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## Species identification of fish shoals using coupled split-beam and multibeam echosounders and two scuba-diving observational methods

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### Abstract :

Species identification remains crucial for interpreting acoustic backscatter delivered by active acoustic methodologies. The study took place in a Marine Protected Area where highly restricted areas were present such as no take zones. We used an innovative methodology coupling split-beam and multibeam echosounders to detect and classify monospecific fish shoals (i.e. schools or aggregations). Species identifications were realised by underwater visual censuses made by scientific divers. Two experimental protocols, where the divers gave the identifications instantaneously thanks to a communication wireframe, were tested: three roving scuba divers locating the shoals or a towed scuba diver directly behind the vessel. Energy responses, 3-D morphological, shape indexes and spatial descriptive variables of multiple independent samples of 4 observed fish species shoals (*Atherina* sp., Boops boops, Chromis chromis and *Spicara maena*) were calculated from the acoustic data. According to their behaviour and feeding strategy, significant differences in the acoustic variables were found between species. The combined use of acoustic data from both echosounders significantly improved the fish species classification. They were well discriminated using a Linear Discriminant Analysis (LDA), including for *B. boops*, *C. chromis* and *S. maena*, which were all observed in aggregations. Finally, we used this LDA model to allocate species to unknown shoals monitored by acoustics methods in the studied site, highlighting the interest of our methodology to predict benthopelagic and pelagic fish distributions in shallow waters. We suggest that these acoustic methods to discriminate fish species could provide valuable insights for marine management and decision-making.

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## Highlights

► A setup coupling split-beam and multibeam echosounders to classify fish shoals. ► Species identifications made in a Marine Protected Area by scientific divers. ► Interest of coupling the acoustic tools shown by comparing three classifier models. ► A case-study application of the classifier was made on unlabeled data.

**Keywords** : Active acoustics, Multibeam echosounder, split-beam echosounder, underwater visual census, species identification

## 1. Introduction

Hydroacoustics tools provide a non-invasive and non-extractive method to estimate the biomass and map the geographical distribution of pelagic fish (Benoit-Bird & Lawson 2016). Essential acoustic tools are split-beam echosounders, which can operate from about 12 kHz up to about 200 kHz with a usually vertical sound beam transmission (Misund 1997), giving quantitative backscatter data. Furthermore, school morphology and shape information are also often provided by the echosounders but there are usually restricted to the appearance inside the vertical echosounder images, giving only partial information on the length, height and surface area of the echotraces (Reid 2002, Paramo et al. 2007). Nevertheless, the use of multibeam echosounders is increasing (Gerotto et al. 2000, Lamouret et al. 2019, David et al. 2022). Especially, they allow to have an entire view of fish shoals (Paramo et al. 2007, Guillard et al. 2006), which is not possible with split-beam echosounders (Brehmer et al. 2002).

Species identification remains a critical requirement in interpreting acoustic backscatters (Horne 2000). Several studies have attempted to identify and classify the echotraces based on the information provided by the echosounders, using single-frequency or multi-frequency information from the echotraces (Scalabrin et al. 1996, Fernandes 2009, Lezama-Ochoa et al. 2011, D'Elia et al. 2014a, Tsagarakis et al. 2015). The frequencies usually used to discriminate fish species were 38, 70, 120 and 200 kHz (Benoit-Bird & Lawson 2016). In coastal shallow waters (< 20 m) and using pole-mounted transducer deployment (Brehmer et al. 2006), 38 kHz is difficult to operate because of the size and weight of the transducer. In

addition, the species composition and behaviour of fish communities of shallow waters differ from offshore communities. They are also more abundant and more diverse (Smith & Brown 2002, Cascão et al. 2019), making species identification challenging. However, in shallow waters, multibeam echosounders have been used for ecological and behavioral studies (Gerlotto et al. 2000, Brehmer et al. 2003) as well as to discriminate species using the 3D morphological characteristics of the echotraces (Guillard et al. 2011). Consequently, coupling different acoustic tools (split-beam and multibeam echosounders) could help identifying species by increasing the number of variables used for species classification (Brehmer et al. 2002).

In addition, direct sampling methods are often necessary to identify species and to validate the acoustics echotraces. Moreover, they provide additional biometric information such as fish length and abundance. Pelagic trawlings have been generally used to describe fish species composition (Doray et al. 2018). However, they are selective fishing gears (Brabant & Nedelec 1988). Furthermore, species identifications using pelagic trawls are not adapted to the constraints of shallow waters and can be forbidden in Marine Protected Areas (MPAs). Alternative sampling methods are then required and could use underwater visual observations by divers or cameras (Brehmer et al. 2019, Minart et al. 2021, Salvetat et al. 2022a). Especially, in shallow waters, the light penetration facilitates the use of these methods.

Coastal shallow waters are connected to many socio-cultural domains, including fisheries or recreational activities. Especially, the Mediterranean Sea is a marine biodiversity hot spot, hosting more than 17,000 marine species, with a high proportion of endemism (Coll et al. 2010), but it is also exposed to increasing direct and indirect human pressures (overexploitation by fisheries, habitat loss, chemical and noise pollution, eutrophication and climate change). MPAs, including various levels of protection such as partial reserve or no-take zones, are increasingly considered as efficient tools to restore ecosystems and to manage

fish populations (Lubchenco et al. 2003). Several studies used underwater visual census to investigate the role of environmental factors such as habitats (Harmelin 1987, Pais et al. 2007, Cheminée et al. 2017, 2021), depths (Milazzo et al. 2011), island sectors (La Mesa et al. 2010) and seasonality (García-Rubies & Macpherson 1995) on juvenile and adult fish in the Mediterranean Sea. All this information can bring insights for MPA managers. However, while underwater visual census methods are well suited to monitor necto-benthic species in coastal habitats (Harmelin-Vivien et al. 1985, Prato et al. 2017), they are not well adapted for crypto-benthic or pelagic species (Thiriet et al. 2016). Complementarily, active acoustic monitoring of these fish populations at small scales is necessary to better understand this fragile ecosystem as well as the role of the MPAs.

Within this context, this study took place in the Calanques National Park (from 5 to 60 m depth), located on the French coast of the Mediterranean Sea. A split-beam and a multibeam echosounders were coupled to monitor different fish shoals. We focused our study on pelagic and benthopelagic species. The species identifications were performed by underwater visual census carried out by scientific divers. Two surveys were carried out in August 2020 and April 2021. Then, different acoustics descriptive variables were calculated to identify fish species.

## **2. Material and methods**

### *2.1. Acquisition of the acoustic data*

Two acoustics surveys were carried out in August 2020 and April 2021 in the Calanques National Park (Mediterranean Sea, France, Figure S1). All samplings were made during daytime in order to i) allow visual census and ii) maximise fish identifications by targeting a

period when pelagic fish species adopt a typical schooling behaviour to reduce the risk of daytime predations (Connell 2000).

Data were collected simultaneously with scientific split-beam echosounders (Simrad EK80) operated at 70, 120 and 200 kHz combined with a multibeam echosounder (Kongsberg M3 Sonar, 922-20007011) operated at 500 kHz, with or without a tilt of 15°. The sampling volume of the M3 was crossing the sampling volume of each EK80 beam. The acoustics emission of the M3 and the three EK80 were synchronized to avoid interferences. The multibeam echosounder provides 128 beams in the imaging mode, 120° swathe, 1.6° angular resolution, 30° vertical beam widths, detection up to 150 m and a pulse duration of 200 µs. The EK80 echosounders were configured to ping simultaneously at a power of 450, 200, and 90 W at 70, 120 and 200 kHz, respectively, with a sample interval of 0.024 ms, ping rate of 2.5 pings per second (pps) and a pulse duration of 0.512 ms for all frequencies. Both acoustic devices were deployed using a side-mounted pole which was specially designed to deploy the acoustic transducers simultaneously on different small boats, including semi-rigid boats (Figure S2) (Brehmer et al. 2003). GPS antennas (GP-01 Sky Traq Venus 8) were used to position the system.

The EK80 echosounders were calibrated *in situ* according to the standard target technique for split-beam echosounders (Foote et al. 1987) using a 38-mm tungsten sphere. The M3 multibeam echosounder was calibrated using a 22-mm tungsten sphere (Perrot et al. 2014, David et al. 2022). As this calibration is difficult to perform, more details are given in the SI for the M3 calibration.

## 2.2. Species identification protocols

Two protocols of underwater visual censuses were implemented and their efficiency to identify species composition of fish shoals detected by acoustics was compared. For both protocols, scientific divers provided detailed descriptions of the observed fish shoals (Minart et al. 2021): fish species, total length ranges, abundance (number of fish) and depth estimates of the fish shoal (in meter). The shoals were classified into two types of fish group structures: aggregation vs. school. Schools refer to a fish group swimming in the same direction in a coordinated manner (Pitcher 1986) whereas aggregations refer to scattered and overlapped fish groups (Charef et al. 2010). Finally, the shoals were considered monospecific if more than 95% of the fish forming it are from the same species. For both protocols, the dives were recorded by underwater cameras (GoPro Hero 5 and Parulenz) to synchronize and manually check the different species identifications (Minart et al. 2021). To reduce observer biases, all divers were previously trained and inter-calibrated by performing previous works together using underwater visual censuses over the study site (Thiriet et al. 2016, Monfort et al. 2021, Cheminée et al. 2021).

The first protocol, called “rebreathing scuba divers”, consisted in a team of three scientific divers equipped with a closed circuit rebreather (CCR). CCR is used to avoid bubbles, which could disturb fish and good acoustic acquisitions (Shabangu et al. 2014). Divers used underwater scooters to move faster to locate shoals of pelagic fish. To coordinate the vessel instrumented by the acoustics system and the divers’ team, a small inflatable boat was used to follow the divers and one of the divers communicated in wireframe with the crew. Once a shoal was located, the first diver launched an underwater parachute to signal their position, so that the vessel can precisely navigate above the observed shoal and proceed to the acoustic acquisition. The second diver noted all the information about the shoal and the goal of the third diver was to transmit this information instantaneously thanks to the communication wireframe.



The second protocol called “towed scuba diver” consisted in towing a diver directly behind the vessel equipped with the echosounders. The line between the boat and the diver was 20 meters long. The diver had a board to control his depth and the vessel speed was low (max 2.5 knots) to ensure the divers’ safety. Similarly, to the first protocol, this diver was able to communicate directly by wireframe with the crew. Once an echotrace was detected on the split-beam and multibeam echosounders, the crew informed the diver by giving the position of the shoal in the water column and the location (middle, port or starboard sides) thanks to the communication wireframe. After that, the diver gave all the required information on the targeted shoal. Given the boat speed, from 20 to 30 seconds separated the acoustic acquisition from the diver observation. Finally, both protocols were used during the survey in August 2020 whereas only the “tower diver” protocol was used in April 2021.

### *2.3. Acoustic data analysis*

Acoustics data were analysed with the open-source Matecho software, which is implemented in Matlab (Perrot et al. 2018). This software is an automated tool that performs shoal extractions using the EK60 echosounder data, as well as the M3 multibeam echosounder data, as described in David et al. (2022). Firstly, raw data were automatically converted into the HDF5 data format. Matecho automatically creates a bottom line, which can be manually corrected. Water column noises can be manually cleaned and noise coming from potential interferences can also be removed when necessary. Then, shoal extractions can be automatically performed and the shoal descriptors calculated. To do that, this algorithm used three thresholds: (i) volume backscattering strength  $S_v$  in dB set to -60 dB, (ii) a maximum along-ping-axis integration distance in m set to 0.5m, and (iii) a depth integration distances in m set to 0.1m for our analyses. The extractions were made up to 0.3m from the bottom and 3m from the surface to avoid blind zone and surface noises. All automatically extracted shoals have been manually checked to avoid false detections. Finally, the GPS coordinates, the

sampling time, the vessel speed and the bottom depth were also recorded. A complete description of the open source Matecho software could be found in Perrot et al. (2018).

All shoal extractions were then manually filtered to only select the shoals having a species identification. Hence, an acoustic database was built keeping the fish shoal descriptors provided by both the EK80 and M3 echosounders as well as the diver's information. Several descriptors were from the EK80 echosounder data (Figure S3) such as the mean volume backscattering strength  $S_v$  in dB ( $S_{v\_70\text{kHz}}$ ,  $S_{v\_120\text{kHz}}$  and  $S_{v\_200\text{kHz}}$ ) and their coefficients of variation at 70, 120 and 200 kHz ( $CV_{S_{v\_70\text{kHz}}}$ ,  $CV_{S_{v\_120\text{kHz}}}$  and  $CV_{S_{v\_200\text{kHz}}}$ ). In addition, the height, length and surface of the shoals at the three frequencies were also calculated ( $H_{70}$ ,  $H_{120}$ ,  $H_{200}$ ,  $L_{70}$ ,  $L_{120}$ ,  $L_{200}$  and  $S_{70}$ ,  $S_{120}$ ,  $S_{200}$ ) as well as the CVs for the height along pings ( $CV_{H_{70}}$ ,  $CV_{H_{120}}$ , and  $CV_{H_{200}}$ ).

Using data from the M3 multibeam echosounder, 3-D morphological descriptors were calculated: the mean height, length, width, maximal surface ( $H$ ,  $L$ ,  $W$ , and  $S$ ) as well as the CVs for the height and width along pings ( $CV_H$  and  $CV_W$ ) (Figure S3). The entire shoal volume ( $V$ ) and the ratio of holes (i.e. samples under the extraction threshold compared to the total number of samples;  $Holes$ ) were also added (David et al. 2022), as well as the CV of this ratio along pings ( $CV_{Holes}$ ). We also added elongation variables: the width:length, the width:height and the height:length ratios (noted  $WL$ ,  $WH$  and  $HL$ , respectively) and shapes variables decomposed into indicators of sphericity, rectangularity, roundness, roughness and flatness ( $Sph$ ,  $Rec$ ,  $Rd$ ,  $Rg$  and  $Flat$ , respectively). Sphericity and rectangularity are measures of the degree of resemblance to a sphere or a rectangle respectively, and they are independent of the size. Roundness is the measure of the sharpness of a form's edges and corners. Symmetry variables along the three axes (length, height, width) were also calculated ( $SymL$ ,  $SymW$  and  $SymD$ ) as well as perimeter variables along these three axes ( $PerL$ ,  $PerW$  and  $PerD$ ). Finally, spatial descriptive variables were calculated from both echosounders. The

altitude in the water column for the M3 and the EK80 was calculated by taking into account the bottom depth to have a relative measure between 0 and 1 (Alt, Alt\_EK80). As the localization of the fish shoals could also be independent of the bottom depth, the absolute distances between the bottoms or the top of the shoals and the seafloor (MinDist, MaxDist, MinDist\_EK80, MaxDist\_EK80) were also taken into account. For the M3 where the entire shoal could be seen, the CVs of these absolute distances (CV\_MinDist and CV\_MaxDist) were added. All descriptors are described in Table 1.

#### *2.4. Statistical analysis*

All statistical analyses were made with the R software (R version 3.6.2) (R Core Team 2021). The descriptive variables were compared using pairwise t-test comparisons. The fish shoal descriptive variables were analysed together through a Linear Discriminant Analysis (LDA), a statistical method that calculates linear combinations of features that find the best separation into groups (here, by species) for a given data set. The packages “MASS” from the R software and the `lda` function was used for the analyses (Venables et al. 2002). The LDA model estimates the mean and variance from our dataset formed by the acoustic variables for each class of species. Independence was checked by using the correlation matrix and the highest correlated variables were withdrawn from the LDA models. To perform LDA, several variables were log-transformed to follow the assumption of normality. Co-linearity was checked within the LDA function. All Q-Q plots and the correlation matrix can be found in the SI (Figures S7 and S8). To avoid biases in the model as LDA is significantly biased towards objects from the majority group, a bootstrap method was used to have the same number of observations per species ( $n = 250$  per class). The accuracy of the model was evaluated by computing the confusion matrix, which compared the model predictions and the species observations.

To assess the interest of using the split-beam EK80 and multibeam M3 echosounders conjointly, LDA models were also run using only the EK80 variables (energetic, morphological and spatial variables) on one hand and the M3 variables (energetic, morphometric, elongation, shape, perimeter, symmetry and spatial variables) on the other hand (Table 1 and Figure S3). Confusion matrices were computed for each model. Statistical metrics such as the accuracies of these LDA models were then compared to the metrics of the LDA model using data from both the echosounders to conclude on the best model.

### *2.5. Application of the LDA model in the Calanques National Park*

For the predictions, we used a dataset of 440 echotraces with no species identification (without observations from divers) (Figure S13). The echotraces were collected in the same conditions, i.e. during the same survey in April 2021. Based on the comparison of the accuracies, we used the best LDA model to predict the species of these unknown echotraces. The method estimates the probability that the new set of acoustics variables belongs to a particular species. The species was predicted only if the echotrace was included in the validity domain of the discriminant functions. In addition, for *Atherina* sp., which had the most remarkable shoal shapes, we visually checked each prediction on the echogram by comparing the shape of the shoals for this species to conclude on the model predictions. Finally, these predictions were used in a case study to compare the acoustic variables of the fish shoals inside and outside of the no-take zones (NTZs) in the Calanques National Park. The same statistical analysis was made including or not the predicted shoals.

## **3. Results**

### 3.1. Diver observations on fish shoals

A dataset totalling 98 independent shoals was built combined information from the EK80 and M3 echosounders and the divers: 20 of juvenile *Atherina* sp., 35 of *Boops boops*, 33 of *Chromis chromis* and 10 of *Spicara maena*. Illustrations of echotraces for each species can be found in Figure S4. Some other species (*Sarpa salpa*, *Oblada melanura*, *Sphyræna viridensis* and *Diplodus sargus*) were observed but, since the number of replicates was too low (only 1 or 2), we excluded them from further analyses.

The three species *B. boops*, *C. chromis* and *S. maena* were observed as aggregations whereas schools were observed for *Atherina* sp. Estimated abundances ranged from 20 to 20000 individuals, depending on species. Higher abundances were observed for *Atherina* sp. Total length ranged from 3 to 17 cm for *B. boops*, *C. chromis* and *S. maena* whereas *Atherina* sp. were smaller (from 1.5 to 5 cm) and they were all observed as juveniles (Table 2).

### 3.2. Fish shoal descriptors

Significant differences in the fish shoal descriptors were observed between species, mostly between *Atherina* sp. and the other species (*B. boops*, *C. chromis* and *S. maena*) (Figure S5 and Table S1). *Atherina* sp. shoals had a significantly higher mean volume backscattering strength  $S_v$  at 70, 120 and 200 kHz compared to the other species. The CVs for the height at the three frequencies (70, 120 and 200 kHz) were significantly lower. The ratio of holes in the shoals was also significantly lower. On the contrary, the elongation ratio (WH) and the roundness index were also significantly higher for *Atherina* sp. compared to the other species. Finally, differences in the spatial variables (altitude and mean absolute distances) were also observed with the shoals of *Atherina* sp. being significantly higher in the water

column compared to the other species. In addition, significantly lower CVs for the absolute distances between the bottom as well as the top of the shoals compared to the seafloor were observed for *Atherina* sp. compared to the other species.

The shoals of *C. Chromis* were significantly closer to the seafloor compared to *B. boops*. The CVs for the height at the frequencies (70, 120 and 200 kHz) were significantly higher for *B. boops* compared to *C. chromis*. There were no significantly different descriptive variables between *S. maena* versus *B. boops* and *C. chromis*. Boxplots of the descriptive variables and statistical results can be found in the SI (Figure S5 and Table S1).

In addition, significant relationships were found between the descriptive variables and the observed abundances. Indeed, the 3-D shoal morphology characteristics (height, width, length, surface and volume) significantly increased with the shoal abundances as well as perimeter variables, the symmetry along the length and the rectangularity. On the contrary, shoal roundness and roughness indexes decreased significantly with abundances. Shoals with larger abundances were significantly higher in the water column. The mean volume backscattering strength  $S_v$  at 70, 120 and 200 kHz significantly increased with shoal abundances as well as the CV at 70 kHz (Figure S6).

### 3.3. LDA model using the variables from both the EK80 and M3 echosounders

The highest correlated variables were withdrawn from the analyses (S, H, L120, S120, H120, L200, S200, H200 and Flat). Percentage of separations achieved by the first, second and third discriminant functions of the LDA model were 85.2, 9.7 and 5.1%, respectively. The accuracy of the model (number of true positives for all species) was estimated to be 89.8% (95% CI: [0.8776; 0.9161]). Especially, the accuracy for each group was 100, 89.5, 88.3 and

95.1% for *Atherina* sp., *B. boops*, *C. chromis* and *S. maena*, respectively. The sensitivity, specificity and precision are also given in Table S2. Indeed, the LDA model discriminated well the group of *Atherina* sp. compared to the three other species (*B. boops*, *C. chromis* and *S. maena*). These last species were also discriminated but they overlapped a little (Figure 1).

For LDA, the coefficients of variables indicated their importance in the different discriminant functions. Here, the CVs of the volume backscattering strengths at the three frequencies (CV<sub>S<sub>v</sub>70</sub>, CV<sub>S<sub>v</sub>120</sub> and CV<sub>S<sub>v</sub>200</sub>) and the percentage of holes (Holes) had the strongest positive and negative loadings on the first discriminant function (Figure 2a). Concerning the second discriminant function, the CV of the volume backscattering strengths at 70 kHz, as well as the WL elongation ratio and the CV of the height and width (CV<sub>H</sub> and CV<sub>W</sub>) had the most important influence (Figure 2b). For the third discriminant function, the roughness index (Rg), the CVs of the volume backscattering strengths at 70 and 200 kHz (CV<sub>S<sub>v</sub>70</sub> and CV<sub>S<sub>v</sub>200</sub>) as well as the WL elongation ratio had the strongest positive and negative loadings (Figure 2c). Other variables also influenced the three discriminant functions like the CVs of the spatial and morphological variables.

#### 3.4. Benefit of a conjoint use of both echosounders

For both models using only one echosounder, the group of *Atherina* sp. was still well discriminated compared to the others. However, the three other species (*B. boops*, *C. chromis* and *S. maena*) overlapped (Figures S9 and S11). The variables having the most important influence for the three discriminant functions were the same as for the model including the data from both echosounders. In particular, the CVs of the volume backscattering strengths at the three frequencies (CV<sub>S<sub>v</sub>70</sub>, CV<sub>S<sub>v</sub>120</sub> and CV<sub>S<sub>v</sub>200</sub>) as well as the altitude and the height, surface and length at 70 kHz (Alt<sub>EK80</sub>, H70, S70 and L70) were important for the

three discriminant functions of the model based on the data from the EK80 (Figure S10). Concerning the model based on the data from the M3, the elongation ratios (HL and WL), the roughness index and volume (Rg and V) and the PerL perimeter variable had a strong influence in the discriminant functions (Figure S12).

The accuracy of the LDA model using only the data from the EK80 was estimated to be 74.0% (95% CI: [0.7116; 0.7669]). Especially, the accuracy was estimated at 97.8, 71.8, 76.8 and 84.2% for *Atherina* sp., *B. boops*, *C. chromis* and *S. maena*, respectively. Furthermore, the accuracy of the LDA model using only the data from the M3 was estimated to be 75.0% (95% CI: [0.7219; 0.7766]). The accuracy was estimated at 97.8, 78.5, 81.5 and 75.5% for *Atherina* sp., *B. boops*, *C. chromis* and *S. maena*, respectively. For both models, the sensitivity, specificity and precision are also given in Tables S3 and S4 and a comparison of the confusion matrices from each LDA model is given in Table 3. As seen from the different metrics, the use of the data from both echosounders in the statistical analysis improved the LDA model performance, especially for the species living in aggregations.

### 3.5. Application of the LDA model in the Calanques National Park

Predictions of the species were made with the dataset without species identification (440 unknown shoals, Figure S13) in the Calanques National Park. The majority of the detections (11 and 30 respectively) were attributed to *B. boops* and *C. chromis*. Fewer detections were attributed to *Atherina* sp. and *S. maena* (10 and 9 respectively) (Figure S14 and Table S5). By visually checking each prediction on the echogram for *Atherina* sp., we found that all predictions were indeed consistent with the shape of the *Atherina* sp. schools (Figure S15). In addition, the localization of the observed and predicted shoals for each species can be



visualized in Figure 3. Larger shoals (higher volumes) were observed near the islands, especially near the Riou, Calseraigne and Jarre islands.

We found significant differences between inside and outside the NTZs for 31 acoustic variables using both predicted and observed shoals. The same statistical analysis using only the observed species data led to 21 variables having significant differences (Table S6). Including the predicted shoals increased the statistical power of the analyses, especially for *Atherina* sp. and *B. boops*, showing that the shoals were significantly larger inside compared to outside the NTZs (larger height, surface and volume) as seen with both the M3 and EK80 data (Figure 4). Significant increase of the sphericity index and the ratio of holes were observed inside the NTZ. On the contrary, the shoals of *C. chromis* were larger outside the NTZ. Concerning *S. maena*, the number of observed shoals inside the NTZs was too low to compare the shoals structure inside and outside the NTZs.

#### 4. Discussion

The combination of the Simrad M3 and EK80 allowed us to have numerous acoustic descriptive variables (energetic, morphologic, elongation, shape, symmetry, perimeter and spatial) providing integrative characterisations of the fish shoals. The configuration using these two different acoustic tools could be used routinely during hydroacoustics surveys. Especially, the side-mounted pole was built to be able to deploy simultaneously the synchronized acoustic devices. This pole could be fixed on different vessels including small boats, allowing to sample shallow water areas. This would be particularly pertinent in some coastal Marine Protected Areas, which are not easily sampled by conventional research vessels (Brehmer et al. 2006). Here, we focused our analysis on only four species for which

we had enough replicates to provide consistent results. By combining acoustic and underwater visual censuses, the analysis has shown the efficiency of the methodology to classify fish assemblages with a high degree of accuracy.

For species identification, the use of a wired communication with the scientific divers enabled to produce a database with a high degree of certainty. Especially, the protocol with the towed scuba diver was found promising for species identification because the implementation of the method was easier and the communication was more direct and efficient. Moreover, this last protocol allowed us to have a larger number of species identifications (Minart et al. 2021) as we could make straight transects for the visual census. This is the reason why we only selected this protocol for the second survey (April 2021). In addition, these diver protocols did not have the limitations of pelagic trawls such as selectivity (Brabant & Nedelec 1988). Nevertheless, unlike visual observations, trawl operations allow to collect biological samples informing the species composition and length distributions of the target detected by the echosounders information which is then used for biomass estimations by echointegration (Doray et al. 2018). In addition, scuba-diving is constrained by other limitations including the requirement of a high degree of expertise, underwater time, maximal diving depth, diver safety as well as observer bias (Williams et al. 2006, Goetze et al. 2019). To overcome these limitations, the use of underwater cameras for species identification (Brehmer et al. 2019, Salvetat et al. 2022) could be a promising alternative to replace divers in similar contexts. In particular, towed cameras are a practical and efficient method to monitor shallow waters (Davis et al. 2019, Cresswell et al. 2021). Nevertheless, underwater cameras also present limitations such as field of view, image quality, battery life and data storage as well as challenge in species identification but the techniques regarding underwater cameras are increasingly progressing (Mallet & Pelletier 2014). Especially, in lowly turbid waters,

stereo underwater cameras could be of great interest as they provide accurate estimates of fish length regardless of user experience (Harvey et al. 2010, Langlois et al. 2020).

Significant differences between species were observed according to the acoustic variables. The variable “ratio of holes” significantly decreased between *Atherina* sp. and the other species, which is consistent with the fact that *Atherina* sp. was observed forming compact schools. Indeed, small individuals could form large schools for foraging purposes and cooperative feeding strategies (Pitcher et al. 1982). Moreno & Castro (1995) explained that juvenile species exert a more prolonged use of the coastal area as they exploit lower trophic levels of the pelagic ecosystem (small-sized zooplankton feeders). Here, *Atherina* sp. had a position in the water column significantly higher compared to the other species and could be easily spotted by predators. This could result in an advantageous strategy of being in large fish schools (Pitcher 1986). The shoals of *Atherina* sp. also exhibited higher mean backscatter strengths at the three frequencies (70, 120 and 200 kHz) compared to species (*B. boops*, *C. chromis* and *S. maena*). The overall received signal is complex to analyze as it could be related to different characteristics such as the abundance and the size distributions of organisms (Benoit-Bird & Lawson 2016). Here, these stronger values in acoustic energies could be related to the larger abundances as observed by the scientific divers for *Atherina* sp. Finally, the CVs for the height along pings at the three frequencies (70, 120 and 200 kHz) were significantly lower for *Atherina* sp. compared to the other species, their schools had a roughly constant height as also observed visually on the echogram. For further improvements, the use of frequency modulation vs continuous wave mode could potentially improve the species discrimination as they increase the amount of information available for spectral characterization of detected targets (Benoit-Bird & Waluk 2020), however it would also cost more extensive data storage and processing.

In addition, the shoals of *B. boops* were found to be significantly higher in the water column compared to *C. chromis* which typically feeds above rocky reefs and seagrass meadows (*Posidonia oceanica*) during the day (Pinnegar 2018). Compared to *Atherina* sp., the three species (*B. boops*, *C. chromis* and *S. maena*) formed loose aggregations in which the individuals are dispersed and swim disorderly (Myrberg et al. 1967, Bottari et al. 2014) which is consistent with the higher values of the “ratio of holes” variable. No difference in the mean height of the shoals at 70, 120 and 200 kHz was found between the two planktivorous species (*B. boops* and *C. chromis*) whereas the CVs of the height were significantly higher for *B. boops* compared to *C. chromis*, suggesting that the distribution of fish within aggregations was more varied for this species.

The LDA model using the descriptive variables from both the EK80 and M3 echosounders easily discriminated the schools of *Atherina* sp., which was logical as several significant differences were found for this species compared to the others. However, the LDA model was also a promising tool to discriminate the three species (*C. chromis*, *B. boops* and *S. maena*) which were all observed as aggregations. The LDA models using the descriptive variables from only the EK80 or the M3 echosounders showed that these species were less well discriminated, the accuracy of the models being lower for *B. boops*, *C. chromis* and *S. maena*. Especially, *B. boops* and *S. maena* are taxonomically and ecologically related sparids (Benhamou et al. 2017). They are both demersal, found over seaweed beds, on sand or muddy bottoms (Froese & Pauly 2022), and omnivorous feeding mainly on copepods (Stergiou & Karpouzi 2002, Benhamou et al. 2017). Consequently, coupling the descriptive variables from both echosounders improved the classification of these closely related species.

In addition, we found that the CVs of the different acoustic variables were important as these variables strongly influenced the discriminant functions of the LDA models. To our knowledge, the use of these CVs is still scarce in classification models (Fernandes 2009,

D'Elia et al. 2014b). These results showed that integrating the CVs of the acoustic variables into the analyses could be of great interest to improve the classification procedures. Indeed, they had a higher discriminant power than the mean backscatter strength variables. Employing a multifrequency approach was also important (Benoit-Bird & Lawson 2016) as the CVs of the volume backscattering strengths did not vary the same way between species and frequencies. Furthermore, variables such as the ratio of holes and the sphericity index calculated with the multibeam echosounder had a significant discrimination power, highlighting the benefit of adding the shape analyses to better classify the shoals (Paramo et al. 2007).

Finally, we highlighted an application of LD<sup>2</sup> models to predict the fish species of unknown shoals sampled in the Calanques National Park. Overall, more shoals were predicted to be of *C. chromis* and *B. boops* which was logical as the populations of these gregarious species are abundant in the Mediterranean Sea (Kalogirou et al. 2010, Pinnegar 2018). The scientific divers also observed more aggregations for these two species. As *C. chromis* was suggested of being a possible indicator species for human disturbance (Pinnegar 2018), predicting the distribution of this species in the Calanques National Park would be of great interest. Furthermore, we showed that the predictions could be used to increase the number of samples (here the shoals) in our statistical analyses to compare the shoal structures inside and outside the NTZs for each species. Indeed, the number of shoals, which can be seen in Supp. Info., was multiplied by 1.4 to 4 depending on the species and the conditions (inside or outside the NTZs). By comparing the statistical analyses using both predicted and observed shoals for each species to the analyses including only the observed shoals, we showed higher significant differences on several acoustic variables like the 3-D morphological acoustic variables (length, height, surface and volume), especially for *Atherina* sp. and *Boops boops*. Indeed, increasing the sample size improves the accuracy of the statistical description of the

acoustic variables of the fish shoals inside and outside the NTZs. However, the number of detections inside and outside the NTZs was not directly compared, as it would be biased because the sampling effort was higher outside than inside the NTZs.

As the 3-D morphological acoustic variables significantly increased with the shoal abundances given by the divers, larger shoals in the NTZs could present higher abundances. Similar results were found by Marshak et al. (2020) as fish species (*B. boops*, *C. chromis*, *O. melanura*, and *S. salpa*) were observed at the greatest densities in the most protected areas of the Tabarca reserve in the Mediterranean Sea. More generally, higher fish density in no-take zones was shown to be positively correlated with the level of MPA enforcement, age and size with fish densities being higher in MPAs of smaller size (Giakoumi et al. 2017). In addition, the volume of the shoals of *Atherina* sp., *B. boops* and *C. chromis* in some specific localizations like near the islands (Riou, Caleraigne and Jarre) was higher. The seagrass meadows of *Posidonia oceanica*, that are extent around these islands, are thought to represent suitable habitats for juveniles and adults of numerous species (Kalogirou et al. 2010, Cheminée et al. 2021). Hence, taking account of the habitats versus shoal localizations and characteristics would improve the understanding of the NTZs effects on fish distribution patterns and conservation.

Finally, the model was used here to predict the species composition of shoals by using acoustic methods and direct observations that were collected during the same survey in April 2021. The performance of the model would likely decrease for *Atherina* sp., as the juveniles grow and their strategies of food exploitation and space occupation evolve (Moreno & Castro 1995). Overall, as the costs and benefits of being in schools can vary extensively through life depending on species, more species identifications over different seasons should be obtained to use the model at different periods and investigate the temporal variations of the acoustic descriptive variables. Moreover, all data were used to train the LDA model due to the small

database. Hence, cross-validated procedures were not possible but they should be taken into account with a larger database to limit over-fitting results. In addition, the majority of the predictions were undetermined, which was not surprising since the LDA model was trained on only four species. Among the undetermined species, we could assume that the species (*Sarpa salpa*, *Oblada melanura*, *Sphyraena viridensis* and *Diplodus sargus*) that were observed by the divers but excluded from the analysis (too low number of observations), could be present. Hence, enriching the database and integrating other species would also be of great interest to test the LDA performance in a broader context. For example, it would be of high interest to apply this methodology to indicator species such as the common dentex (*Dentex dentex*) and the brown meagre (*Sciaena umbra*), as their abundance is an indicator of pressure exploitation of both professional and recreational fisheries (Harmelin 1991, Marengo et al. 2014, Harmelin-Vivien et al. 2015).

## 5. Conclusion

We demonstrated the interest of combining split-beam and multibeam echosounders to better characterize the fish schools and aggregations and therefore improve the ability to discriminate species. We found that both underwater visual census protocols for species identification provided robust information on fish composition with high certainty, although the towed scuba diver protocol was more efficient. The differences in the descriptive acoustic variables used were highly informative to discriminate species as they reflected their different behavior, ecology and feeding strategy. The LDA achieved good performance in species discrimination even if they were biologically and ecologically similar. Model predictions could allow to improve the understanding of current management strategies, such as NTZs, by extrapolating species to unknown shoals and analyzing their characteristics and distributions.

Promising perspectives would be to use the method for a larger number of fish species, sites and seasons. Finally, we recommend such a method in any non-turbid waters where fish species distribution mapping is needed to acknowledge marine management decisions.

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## 9. Figures and tables

**Table 1.** List of the variables provided by the split-beam and multibeam echosounders. For the calculations, a, b and c represent the height, width and length listed in the order: maximum, medium and minor lengths, respectively. CV: coefficients of variation.  $S_v$  volume backscattering strength in dB.  $N_{echos}$ : Number of echos. Threshold: threshold used for the shoal extractions in dB.  $B_{depth}$ : bottom depth and  $shoal_{depth}$ : mean depth of the shoal.

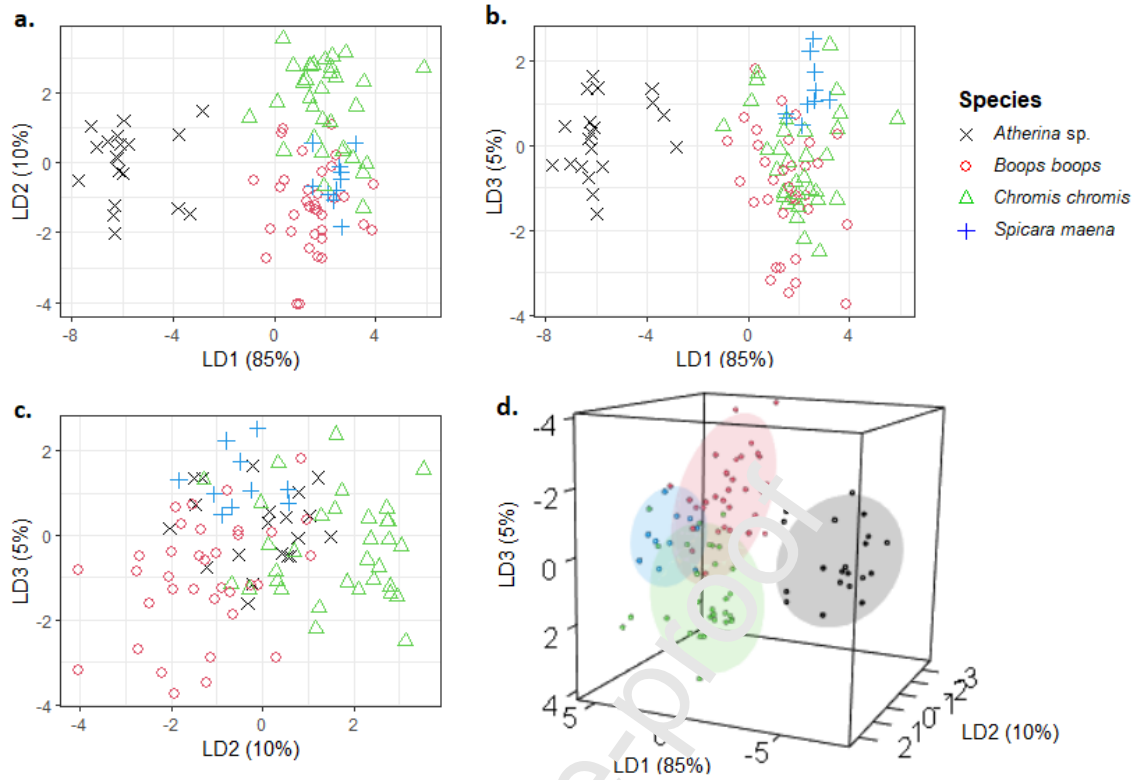
Variable type	Variables	Abbreviation	Acoustics device	Computations	Unit	Reference	
Energetic	$S_v$ mean weighted 70 dB	$S_v_{70kHz}$	EK80		dB		
	$S_v$ mean weighted 120 dB	$S_v_{120kHz}$	EK80		dB		
	$S_v$ mean weighted 200 dB	$S_v_{200kHz}$	EK80		dB		
	CV of the $S_v$ mean weighted 70 dB	CV $S_v_{70kHz}$	EK80		-		
	CV of the $S_v$ mean weighted 120 dB	CV $S_v_{120kHz}$	EK80		-		
	CV of the $S_v$ mean weighted 200 dB	CV $S_v_{200kHz}$	EK80		-		
Morphometric	Length at 70 kHz	L70	EK80		m		
	Length at 120 kHz	L120	EK80		m		
	Length at 200 kHz	L200	EK80		m		
	Height at 70 kHz	H70	EK80		m		
	Height at 120 kHz	H120	EK80		m		
	Height at 200 kHz	H200	EK80		m		
	Surface at 70 kHz	S70	EK80		m <sup>2</sup>		
	Surface at 120 kHz	S120	EK80		m <sup>2</sup>		
	Surface at 200 kHz	S200	EK80		m <sup>2</sup>		
	CV Height at 70 kHz	CV_H70	EK80		-		
	CV Height at 120 kHz	CV_H120	EK80		-		
	CV Height at 200 kHz	CV_H200	EK80		-		
	Height	H	M3		m		
	Width	W	M3		m		
	Length	L	M3		m		
	Surface	S	M3		m <sup>2</sup>		
	Volume	V	M3		m <sup>3</sup>		
		Ratio of holes	Holes	M3	$\frac{N_{echos} < threshold}{Total N_{echos}}$	-	Paramo et al. (2007) ; Guillard et al. (2011)
		CV Height (along pings)	CV_H	M3		-	
		CV Width (along pings)	CV_W	M3		-	
	CV Ratio of holes (along pings)	CV_Holes	M3		-		
Elongation	Width by length	WL	M3	$\frac{W}{L}$	-		
	Height by length	HL	M3	$\frac{H}{L}$	-	Weill et al. (1993)	
	Width by height	WH	M3	$\frac{W}{H}$	-		

Shape	Sphericity	Sph	M3	$\sqrt[3]{\frac{c^2}{a \times b}}$	-	Cruz-Matías et al. (2019)
	Rectangularity	Rec	M3	$\frac{a \times b \times c}{V}$	-	Cruz-Matías et al. (2019)
	Roundness	Rd	M3	$\frac{V}{S \times \sqrt[3]{a \times b \times c}}$	-	
	Roughness	Rg	M3	$\frac{S}{V}$	m <sup>-1</sup>	Paramo et al. (2007)
	Flatness	Flat	M3	$\frac{a + b}{2 \times c}$	-	
Perimeter	Perimeter along the length axis	PerL	M3		m	
	Perimeter along the width axis	PerW	M3		m	
	Perimeter along the depth axis	PerD	M3		m	
Symmetry	Symmetry along the length axis	SymL	M3		-	
	Symmetry along the width axis	SymW	M3		-	
	Symmetry along the depth axis	SymD	M3		-	
Spatial	Mean altitude	Alt, Alt_EK80	M3, EK80	$\frac{B\_depth \cdot shoal\_depth}{B\_depth}$	-	
	Minimal distance between the bottom of shoal and the seafloor	MinDist MinDist_EK80	M3, EK80		m	
	CV distance from this minimal distance	CV_MinDist	M3		-	
	Maximal distance between the top of shoal and the seafloor	MaxDist MaxDist_EK80	M3, EK80		m	
	CV distance from this maximal distance	CV_MaxDist	M3		-	

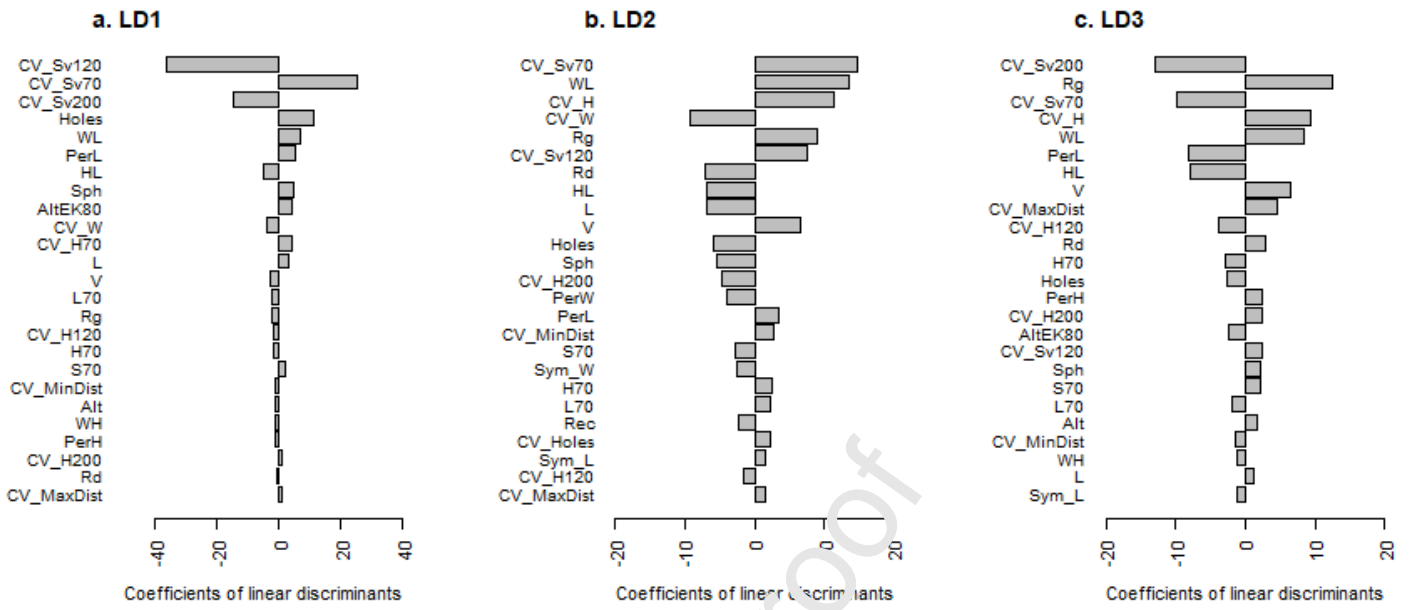
**Table 2.** Characteristics of the shoals observed by the divers (species, abundance, length and fish group structure, i.e. school or aggregation (Pitcher 1986)) and kept for the analyses.

Species	n	Abundance (median, 95% CI)	Total length (cm) [min., max.]	Fish structure
<i>Boops boops</i>	35	500 [40; 5 000]	[5.0-17.0]	Aggregation
<i>Chromis chromis</i>	33	350 [25; 10 000]	[3.0-9.0]	Aggregation
<i>Spicara maena</i>	10	375 [154; 1 891]	[5.0-15.0]	Aggregation
<i>Atherina</i> sp.	20	3 167 [198; 16 100]	[1.5-5.0]	School





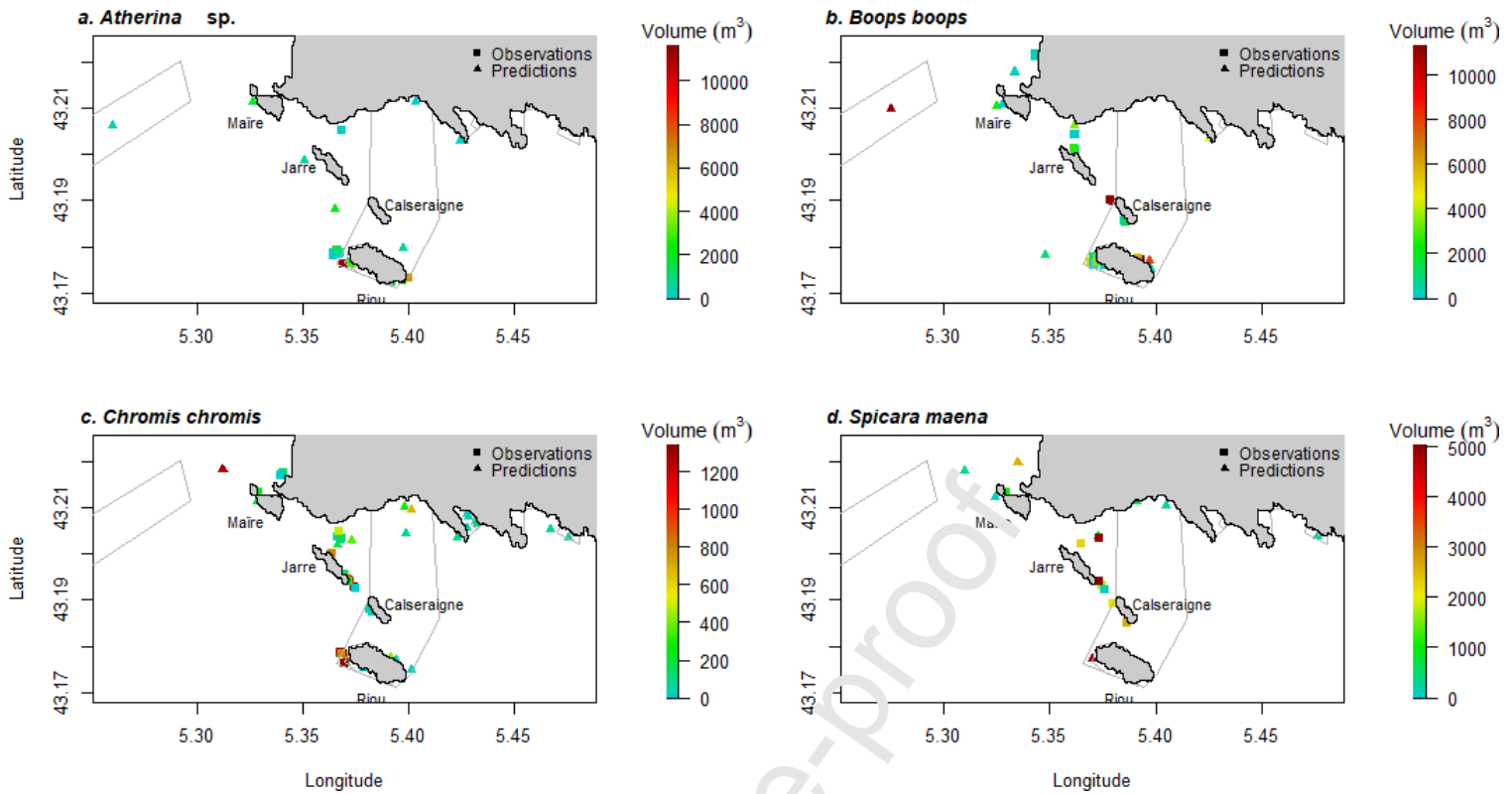
**Figure 1.** Linear discriminant analysis “LDA” plots for classification of the different fish species observed using fisheries acoustics tools. (a) LD1–LD2 plane of the plot; (b) LD1–LD3 plane of the plot; (c) LD2–LD3 plane of the plot; (d) 3D plot with ellipsoids having an 80% interval.



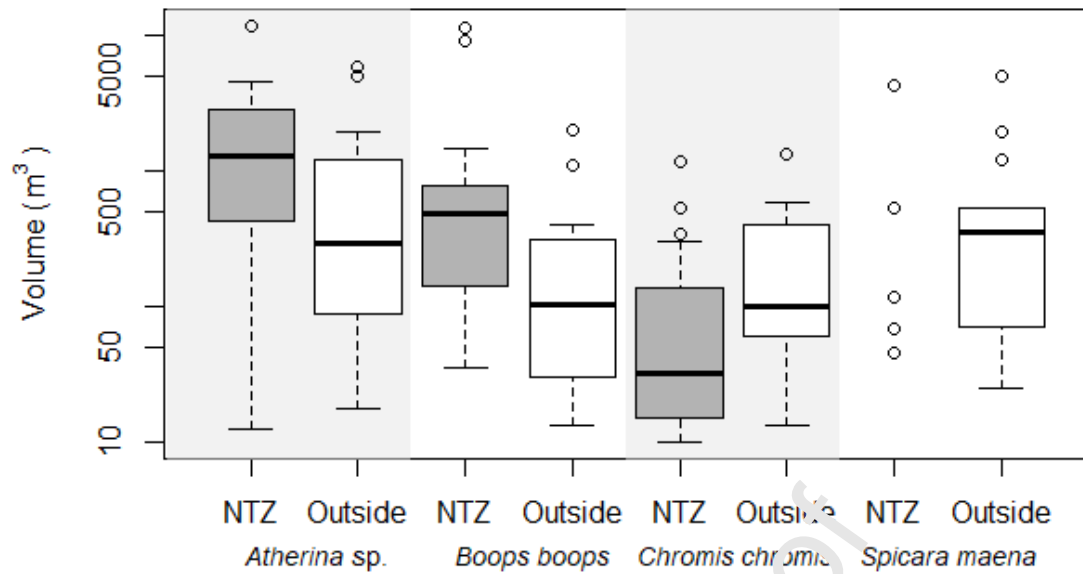
**Figure 2.** Coefficients of the variables in the first (a), second (b) and third discriminant (c) functions from the Linear Discriminant Analysis. Only the 25 first variables having the most importance are represented.

**Table 3.** Confusion matrices for each model using data from both echosounders, only the EK80 or only the M3 echosounder.

<b>Predicted</b>	<b>Observed</b>											
	<i>Atherina</i> sp.			<i>B. boops</i>			<i>C. chromis</i>			<i>S. maena</i>		
	Both EK80	M3		Both EK80	M3		Both EK80	M3		Both EK80	M3	
<i>Atherina</i> sp.	250	239	239	0	0	0	0	0	0	0	0	0
<i>B. boops</i>	0	0	0	203	115	154	17	18	13	0	0	21
<i>C. chromis</i>	0	11	11	11	82	41	195	174	198	0	38	70
<i>S. maena</i>	0	0	0	36	53	55	38	58	39	250	212	159



**Figure 3.** Map of the observed and predicted shoals for (a) *Atherina* sp., (b) *Boops boops*, (c) *Chromis chromis* and (d) *Spicara maena* in April 2021. The shoals having a species identification are represented by squares whereas the shoals for which the species was predicted by the model are represented by triangles. The grey lines represent the limits of the no-take zones.



**Figure 4.** Comparison of the volume of the shoals (in  $m^3$ ) for different species (*Atherina sp.*, *Boops boops*, *Chromis chromis* and *Spicara maena*) inside (grey boxplots) and outside (white boxplots) the no-take zones (NTZ), using the observed and predicted species by the Linear Discriminant Analysis model. The boxplots present the median, the 25<sup>th</sup> and 75<sup>th</sup> percentiles with a 1.5 interquartile range and the outliers.

**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Journal Pre-proof

## Highlights

- A setup coupling split-beam and multibeam echosounders to classify fish shoals
- Species identifications made in a Marine Protected Area by scientific divers
- Interest of coupling the acoustic tools shown by comparing three classifier models
- A case-study application of the classifier was made on unlabeled data