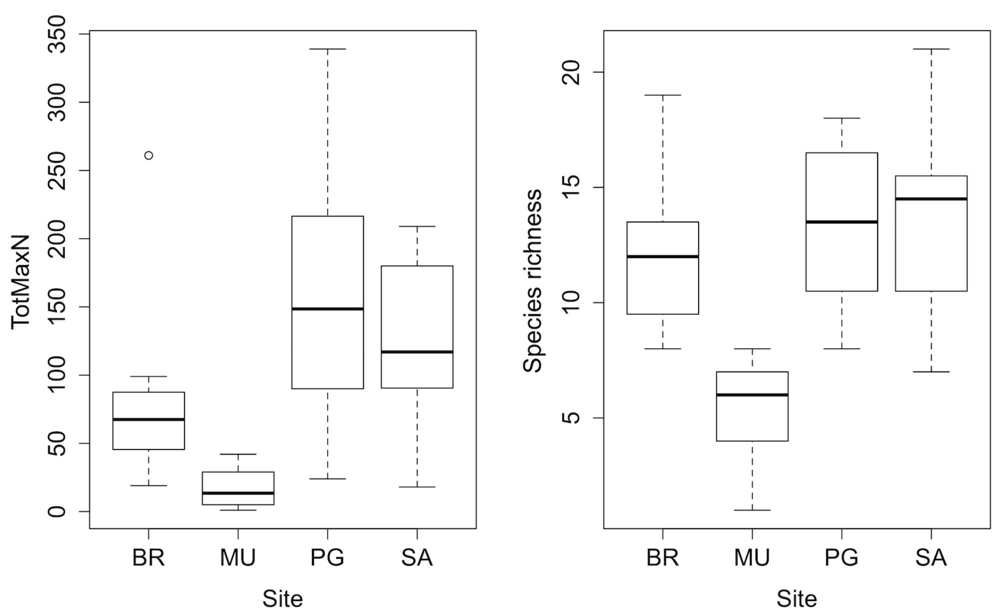

Fig. 2: Summary of the ANOVAs showing only the significant factors, abundance (TotMaxN) and species richness, observed by BRUV. The thick black lines represent the medians, the boxes encompass the 25% and 75% quartiles, the whiskers extend to the most extreme data points within 1.5 x the interquartile range outside the box, and the circles show data points beyond the whiskers.

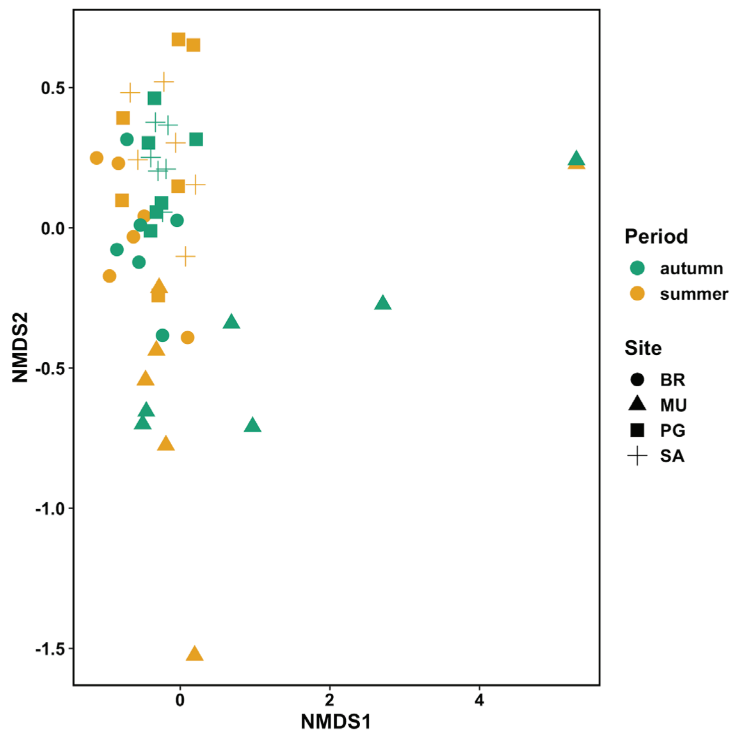

Fig. 3: Multidimensional scaling plots showing the similarity of species composition (detected by BRUV) among sites, grouped by period.

Soak time

The mean time of first seen at the BRUV and the mean time to reach the MaxN varied greatly depending on the species (Table 3), but the mean values for any species were all below 31 min 37 sec ± 15 min 18 sec and 36 min 5 sec ± 13 min 13 sec, respectively. The first species observed in the camera's field of view were the planktivorous *Chromis chromis* and *Oblada melanura*, which appeared within 4 min on average. They were also the fastest to reach the MaxN (below 24 minutes). Subsequently, 19 carnivorous species belonging to the families Labridae, Serranidae and Sparidae were observed in a mean time ranging between 6 and 21 min, but the mean time to reach the MaxN increased up to 37 min. Lastly, four species of high-level predators (*Dentex dentex, Seriola dumerili, Sphyraena viridensis, Dicentrarchus labrax*), Mugilidae and *Mullus surmuletus* arrived in the camera's field of view on average within 22 min, and took a mean of 28 min to reach the MaxN. The maximum species richness and the TotMaxN were reached in all deployments between 39 and 50 min, apart from four, where 25 min were enough (Fig. 4). The cumulative species richness increased generally up to about 30 min (Fig. 5).

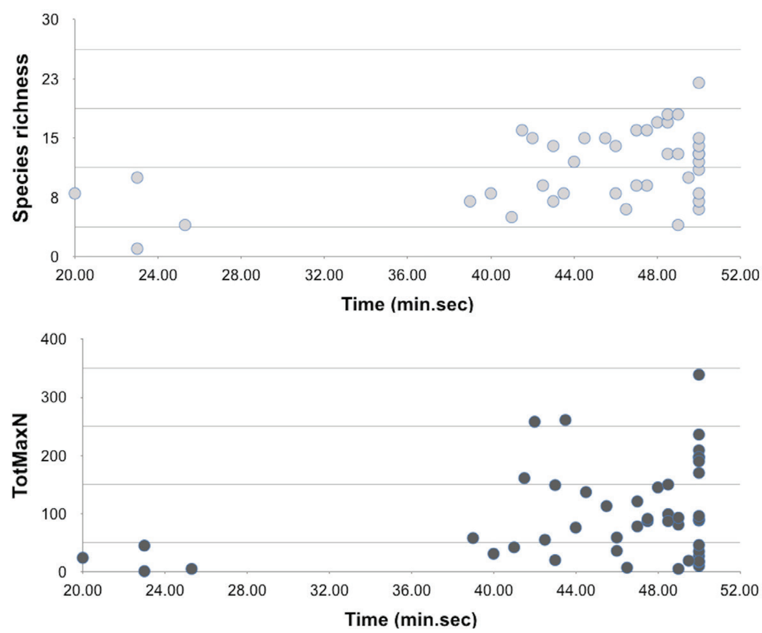

Fig. 4: Time necessary to reach species richness and TotMaxN in each BRUV deployment.

Table 3. Mean and standard deviation of the time of first seen and the time to MaxN per species. Trophic level: CA = carnivore; HE = herbivore; PL = planktivore; HP = high-level predator. Species are listed in order of mean time of first seen.

Trophic level	Family	Species	Mean Time of first seen	SD	Mean Time to MaxN	SD
PL	Pomacentridae	C. chromis	0:02:54	0:03:48	0:24:21	0:16:13
PL	Sparidae	*O. melanura*	0:03:45	0:05:18	0:22:34	0:15:57
CA	Labridae	*C. julis*	0:04:28	0:07:46	0:22:11	0:15:40
CA	Sparidae	*D. vulgaris*	0:06:27	0:10:33	0:18:24	0:15:48
CA	Sparidae	*D. sargus*	0:07:40	0:08:40	0:37:09	0:14:06
CA	Sparidae	*D. annularis*	0:07:54	0:08:13	0:19:17	0:14:09
HE	Sparidae	*S. salpa*	0:08:01	0:10:15	0:23:42	0:14:26
CA	Serranidae	*S. cabrilla*	0:08:39	0:12:52	0:12:51	0:12:15
CA	Sparidae	*S. cantharus*	0:09:22	0:08:35	0:13:22	0:08:36
CA	Labridae	*S. rostratus*	0:11:09	0:06:00	0:15:09	0:06:00
CA	Labridae	*L. viridis*	0:11:15	0:08:00	0:15:15	0:08:01
HE	Blennidae	*P. rouxi*	0:11:20	0:15:53	0:15:20	0:15:54
CA	Labridae	*S. tinca*	0:12:29	0:13:07	0:24:26	0:15:47
CA	Serranidae	*S. scriba*	0:13:18	0:14:17	0:19:27	0:14:29
CA	Labridae	*T. pavo*	0:13:50	0:12:29	0:25:10	0:16:48
CA	Labridae	*S. roissali*	0:14:57	0:15:31	0:26:06	0:16:50
CA	Labridae	*S. melanocerus*	0:15:30	0:14:05	0:25:17	0:14:15
CA	Labridae	*S. mediterraneus*	0:15:43	0:10:58	0:21:38	0:14:23
CA	Sparidae	*D. puntazzo*	0:16:13	0:16:52	0:26:31	0:17:58
CA	Labridae	*S. ocellatus*	0:17:24	0:16:36	0:23:19	0:15:31
CA	Apogonidae	*A. imberbis*	0:18:30	0:23:58	0:24:30	0:22:05
CA	Labridae	*L. merula*	0:19:39	0:15:00	0:23:39	0:15:00
CA	Sparidae	*S. aurata*	0:19:52	0:13:22	0:23:55	0:11:41
CA	Labridae	*S. doderleini*	0:20:38	0:14:53	0:27:17	0:16:59
CA	Mullidae	*M. surmuletus*	0:21:42	0:16:25	0:33:42	0:14:50
HP	Sphyraenidae	*S. viridensis*	0:22:09	0:13:22	0:28:36	0:15:07
HP	Serranidae	*D. labrax*	0:22:43	0:14:25	0:30:36	0:17:22
HP	Sparidae	*S. dumerili*	0:24:45	0:14:08	0:34:16	0:16:25
CA	Muggillidae	*Muggillidae spp*	0:27:37	0:15:42	0:36:05	0:13:13
HP	Sparidae	*D. dentex*	0:31:37	0:15:18	0:35:37	0:15:19

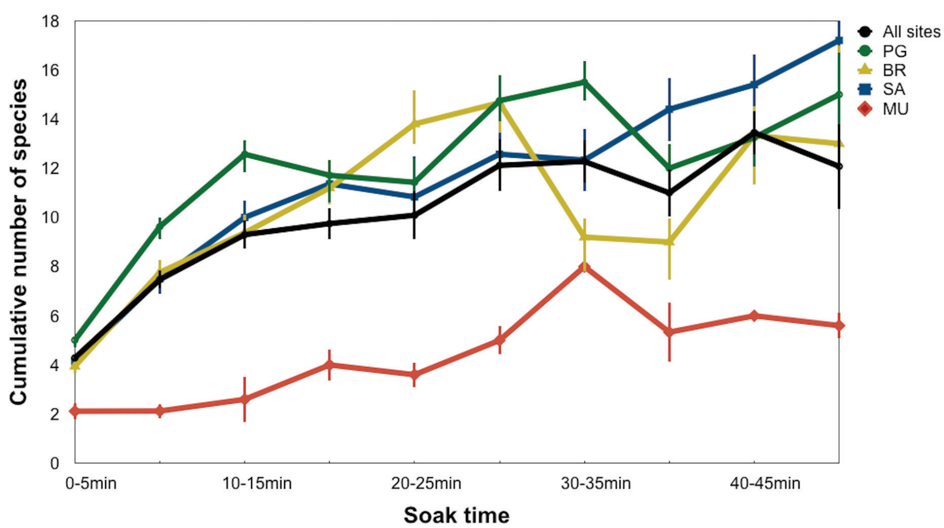

Fig. 5: Cumulative species richness against soak time (in blocks of 5 min), for each site and all sites combined.

At the sandy site (MU), 35 min was enough to reach the maximum species richness while in the rocky sites more than 45 min was necessary (Fig. 5).

BRUV-UVC comparison

Overall, 46 different taxa and a total of 3620 individuals were observed by BRUV, while 36 taxa and a total of 2995 individuals were observed by UVC. Seven and 16 species were observed exclusively by UVC and BRUV, respectively. Considering the trophic levels, the number of planktivorous and herbivores species was approximately the same between UVC (4 and 2 respectively) and BRUV (3 and 2, respectively). BRUV observed a higher number of carnivorous and high-level predators (30 and 7 respectively) in respect to UVC (27 and 2, respectively - Tables 4). The technique and site were found to be significant sources of variability (independent of one another) in species richness (Table 5). Overall, the nMDS indicated a separation between techniques and sites (Fig. 6).

To verify if the number of samples (deployments and transects) were suitable in investigating the species richness a species accumulation curve per each technique was

Table 4. Presence of species observed by UVC and BRUV. In bold the species observed exclusively by one of the two techniques. CA = carnivore; HE = herbivore; PL = planktivore; HP = high-level predator.

Trophic level	Family	Species	UVC	BRUV	Trophic level	Family	Species	UVC	BRUV
PL	Pomacentridae	*C. chromis*	+	+	CA	Sparidae	*D. puntazzo*	+	+
PL	Sparidae	*O. melanura*	+	+	CA	Labridae	*S. roissali*	+	+
PL	**Sparidae**	***S. maena***	**-**	**+**	**CA**	**Gobiidae**	***P. marmoratus***	**-**	**+**
PL	**Atherinidae**	**Atherina spp.**	**+**	**-**	CA	Labridae	*S. melanocercus*	+	+
PL	**Sparidae**	***S. smaris***	**+**	**-**	CA	Serranidae	*S. scriba*	+	+
HE	Sparidae	*S. salpa*	+	+	CA	Labridae	*S. mediterraneus*	+	+
HE	**Blennidae**	**Blennius spp.**	**-**	**+**	**CA**	**Scorpaenidae**	***S. notata***	**-**	**+**
HE	Blennidae	*P. rouxi*	+	+	**CA**	**Labridae**	***S. doderleini***	**-**	**+**
CA	**Mugilidae**	**Mugilidae**	**-**	**+**	CA	Sciaenidae	*S. umbra*	+	+
CA	Sparidae	*D. sargus*	+	+	CA	Labridae	*L. merula*	+	+
CA	Sparidae	*D. vulgaris*	+	+	CA	Tripterygiidae	Trypterigion spp.	+	+
CA	Labridae	*C. julis*	+	+	CA	Sparidae	*S. cantharus*	+	+
CA	Sparidae	*D. annularis*	+	+	CA	Labridae	*L. viridis*	+	+
CA	Labridae	*S. tinca*	+	+	CA	Scorpaenidae	*S. maderensis*	+	+
CA	Mullidae	*M. surmuletus*	+	+	CA	Labridae	*S. cinereus*	+	+
CA	Serranidae	*S. cabrilla*	+	+	CA	Labridae	*S. rostratus*	+	+
CA	Sparidae	*S. aurata*	+	+	**CA**	**Sparidae**	***D. cervinus***	**-**	**+**
CA	Callionymidae	Callionymus spp.	-	+	**CA**	**Scorpaenidae**	***S. scrofa***	**+**	**-**
CA	**Gobiidae**	**Gobius spp**	**-**	**+**	**HP**	**Sparidae**	***S. dumerili***	**-**	**+**
CA	**Gobiidae**	***G. geniporus***	**+**	**-**	**HP**	**Sphyraenidae**	***S. viridensis***	**-**	**+**
CA	**Gobiidae**	***G. incognitus***	**+**	**-**	**HP**	**Serranidae**	***D. labrax***	**-**	**+**
CA	Apogonidae	*A. imberbis*	+	+	HP	Serranidae	*E. marginatus*	+	+
CA	Labridae	*S. ocellatus*	+	+	**HP**	**Muraenidae**	***M. helena***	**-**	**+**
CA	**Serranidae**	***S. epatus***	**-**	**+**	**HP**	**Sparidae**	***D. dentex***	**-**	**+**
CA	**Labridae**	***X. novacula***	**-**	**+**	**HP**	**Scombridae**	***T. thynnus***	**-**	**+**
CA	Labridae	*T. pavo*	+	+	**AP**	**Scombridae**	***E. alletteratus***	**+**	**-**

Table 5. Output of the ANOVAs run on species richness as a function of the factors technique and site, and their interactions (":"). Significant values in bold.

	Df	Sum Sq	Mean Sq	F value	P value
SPECIES RICHNESS					
Technique	1	133.4	133.39	75.622	**0.01297**
Site	2	1220.2	610.10	89.581	**<0.0001**
Technique: Site	2	3.53	1.76	0.259	0.77261
Residuals	66	449.50	6.81		

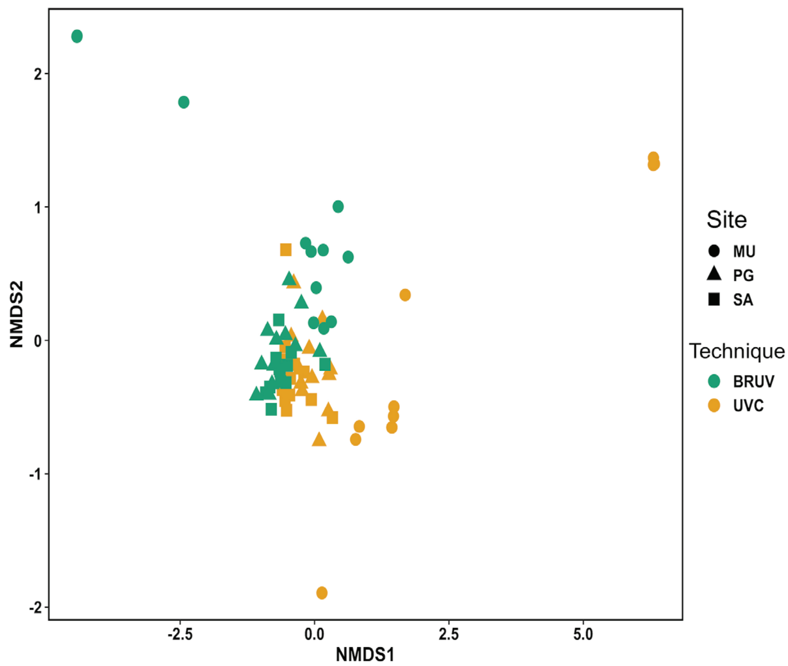

Fig. 6: Multidimensional scaling plots showing the similarity of species composition among sites grouped by technique.

calculated. With 10 samples, UVC and BRUV identified 69% and 65% of the total species, respectively. With 25 samples they identified 83% and 87% of the total species, respectively. For both techniques, the curve never tended to zero, indicating the need for more deployments and transects to identify the rare species. However, with the same number of samples, BRUV found a higher number of taxa than UVC (Fig. 7).

At the end, cost/benefit ratio analysis indicated that BRUV had a higher precision, but UVC had a better cost/benefit ratio, due to the relative lower total costs (Table 6). Even if both techniques required three days of field work and three days for data entry, analysis and reporting, another 12 days were necessary for BRUV video analysis. In particular, this corresponds to a cost per sampling unit (transect and deployment) of 121.59 € and 168.02 €, for UVC and BRUV respectively.

Table 6. Cost/benefit ratio analysis for the two techniques, BRUV and UVC. C_t: total cost; C_n: cost per sampling unit = C_t/n; n: number of sampling unit; *p*: precision = SE/X; SE: standard error; X: mean species richness; CB: cost/benefit ratio = $(C_t/1\text{-}p)/1000$. Costs in euro.

	BRUV	UVC
C_t	6,048.89 €	4,377.39 €
C_n	168.02 €	121.59 €
p	0.007	0.012
CB	**6.09**	**4.43**

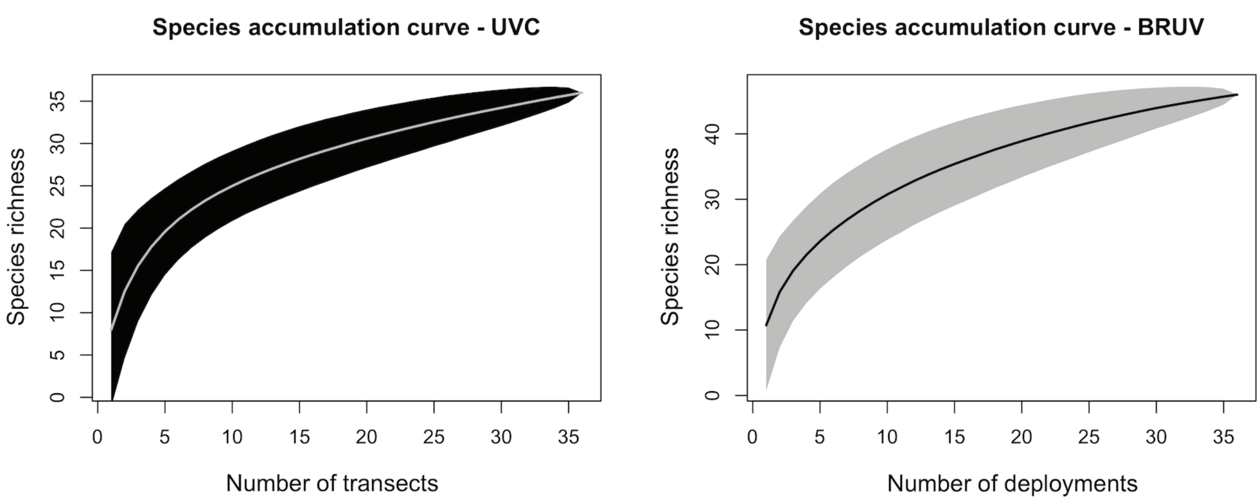

Fig. 7: Species accumulation curve for UVC (sx) and BRUV (dx).

Discussion

This study represents one of the few attempts to verify the performance of a customized BRUV system for assessing the fish assemblage structure in a Mediterranean area and to compare its effectiveness with UVC, the most used technique in temperate reefs. In comparison with video-based techniques, UVC is considered more efficient and cost effective in the shallow areas where it is usually performed (below 30-40 m; Gambi & Dappiano, 2004), and is the most widespread technique in MPA fish fauna monitoring programs (Tessier *et al.*, 2013; Prato *et al.*, 2017).

Given the small number of BRUV-based monitoring trials in the Mediterranean Sea (but see Stobart *et al.*, 2007; Stobart *et al*., 2015; Aglieri *et al*., 2020; Torres *et al*., 2020; Cattaneo *et al*., 2021), we assessed the performance of the technique based on the protocol guidelines suggested for other geographic contexts. Since these customised BRUV systems use low-cost underwater cameras, limited by the duration of the battery life, first it was necessary to verify whether a soak time of 50 min could be sufficient to identify the species present. The times of first seen in the camera's field of view, considering all species, were less than 31 min, well below the scheduled soak time. The shortest arrival times were recorded for planktivorous species, while the longer ones were found for the high-level predators. The time necessary to reach the maximum number of species and the maximum abundance in a sample was between 39 and 50 min, less than 25 min in only four samples (in the sandy site), indicating that the soak time was sufficient, but in general it should not be less than 30-50 min. This result is not consistent with Willis & Babcock (2000) and Cappo *et al.* (2004), who suggested a soak time between 23 and 30 min for monitoring fish fauna in New Zealand and Australia, but in agreement with the results found by Stobart *et al.* (2007) in the Mediterranean Sea. In fact, the latter suggested a minimum soak time between 15 and 30 min for the most reactive families (Pomacentridae, Sparidae and Serranidae) and a longer soak time for the high-level predators, finding a similar time of first seen and time to reach the MaxN with the present study (Stobart *et al.*, 2007). In the sandy areas a shorter time to reach the maximum number of species and the maximum abundance was found, likely due to the absence of high-level predators and the presence of species that react faster to the bait (namely Pomacentridae, Sparidae and Serranidae). Even if Harasti *et al.* (2015) found that BRUV's soak time of 30 min may be sufficient for monitoring species richness and relative abundance of key fishery species on rocky reefs, while a time up to 60 min would increase time and monetary costs without relevant benefit, a recent study (Birt *et al.,* 2021) highlighted that 60 min are necessary to characterize fish assemblage in temperate water. These apparently contrasting results show that soak times strongly depend on the target species and the habitat monitored, and that it should be planned based on the geographic context and previous knowledge on local fish community.

The effectiveness of the BRUV technique was tested in two habitat types (rocky reef and sandy bottom), with different levels of protection, as a function of different periods. During 48 deployments, at four sites and during two periods (summer and autumn), BRUV recorded 46 taxa, belonging to 18 families. The sites with the highest species richness and abundance were the rocky ones. Abundance was higher in the B zone (general protection) rather than the A zone (integral protection). The sandy site had the lowest abundance, species richness and Shannon-Wiener index values, consistent with the lower biodiversity, three-dimensional heterogeneity and complexity that characterizes these habitats compared to the rocky ones in the Mediterranean Sea (García-Charton & Pérez-Ruzafa, 2001; García-Charton *et al.*, 2004). No differences in the fish assemblage structure were found according to the period (even if in autumn the water temperature was 5-7° C lower than in summer), giving a first indication that the temporal stratification of the monitoring program by BRUV, usually adopted in UVC-based monitoring to obtain a more exhaustive description of the species diversity (Desiderà *et al.*, 2019), may not be necessary in Mediterranean temperate waters. Nevertheless, further investigations are necessary in other geographical contexts and with a larger sample size to clarify this particularly relevant aspect. In fact, the resources available to protected areas for monitoring are often limited, and unnecessary replication of surveys that do not lead to greater ecological knowledge could be avoided.

BRUV observed mainly carnivorous species (*Coris julis, Symphodus tinca, Diplodus sargus, Diplodus vulgaris, Diplodus annularis)* and some planktivores and herbivores *(Chromis chromis, Oblada melanura, Sarpa salpa),* allowing for a count of both the species attracted by the bait and those attracted by the movement of many fishes around the system. Even if there is an upper limit to the number of fish that can enter the camera's field of view at any given time, and this limit can underestimate the abundance of schooling fish where their density is very high (Willis *et al.*, 2003), BRUV detected high planktivore abundance. Other than carnivores and some planktivores, BRUV recorded high-level predators and some cryptic species, the latter in low percentages. Moreover, similar to other studies (Watson *et al.*, 2005; Colton & Swearer, 2010), some large high-level predators observed by BRUV, such as *Thunnus thynnus*, *Sphyraena viridensis*, *Seriola dumerili, Dentex dentex* and *Dicentrarchus labrax*, were absent in the monitoring by UVC. Species that are highly mobile and targeted by fishing may be better observed by BRUV than UVC, likely due to the escape response of these species to diver and human presence (Willis & Babcock, 2000; Watson & Harvey, 2007, Dickens *et al.,* 2011, Davis *et al*., 2019), or the larger area surveyed by BRUV compared to that sampled by UVC. Even if the low replication of the present study could have influenced the low number of high-level predators detected by UVC, this result, if confirmed by the ongoing study and the increase in sample size, may be extremely relevant for MPA monitoring since these species represent a target for both recreational and commercial fisheries and usually occur in low density (Peters *et al.*, 1983).

UVC has been considered more efficient than cameras in detecting cryptic species (Watson *et al.*, 2005; Stobart *et al.*, 2007) since scuba divers can search fish in complex habitats, including cracks and crevices. However, in the present study, some small cryptic fishes such as *Blennius* spp., *Callionymus* spp. and *Gobius* spp., as well as some more mobile fishes such as Mugilidae and *Serranus hepatus*, were sampled by BRUV, but not by UVC. In contrast, other species, such as Atherinidae, *Spicara smaris* and *Scorpaena scrofa*, were counted by UVC only. Strongly substrate-attached and territorial species, like some *Scorpaena* spp., may have not been detected by BRUV because the devices were deployed outside of their home range. Nevertheless, it is complex to explain the response to BRUV by the other more mobile species. Some species may be underestimated by BRUV either because they are less attracted by the bait since they are planktivorous, such as *Spicara smaris*, or because they are less bound to the sea bottom and easily outside the camera's field of view, such as the Atheniridae. However, as there are many factors that can influence the species response to the two monitoring techniques, such as seasonal and reproductive cycles, swimming speed, behavioral state of fish and their appetite, individual attraction and curiosity, life history, dietary preference, presence or absence of predators and size of the home range (Newman & Williams, 1995; Colton & Swearer, 2010; Phenix *et al*., 2019), and these complex and interacting factors have been little studied, especially in the Mediterranean Sea, further investigations are necessary to avoid speculative interpretations.

Fish assemblages depended on the technique used. Species richness was higher if identified by BRUV rather than by UVC, particularly in the site characterised by sandy bottom likely for the low fish abundance and species richness characterizing the MPA sandy habitat. In this habitat, with such poor resources, the bait may be more attractive than in other habitats, allowing the observation of species distributed over a wider area than that sampled by the UVC (Phenix *et al*., 2019). Furthermore, the scarce presence of predators in this habitat could have made the species observed by BRUVs less reluctant to approach the cameras.

The consistency in findings between BRUV and UVC and the better performance of BRUV in detecting some species, mainly high-level predators, supports the use of BRUV as an efficient complementary technique for describing and quantifying species richness and abundance in Mediterranean MPAs. These results are consistent with those found in other studies which compared BRUV to UVC, highlighting the greater ability of BRUV in terms of detecting species diversity (Willis *et al.*, 2000; Watson *et al.*, 2005; Aglieri *et al.*, 2020). In a similar geographic context as the Mediterranean Sea, one of the few studies where BRUV and UVC were compared, found that the latter technique recorded higher diversity and greater abundance of many species (Stobart *et al.,* 2007). The inconsistency with this study could be due to i) the use of a different bait (crushed sardines and effervescent bait pellet) which could have affected the type and abundance of the species attracted (Harvey *et al.*, 2007); ii) the soak time generally lower than 35 min and iii) the possibility of counting only the fish within 1.5 m of the camera, reducing the possibility of observing some more shy species, such as the high-level predators; iv) a bias due to the different sampling times for each technique within the same season, while in the present study BRUV and UVC samples were collected within a few weeks.

Concerning the adequacy of the number of deployments, the accumulation curves of the species showed that after 25 deployments, BRUVs detected 40 of the 46 total species, while with UVC, the 36 transects were not sufficient to equal the number of species observed by BRUV. However, the samples of the two techniques cannot be considered as equal (Colton & Swearer, 2010). Neither of the two species accumulation curves reached the asymptote, indicating that neither of the two techniques had the ability to identify the less common species with the number of samples (deployments and transects) collected, suggesting that a high replication would be necessary.

Based on the results of this study, we can outline shortcomings, suggestions and general resources needed for the two techniques (Tables 6 and 7). The cost/benefit ratio analysis, while indicating a higher precision using the BRUV technique (concerning species richness estimate), found a better cost/benefit ratio with the use of UVC due to its lower total costs. In about the same amount of time in the field (6 hours), four BRUV units can collect the same number of samples (12) as UVC performed by two scientifically trained divers. The time in the field is consistent with that reported by Stobart *et al.* (2007) and, even if the timing changes from one working group to another, it can be considered realistic. However, the number of samples that can be collected by BRUV decreases if there are fewer units available. In the latter case, the time in the field becomes considerably greater than that of the monitoring by UVC. The time and cost of BRUV data analysis is significantly higher than that required for UVC data entry and analysis. However, the lack of scientifically trained divers needed for UVC data collection, the relatively cheaper personnel costs needed for BRUV sampling, the lower risk in the field and the different use of the video and relative data collected by BRUV can balance its extra budget. In fact, the latter aspects are often cited among the main benefit of BRUV (Whitmarsh *et al.*, 2017).

The type of data collected by BRUV includes both the structure of fish assemblages and behavioural data, as well as a large amount of video footage that can also be used for educational or communication purposes. However, when the objective of monitoring concerns the evaluation of fish biomass, for example to measure the effectiveness of fishery protection in marine reserves (Russ & Aicala, 1996; Willis *et al.*, 2003), UVC is the fastest and least expensive technique. In fact, biomass data can only be obtained by BRUV equipped with calibrated stereo cameras, with a considerable increase in time and financial resources required. Furthermore, it is not possible to obtain fish density measurements with BRUV unless the area of bait dispersion can be properly estimated. Nev-

Table 7. Comparison between BRUV and UVC techniques. "+": higher compared to the other technique; "-": lower compared to the other technique.

		BRUV	UVC	NOTE
Sampling time		+	-	BRUV: up to 12 replicates (deployments) in 6 hours with 4 BRUV units. UVC: Up to 12 replicates in 4-6 hours with two scientifically trained divers. These times can change according to the working group, employer safety rules, distance between sampling sites, etc.
Analysis time		+	-	BRUV: from 100 min up to 240 min per video (soak time 50 min), depending on the fish abundance. UVC: about 20 min to enter the data collected in each transect into the database
Equipment		+	-	BRUV: cameras and frames; software for analysis; hard disk for data storage. Calibration system for obtaining fish length measurements and biomass. UVC: SCUBA equipment
Type of data	Structure of fish assemblages	yes	yes	Both methods are suitable for measuring the structure of fish assemblages.
	Fish behavior	+	-	The long videos collected by BRUVs can be more suitable for behavioural studies than the video collected during UVC monitoring, but this strongly depends on the study objectives. By BRUV complementary data and video for communication purpose can be also available.
Results	Species richness	yes	yes	BRUV and UVC used together can provide more extensive knowledge on species richness.
	Abundance	yes	yes	Both techniques are suitable for measuring fish abundance. Nevertheless, the differences between the two techniques, in terms of surveyed areas, make difficult comparisons of the results among them.
	Density	no	yes	BRUV does not allow density estimation because the dispersion of the bait odours is strongly influenced by many factors (for example, currents direction and strength) which can hardly be kept under control.
	Length measurement and biomass	-	+	Both techniques allow fish lengths and biomass estimation, but BRUV requires calibrated stereo camera systems, which considerably increases its costs.
Limits	Need of SCUBA divers	-	+	Scuba divers are essential to UVC. While they may be useful (but not necessary) when BRUVs were deployed in shallow water, to verify the correct position of the cameras
	Depth	-	+	UVC maximum depth is limited by safety reason. No limits for BRUV.
	Climatic condition and sea temperature	-	+	Seawater temperature and climatic condition can strongly influence UVC monitoring due to safety requirements for divers. However, extreme weather conditions also hinder monitoring by BRUV.
	Effect of the method on fish behavior	yes	yes	Scuba divers' presence can cause attraction or avoidance reactions on fish that depend on the species and how much they are accustomed to divers' presence. On the other hand, the presence of bait in BRUV monitoring influences fish attraction and the interaction between species.

ertheless, the BRUV technique may reduce some of the limitations of UVC, for example, related to the maximum operational depth, weather conditions (except for extreme events), sea temperature and some species response induced by the presence of diver (Table 7).

In conclusion, the choice of the sampling technique depends mainly on the study objectives, the type of data to be obtained, budget, time, and human resources. A sampling strategy based on a species-by-species approach may be the most appropriate for fish monitoring in MPAs to reduce the biases associated with the technique and increase the statistical power, even if this strategy lengthens time, increases personnel and financial resources required (Willis *et al.,* 2003). Particularly, by the present results, the use of both techniques, UVC and BRUV, should be recommended when a detailed inventory of the fish biodiversity is needed or when the evaluation of the response to the protection of different groups/species is required (Prato *et al.*, 2017). For these reasons, data obtained through the coupling of these different techniques can provide more comprehensive information on the structure of fish assemblages. However, when measuring fish biomass and density is the main goal of monitoring and the budget is limited or BRUVs with calibrated stereo cameras are not available, UVC should still be considered the least expensive and most efficient technique.

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