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Detecting changes in cetacean distributions in the North-West Mediterranean Sea: testing methods of data analysis

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Mestrado em Biologia Marinha

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Abstract

When reporting under the EU Directives (Habitat Directive and Marine Strategy Framework Directive), species distribution and its trends are required. The research aimed to investigate the performance of three grid resolutions (5x5, 10x10 and 50x50 km) with two indexes (proportion of area occupied; shift) and an occupancy model to study the distribution of 4 species occurring in the Ligurian Sea (striped dolphin, fin whale, sperm whale and Cuvier's beaked whale) along 3 periods (2008-2012; 2013-2018; 2019, 2021, 2022) corresponding to the HD reporting cycles. Data was collected onboard ferries as platform-of-opportunity. Detection/non-detection maps and the proportion of area occupied showed the striped dolphin as a species with generalized use of the study area, whereas the fin whale preferred the open sea and the sperm whale and Cuvier's beaked whale favoured slopes and seamounts. Changes in the area used were denoted for the sperm whale, which had a complete non-detection in the southwestern part in the last period, and for the Cuvier's beaked whale, describing an expansion movement in the eastern part from the 1st to the subsequent periods. Encounter rate maps showed a decrease in detections for all the species from the 2nd to the 3rd period, being most prevalent in the western part, indicating a decrease in their presence. The occupancy model described similar use (%) of the study area, except for higher values for the sperm whale. Compared to coarser resolutions, the use of a finer resolution allowed, to a greater extent, the detection of changes in all methods. The model performed better when more surveying occasions (14) were given. This study supports the use of finer resolutions when reporting the distribution of cetaceans under the EU Directives, as well as the importance of long-term monitoring programmes for detecting species distribution changes.

Key-words: distribution, cetaceans, occupancy, detection, Habitat Directive

Resumo

De modo a compreender o estado de conservação das espécies e atuar em direção à sua preservação, as Diretivas Europeias (Diretiva Habitat (DH) e Diretiva Quadro Estratégia Marinha (DQEM)) requisitam aos Estados-Membros a composição de um relatório, com a periodicidade de 6 anos, onde informação relativa à distribuição das espécies e possíveis alterações ao longo de séries temporais é necessária. Tendo em consideração que as variadas possibilidades de análise de dados descritas por parte das Diretivas são apenas recomendações, diferentes grupos de investigação tendem a usar diversos métodos de análise de dados, inclusive métodos não recomendados pelas Diretivas, justificando serem mais adequados dada a finalidade do estudo. Deste modo, torna-se difícil a comparação entre resultados, estando em falta um método de análise de dados padronizado a ser utilizado ao dar resposta às Diretivas. Este estudo teve como principal objetivo proporcionar uma perspetiva sobre a performance de vários métodos de análise de dados relativos à deteção de alterações na distribuição de espécies bastante móveis, como é o caso dos cetáceos. Os métodos testados foram aplicados para estudar a distribuição de 4 espécies que ocorrem no noroeste do Mar Mediterrâneo: golfinho-riscado (*Stenella coeruleoalba* (Meyen 1833)), baleia-comum (*Balaenoptera physalus* (Linnaeus 1758)), cachalote (*Physeter macrocephalus* Linnaeus 1758) e baleia-bicuda-de-Cuvier (*Ziphius cavirostris* G. Cuvier 1823). Os dados utilizados na análise foram obtidos através de programas de monitorização de cetáceos promovidos entre 2008 e 2022 no mar da Liguria, utilizando ferries de passageiros como plataforma-de-oportunidade, seguindo um protocolo fixo.

Ao reportar a distribuição de espécies de cetáceos, a DH recomenda como data análise a utilização de grelhas com resolução de 10x10 km, enquanto que a DQEM propõe uma resolução de 50x50 km. Ambas foram testadas, juntamente com uma resolução mais elevada

(5x5 km) que, dada a complexa fisiografia que compõe a área de estudo, se espera ser mais adequada para descrever a variabilidade de habitats presente na área. Por forma a analisar possíveis alterações na distribuição, as Diretivas sugerem o estudo entre diferentes séries temporais (curtas, de 12 anos, ou longas, de 24 anos). Assim, os dados foram divididos em três períodos, correspondendo aos ciclos de relato da DH: 2008-2012; 2013-2018 e 2019-2022. O ano de 2020 não foi incluído na análise dado que as campanhas de monitorização foram suspensas pelas restrições relativas à COVID-19. Duas metodologias foram aplicadas, uma relativa à ocupação observada e outra em relação à ocupação modelada. Focando na primeira, de modo a ser possível comparar a distribuição das espécies entre períodos, apenas células visitadas em comum em todos os anos que compõem os mesmos foram utilizadas. Para todas as resoluções, mapas de deteção/não-deteção foram criados e a proporção de área ocupada (PAO) calculada, permitindo obter uma imagem da distribuição e a percentagem de células usadas pelas espécies em cada período. Um índice de deslocação calculado possibilitou, de um ponto de vista espacial, perceber se as espécies utilizaram as mesmas ou diferentes células entre períodos. Além disso, mapas de taxa de encontro foram concebidos com o intuito de demonstrar se se sucederam variações no número de deteções ao longo dos períodos. De seguida, um modelo de ocupação, que tem em conta imperfeições na deteção, criado através do pacote *unmarked* para o software *RStudio*, foi também testado, o que permitiu obter valores de ocupação relativos a uma maior parte da área de estudo e sem a restrição de utilização apenas de células visitadas em comum entre os períodos. Para tal, históricos de deteção foram criados para cada célula em que “0” representa não-deteção e “1” deteção em cada ano, primeiramente para os 14 anos de estudo e, posteriormente, reduzido para os anos referentes aos dois primeiros períodos (5 e 6 anos), por forma a testar os limites do modelo em termos de número de visitas necessárias.

Como resultados, relativamente à ocupação observada, os mapas e o índice de PAO descreveram o golfinho-riscado como a espécie com maior uso da área de estudo, seguidamente a baleia-comum e, por fim, o cachalote e a baleia-bicuda-de-Cuvier com preferências por zonas de talude continental e montes submarinos. Foi também possível observar, conjuntamente com o índice de deslocamento, alterações na distribuição destas duas últimas espécies ao longo dos períodos, com a não-deteção do cachalote a sudoeste no 3º período e uma expansão do uso a este da área de estudo pela baleia-bicuda-de-Cuvier do 1º para o 2º período. Os mapas de taxa de encontro descreveram, para além de um ponto de vista qualitativo de deteção das espécies, uma perspetiva quantitativa, onde foi possível observar preferências por zonas de mar aberto pela baleia-comum, bem como um decréscimo generalizado do número de encontros de todas as espécies do 2º para o 3º período, sendo mais evidente na zona oeste. Este resultado indica uma diminuição na presença das 4 espécies nos últimos anos na área de estudo. No que diz respeito à ocupação modelada, o golfinho-riscado foi novamente a espécie descrita com maior área de uso e maior taxa de deteção. No entanto, contrário à diferença obtida entre a baleia-comum e o cachalote obtida através do cálculo da PAO, o modelo sugere uma ocupação similar entre estas duas espécies, com uma taxa de deteção mais elevada para a primeira. A baleia-bicuda-de-Cuvier obteve os valores mais baixos de uso da área de estudo, mas com um valor de taxa de deteção semelhante ao cachalote – resultados esperados dado serem espécies inconspícuas e elusivas, contrariamente a espécies como o golfinho-riscado e a baleia-comum. O modelo funcionou com maior sucesso quando os históricos de deteção foram compostos pelos 14 anos do estudo, diminuindo a sua capacidade à medida que o número de visitas à célula se reduziu (6 e 5 anos). Relativamente às escalas de resolução testadas, comparando com resoluções mais baixas (50x50 e 10x10 km), a resolução de 5x5 km permitiu, numa maior medida, a deteção de alterações na distribuição das espécies em todos os métodos aplicados.

Este estudo demonstra a importância das campanhas de monitorização promovidas a longo prazo, que permitem detetar alterações na distribuição das espécies, e suporta a utilização de resoluções mais elevadas na análise da distribuição de cetáceos ao responder às Diretivas Europeias.

Palavras-chave: distribuição, cetáceos, ocupação, deteção, Diretiva Habitat

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“*After the magical moment when my eyes were opened to the sea,*

I was no longer possible to see, think and live as before.”

– Unknown source

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List of abbreviations, acronyms and symbols

$\hat{c}$	Overdispersion parameter
ECC	Eastern Corsica Current
ER	Encounter Rate
EU	European Union
Fig.	Figure
GIS	Geographic Information System
GPS	Global Positioning System
HD	Habitat Directive
i.e.	That is
IUCN	International Union for Conservation of Nature
km	Kilometre
$\mathcal{L}(\Theta \mid x)$	Likelihood function of the model parameters, given the data x
MLE	Maximum Likelihood Estimator
MMO	Marine Mammal Observer
MSFD	Marine Strategy Framework Directive
NC	Northern Current
ODS	Observed Distributional Range
PAO	Proportion of Area Occupied
QGIS	Geographic Information System desktop application
SE	Standard Error
SDM	Species distribution models
Θ	Generic parameter vector (parameters such as ψ and p)
WCC	Western Corsica Current
α	Significance level
$\sim$	Distributed as
$\approx$	Approximately equal to

Chapter I

1. General Introduction

The study of the distribution of species plays a key role in understanding the ecology and possible implementation measures concerning species conservation [1], [2]. Consistent monitoring through the years allows, therefore, a constant update of this knowledge alongside an adaptative improvement on management matters [3].

This thesis is focused on the spatial distribution of four cetacean species that regularly occur in Ligurian waters, in the North-west of the Mediterranean Sea: striped dolphin (*Stenella coeruleoalba* (Meyen 1833)), fin whale (*Balaenoptera physalus* (Linnaeus 1758)), sperm whale (*Physeter macrocephalus* Linnaeus 1758) and Cuvier's beaked whale (*Ziphius cavirostris* G. Cuvier 1823).

In this first chapter, the conceptual idea of distribution is approached, as well as a rough overview of commonly used methods applied to measure it in a wide scope of taxa and, afterwards, specifically in relation to cetaceans. Lastly, the objectives of the thesis are detailed.

1.1. Distribution definition

In 1920, Henderson and Henderson [4] defined distribution as "the range of an organism or group in the biogeographical divisions of the globe". Research on species distribution has considerably increased over the years, and different methodologies and tools have been developed. Standardized measurement units and a further precise description regarding the "divisions of the globe" are needed to effectively compare and analyze differences on spatial and/or temporal scales. Therefore, constant definitions have arisen to more and more systematize the concept of distribution. The fundamental basis of the contemporary concept of distribution can be summed up by the definition applied by the European Union (EU) INSPIRE Directive (2007/2/EC): “geographical distribution of occurrence of animal and plant species aggregated by grid, region, administrative unit or other analytical unit”. As an example, the EU Habitat Directive (HD, 92/43/EEC), which aims to promote the maintenance of biodiversity, and the EU Marine Strategy Framework Directive (MSFD, 2008/56/EC), which aims to achieve a Good Environmental Status of EU Marine waters, recommend the appliance of grids when reporting the distributional range of species.

Accordingly, the definition of distribution followed in this thesis is encompassed within the EU INSPIRE Directive definition.

1.2. Methods to measure distribution

Distribution studies encompass a wide diversity of taxa, including terrestrial and aquatic mammals [5], [6], birds [7], [8], fish [9], [10], reptiles [11], amphibians [12], [13], invertebrates [14], [15] and plants [16], [17], employing a broad variety of methods for data collection and analysis. Acoustic methods were applied to study the distribution of birds [7], seagrass [18], fish [10] and crabs [14]. Camera traps were employed to detect birds [19], minks [20] and deer [21]. Other methods, such as satellite tags, were applied to study fish [22] and seals [23]; and human observations were used to study deer [21] and koalas [24]. Distribution can be associated with and measured by the presence of species. From the aforementioned methods, presence is the primary image that may be retrieved and can be used to investigate the distribution of a species in a specific location. When the only information available is geographical coordinates of locations where the species was observed, but no information on where it was absent, it is defined as presence-only data [26] and it can be found in atlas and museum records or as opportunistic datasets (e.g. citizen science). Whereas, presence/absence data is collected through dedicated surveys, concerning and recording information on species presence and absence [27], as performed in the data collection method of the present thesis. When working with presence/absence data, it is important to remember that a species' detectability can hardly be 100%. Presence is confirmed when detected, but its absence may not be effectively stated, given the fact that, during a survey, although while using standardized protocols, one is to expect that the species is not always detected, even if present at the site [28], [29]. Therefore, in order to not underestimate the true occupancy, it is important to take into account the imperfection in detectability when employing a method to study the distribution of a species.

Regarding data analysis, a common practice is the use of species distribution models (SDMs) (also frequently mentioned with different meanings as habitat models and ecological niche models, as discussed by Peterson & Soberón [30] and Elith & Leathwick [31]) to predict the current and potential spatial distribution of species [8], [12], [25], where unsurveyed areas and predictive shifts on current distributions can be analysed. SDMs are one of the preferred methods for studying species distribution that may take into consideration the imperfections in detection. These models are interesting in the point that they relate the occurrence of a species with the environmental information in a certain location [31], allowing the distinction of the more suitable areas for the species. In the vast field of species distribution studies, such models were previously applied to study the distribution of trees [32], birds [25], cetaceans [8], [33], [34], snakes [35], amphibians [12], [13] and fish [9]. However, the main objective of the referred types of models, as previously mentioned, is evaluating how environmental variables may influence the distribution of species and, accordingly, infer its distribution in new areas of potentially suitable habitat, which is not the aim of the present thesis. Here, the intent is not to relate the presence/absence of the species to characteristics of the sampling sites but to portray an image of where the species is present/absent in a given area, while addressing the uncertainty of true absence or non-detection. Hence, the most suitable option to model distribution data, in our case, might be to use instead occupancy models [28], [36], which estimate occupancy and detectability from presence/absence data.

Occupancy is generally described as the probability of a species using a sampling unit (i.e., quadrats) at a certain period of time [37], and it can result in a proportion of area (sample units) occupied [28]. Occupancy models are applied based on data composed of observed occupancy states of sampling units (s), that were surveyed frequently during a 'season' (time interval where the sampling units are closed to changes regarding occupancy state), to provide estimates for the whole area of interest (S). A basic occupancy model measures two parameters simultaneously: ψ, probability of species presence, with each sampling unit being classified as occupied or unoccupied ($1 - \psi$), and p, probability of detecting the species (given presence) (undetected being $1 - p$) [27], [28], [36]. For further complexity, site- and survey-specific covariates may be added to the model, to represent unequal probabilities of a unit being occupied and of detecting the species in a survey [27], and occupancy maps may be assembled. Basic assumptions of the model, in addition to the closure assumption, are i) independency between and within surveys and sampling units; ii) no false detections resulting in false presence (i.e., misidentification); and iii) all parameters being constant across sites or modelled by covariates at any given time [36]. Nonetheless, some assumptions may be relaxed as explained by MacKenzie et al. [27], such as the closure assumption, where, in case of violation, the proportion of area occupied should be interpreted as proportion of area "used". Also, occupancy models allow an unequal survey effort at different sampling units, which is a typical concern in realistic study designs. Such models were only recently used to study cetacean species [38]–[41].

1.3. Distribution of cetaceans

In marine environments, the increase in knowledge regarding cetacean species is relatively slower than in other taxa, as a result of their wide-range distribution, natural diving periods (that promote a species to go undetected), and difficulty and high cost in accessing their environment [3], [33]. Common methods used to collect data on the distribution of cetaceans are performed via acoustic detection [42]–[44], telemetry tags [45]–[47], photo-identification [48]–[50] and aerial [49]–[51] and ship [1], [52]–[54] observations.

Depending on the method intended to be employed, as well as the objectives of the study and financial availability, different types of observation platforms can be used to study cetaceans, i.e., land-based stations or moving platforms, such as aircraft and ships. The latter encompasses both dedicated research vessels [41], [57], [58] and platforms of opportunities, such as whale watching [55], [56] and commercial ships (cargo ships and passenger ferries) [54], [59], [60]. Being the offshore areas scarcer in research regarding cetacean distribution, platforms of opportunity that provide the possibility of reaching the open ocean, such as passenger ferries, are highly valued. Equally, characteristics like its constant speed, fixed routes and cost-effective method allow the performance of large-scale long-term monitoring programs [61], [62].

EU Member States are required to develop and deliver reports answering to the EU Directives' exigencies. Both the HD and the MSFD have a 6-year cycle report, where the evaluation of the current distribution of species and its trends are requested. However, the reports already delivered by several countries have stated that there is not enough data to draw conclusions, leaving the questions unanswered. Therefore, a proper debate about methodologies is still in need. Regarding the HD, all cetacean species are protected under Annex IV and have their distribution recommended to be analysed by applying a grid of 10x10 km resolution. Whereas, for the MSFD, which considers cetaceans as functional groups within the Descriptor 1 – Biodiversity, the recommended methods comprise the appliance of grids of 50x50 km resolution or the use of regression methods (generalised linear and generalised additive models) to study distribution, together with power analysis for analysing trends. As no standard method is demanded, with an ample variety of methods for data analysis being recommended, different research groups employ distinct methods, which contributes to disparate results. Also, researchers frequently find other approaches to be more suitable for studying cetaceans' distribution and, therefore, apply other than the recommended methods, including the maximum entropy approach [34], [63], [64], and likelihood- [38], [39] or Bayesian-based occupancy models [40], [41]. Hence, a standardized method to be applied when reporting under the EU Directives is still lacking.

1.4. Thesis objectives

In the Mediterranean Sea, a few studies on cetaceans have been done over the past centuries, but, only after the 1980s, extensive research has been committed to enhancing the knowledge of such species in the waters around Italy [65]. Although big progress has been made regarding cetaceans' distribution, a lack of information regarding distribution trends over the years is still occurring. Therefore, in order to reduce this gap, further studies on this matter are needed, especially applying a standardized method, which will allow comparisons between results. For that matter, the focus of this thesis was to test and compare different methods of data analysis regarding trends in cetacean distribution.

The main objective of this study was to analyse possible changes in the spatial distribution of four cetaceans occurring in the Ligurian Sea, with data collected from passenger ferries between the years of 2008 and 2022, by applying different methods of data analysis. All methods were reproduced for the two grid resolutions requested by the EU Directives, as well as for a finer resolution of 5x5 km, with the aim of assessing the most suitable resolution which allows the detection of changes in distribution regarding the species in question. To test the hypothesis that the distribution of the aforementioned cetacean species did not change between periods (corresponding to the 6-year cycle report of the HD) in the study area, analyses were performed where imperfections in detectability were not considered, such as the proportion of area occupied and a shift index. In order to address imperfect detection in our study, the probability of occupancy and detection were further obtained from the appliance of an occupancy model, where the *unmarked* package built for *R* software, which allows the estimation of site occupancy by organisms that cannot be detected with certainty, was used.

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Chapter II

Detecting changes in cetacean distributions in the North-West Mediterranean Sea: testing methods of data analysis

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Key-words: distribution, cetaceans, occupancy, detection, Habitat Directive

Abstract

When reporting under the EU Directives (Habitat Directive and Marine Strategy Framework Directive), species distribution and its trends are required. The research aimed to investigate the performance of three grid resolutions (5x5, 10x10 and 50x50 km) with two indexes (proportion of area occupied; shift) and an occupancy model to study the distribution of 4 species occurring in the Ligurian Sea (striped dolphin, fin whale, sperm whale and Cuvier's beaked whale) along 3 periods (2008-2012; 2013-2018; 2019, 2021, 2022) corresponding to the HD reporting cycles. Data was collected onboard ferries as platform-of-opportunity. Detection/non-detection maps and the proportion of area occupied showed the striped dolphin as a species with generalized use of the study area, whereas the fin whale preferred the open sea and the sperm whale and Cuvier's beaked whale favoured slopes and seamounts. Changes in the area used were denoted for the sperm whale, which had a complete non-detection in the southwestern part in the last period, and for the Cuvier's beaked whale, describing an expansion movement in the eastern part from the 1st to the subsequent periods. Encounter rate maps showed a decrease in detections for all the species from the 2nd to the 3rd period, being most prevalent in the western part, indicating a decrease in their presence. The occupancy model described similar use (%) of the study area, except for higher values for the sperm whale. Compared to coarser resolutions, the use of a finer resolution allowed, to a greater extent, the detection of changes in all methods. The model performed better when more surveying occasions (14) were given. This study supports the use of finer resolutions when reporting the distribution of cetaceans under the EU Directives, as well as the importance of long-term monitoring programmes for detecting species distribution changes.

2.1. Introduction

The study of species distribution is an elemental and critical component of ecology, being part of other research areas and allowing its further understanding, such as conservation biology, biogeography, climate change ecology, and trend assessments [1]. Distribution studies have been performed concerning several taxa (i.e.: plants [2], [3], invertebrates [4], [5], vertebrates [6]–[11]) with more or less emphasis, depending on factors such as financial availability and the ecology of species itself. Species that are highly mobile and habit hard-to-reach environments, such as cetaceans, tend, for those matters, to be less studied.

Within the Mediterranean Sea, the Ligurian Sea is known to be a permanent summer, but also winter [12]–[15], ground for several cetacean species [13], [14], [16], [17]. Its complex topography, together with the oceanographic characteristics that lead to the high levels of primary production found in this region [16], [18], [19], constitute a preferential habitat for such species [20] and turn the Ligurian sea into an area of cetacean concentration. For that matter, with the will to protect these species and their habitats, the establishment of the Pelagos Sanctuary, an agreement signed between France, Monaco and Italy in 1999, occurred [21].

The striped dolphin (*Stenella coeruleoalba* (Meyen 1833)) is the commonest cetacean species found in the Mediterranean Sea, being currently classified in this basin as ‘Least Concern’ by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species [22]. This pelagic species is predominantly present in deep waters (>1000 m) and steep slopes, occurring regularly in the entire basin, except the northern Adriatic Sea [23]–[27].

The fin whale (*Balaenoptera physalus* (Linnaeus 1758)) is a regular species of the Mediterranean Sea, mainly occurring in the western and central parts of the basin, in deep offshore areas (~2000 m depth) [18], [23], [25]. A recent assessment of this mysticete in the Mediterranean Sea reported this species to have its conservation status upgraded from ‘Vulnerable’ to ‘Endangered’ [28], given the low estimates of mature individuals of this subpopulation.

The sperm whale (*Physeter macrocephalus* Linnaeus 1758), the largest odontocete [29], is currently classified in the Mediterranean as ‘Endangered’, following the IUCN Red List of Threatened Species criteria [30]. This species shows a preference for the continental slopes and submarine canyons, where mesopelagic cephalopods, the species’ main prey, mainly occur [23], [25], [31], [32]. This species is also known for its deep long dives (40-50 min) when foraging with little surface periods (~9 min) [33], [34], making the species harder to detect when only human-observation methods are employed.

Cuvier’s beaked whale (*Ziphius cavirostris* G. Cuvier 1823), the species with the current deepest and longest mammalian dive records (2992 m [35]; 222 min [36]), has a conservation status classified as ‘Vulnerable’ by the IUCN Red List of Threatened Species [37] in the Mediterranean Sea. Like the previous species, this cetacean is relatively harder to observe at the surface given its long deep dives. Nevertheless, this species is associated mainly with areas of slopes, submarine canyons and seamounts [23], [25], [32].

Common bottlenose dolphin (*Tursiops truncatus truncatus* (Montagu 1821)) is also present along the entire Mediterranean basin, being regular in areas where continental shelf (<200m depth) is predominant, whereas Risso’s dolphin (*Grampus griseus* (G. Cuvier 1812)) and long-finned pilot whale (*Globicephala melas* (Traill 1809)) are present mainly in the north-western portion, in deeper waters [16], [27], [38]. The common dolphin (*Delphinus delphis delphis* Linnaeus 1758), which was present widely in the Mediterranean Sea until the 1960s, in coastal and offshore areas, is now vagrant in the basin except the Alboràn Sea [27], [39].

Given the difficulty in studying cetaceans' distribution, platforms of opportunity that allow cost-effective data collection to be done along repetitive routes for extended periods are favoured in comparison to costly dedicated surveys at sea. As presented by the literature, passenger ferries represent a thoroughly used moving platform for such objectives [40]–[42], with positive points on reaching open ocean areas and allowing the replication of the same transects to the detriment of other types of platforms.

Such systematic surveys allow the development of, among others, long-term spatial distribution studies, which are required by the European Union (EU) Habitat Directive (HD, 92/43/EEC) and Marine Strategy Framework Directive (MSFD, 008/56/EC). All cetacean species that occur in the Mediterranean Sea are protected under both Directives (Annex IV and Descriptor 1 – Biodiversity, respectively), being required the Member States to issue regular assessments and monitorization activities of several parameters, among which is the distributional range of the species. In order to answer to the EU Directives, distinct methods of data analysis may be performed, with both Directives recommending the appliance of grids or regression methods, but no standardized approach, which leads to differing analyses between researchers. Hence, it is of extreme importance the implementation of a standardised method when reporting under the Directives. It is, therefore, with this in mind that the purposes of this study compared different methods of data analysis, such as the proportion of area occupied and a shift index [43], where no imperfections in detectability are taken into account. To address this issue, an occupancy model, which accounts for imperfect detection [44], was, afterwards, applied to obtain probabilities of occupancy and detectability in the study area. The model estimates two parameters, occupancy (ψ) and detection (p) probabilities, using a maximum likelihood [44] or Bayesian [45] approach, retrieving the probabilities of each sampling unit being occupied by the species and for the species to be detected in that unit. Occupancy estimates regarding series of years can be retrieved, which may be used to analyse distribution trends between periods, as requested by the EU Directives. Such occupancy models were only recently applied to model cetacean distribution, initially by Dwyer [46], followed by Vilela *et al*., [47], Pennino *et al*., [48] and Currie *et al*. [49].

In this study, data collected onboard passenger ferries as platforms of opportunity for monitoring cetacean presence from 2008 to 2022 was used. Given its request by EU Directives and the current lack of information, inter-period trends on cetacean distributions are in need to be analysed. The study aimed, therefore, to compare different methods that, taking into account the data collected onboard ferries, allow the detection of possible changes in the spatial distribution of cetaceans occurring in the Ligurian Sea. To test the hypothesis that the distribution of 4 cetacean species (striped dolphin, fin whale, sperm whale and Cuvier's beaked whale) did not change between periods in the study area, occupancy obtained during the HD reporting periods was compared by applying different methods of analysis.

2.2. Material and Methods

2.2.1. Study area

The study area is located in the Ligurian Sea, at the North-west Mediterranean Sea, which connects westerly with the Gulf of Lion and the Algero-Provençal basin and eastern with the Tyrrhenian Sea by the Corsica Channel [50]. Its complex topography is composed of predominantly continental shelves (200 m depth) and slopes cut by several canyons and a seamount in the eastern region, with an abyssal plain (>2000 m depth) dominating the western area [50] (Fig. 2.1).

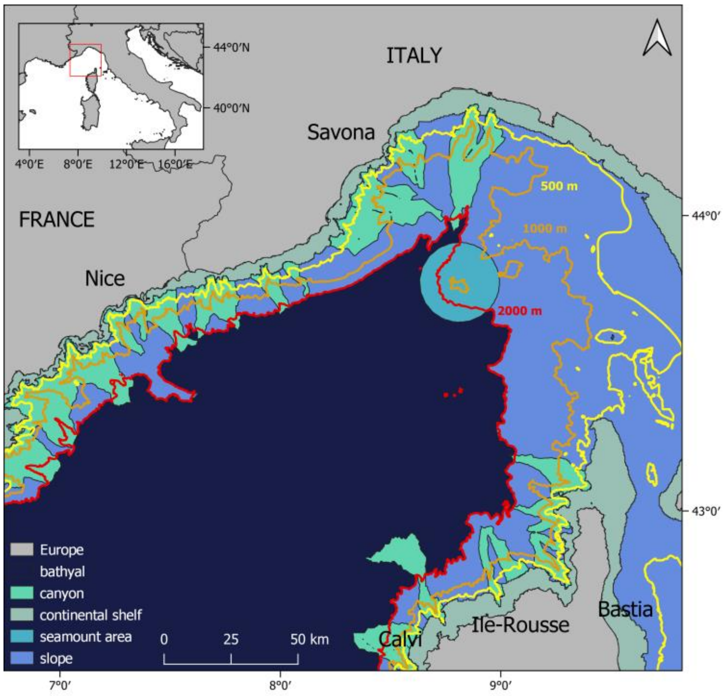

Fig. 2.1. Contoured bathymetry and habitats that compose the study area [51].

Three main currents occur in the area: Eastern Corsica Current (ECC), and Western Corsica Current (WCC), which join northerly and create a third current named Northern Current (NC) (Fig. 2.2). A seasonal cyclonic circulation is described by WCC and NC in the Liguro-Provencal front [52]–[54], which originates a permanent frontal system between the coast and offshore waters. This water mass boundary, jointly with vertical mixing and coastal upwelling, enhances the primary productivity levels of this region, increasing the presence of zooplankton, such as krill (*Meganyctiphanes norvegica*, the main prey of the Mediterranean fin whales [55]–[57]), and squids (main prey of sperm whales [58], [59] and Cuvier’s beaked whales [60], [61]).

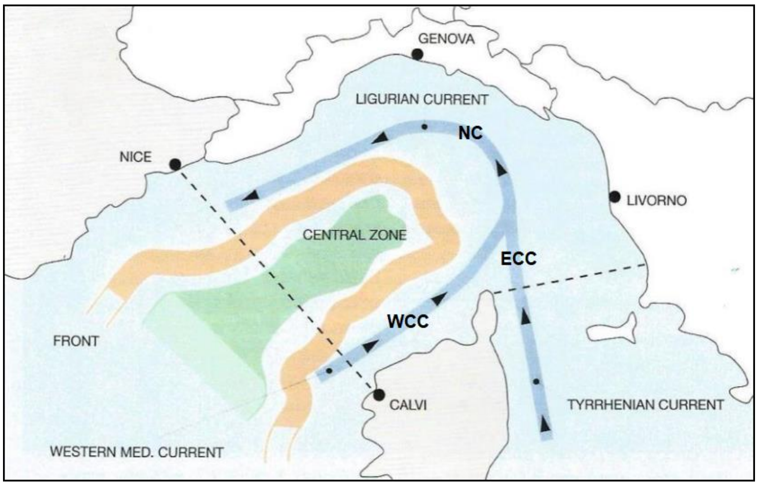

Fig. 2.2. The three major currents (ECC, WCC and NC) and dynamic structures of the Ligurian Sea. Adapted from [57].

2.2.2. Data collection

The present study has been developed within the project LIFE20 NAT/IT/001371 CONCEPTU Maris "CONservation of CEtaceans and Pelagic sea TUrtles in Med: Managing Actions for their Recovery In Sustainability" (www.lifeconceptu.eu), which aims to increase knowledge on cetacean and sea turtle species' distribution on the Mediterranean Sea in order to create new tools that will allow more confident choices in conservations matters. Several international institutes are joining the effort in this project, one of them being the *CIMA Research Foundation* [51], which has been collecting data using ferries as a platform of opportunity for dedicated surveys since 2008, whose data is the one being explored in the present work.

The data analysed in this study was obtained from data collected onboard ferries along fixed-line transects across the Pelagos Sanctuary area, following the method described by [62], hereafter detailed, during summer months (May to September) from 2008 to 2022. The surveys were conducted aboard passenger ferries of the company *Corsica-Sardinia Ferry,* that crossed the study area from the Ligurian (Savona) or French

(Nice) coast to the Corsica island (Bastia; Calvi; Ile-Rousse) (Fig. 2.3). Survey effort is distributed in accordance with the availability of routes carried out by the ferries company.

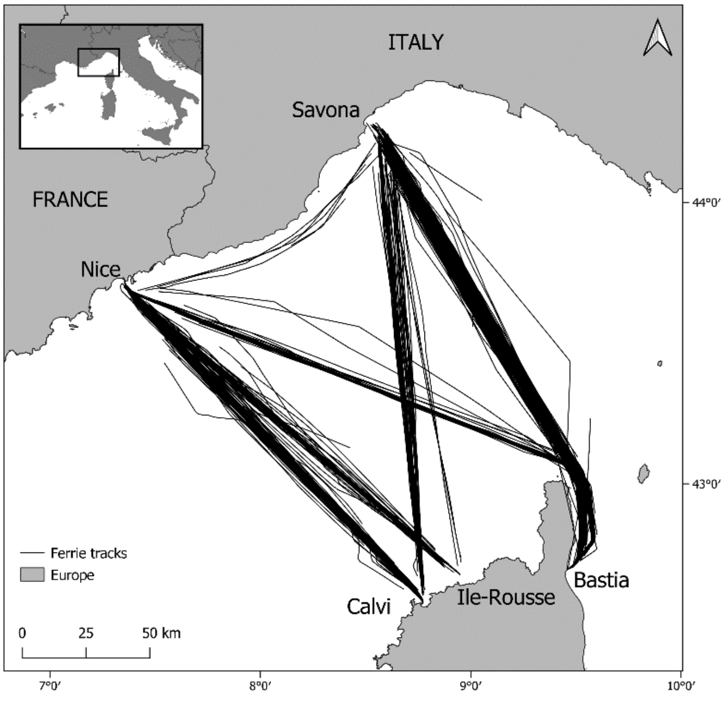

Fig. 2.3. Transects' location performed in the study area of the Ligurian Sea (Mediterranean Sea), in summer months, from 2008 to 2022 (except 2020).

The observations were performed from the ferries' command deck, with two Marine Mammal Observers (MMOs), one on each side of the bridge, continuously scanning the sea with naked eye and binoculars (7x50). A third researcher is assigned to input the information of the sightings detailed by the observers into specific data sheets or a tablet with a designated application assembled to support the activity of cetacean monitoring. The survey data comprise information such as the type of vessel employed, MMOs present, transect performed, weather and maritime traffic, effort and sightings. Within the latter, species, number of individuals, behaviour, distance and angle between the animal and the boat were noted. Transects were georeferenced using the Global Positioning System (GPS) incorporated in the tablet and using a dedicated GPS (Garmin eTrex 10), that functioned as a backup, to mark the beginning and end of each transect and waypoints to indicate the sighting's position. Vessel traffic was registered randomly every hour in the absence of cetaceans and every time a cetacean sighting was noted. Weather conditions were also recorded, at the beginning of the survey and when changes occurred. All observations were performed during daylight hours and under a Beaufort sea state minor or equal to 4, with vessel speed ranging from 14 to 29 knots. To avoid fatigue, every 30 min, since the beginning of the session, a rotation in position between the MMOs occurred. To each survey a specific code was attributed, in order to be easily identified (i.e.: 20180705232 – date: 05/07/2018; number of survey: 2; identifying number of the ferry: 32).

2.2.3. Data processing

All data processing was performed using QGIS software (Version 3.26.2), a free and open-source Geographic Information System (GIS) where it is possible to work with spatial information.

The EU HD and MSFD request the use of a grid when reporting the distribution of cetacean species, with a resolution of 10x10 and 50x50 km, respectively. In order to compare the performance of the different grids, both resolutions, as well as a finer resolution of 5x5 km, were used in the Data Analysis section of this study. All grids were obtained based on the European Environmental Agency's INSPIRE compliance guidelines.

The GPS points collected in each survey during the summer months were imported into the software and transformed into track lines. Each grid was laid over the whole Pelagos Sanctuary area and, applying the tool "Sum line lengths" from the QGIS processing toolbox, the number of tracks (visits) was associated with each cell per year. The year 2020 was not included in the analysis as surveys were stopped due to the COVID-19 restrictions. The sightings data regarding each species was afterwards imported into QGIS and the number of sightings per visited cell per year was counted by applying the tool “Count points in polygon”.

2.2.4. Data analysis

As required by the Directives, estimates of trends, in order to be statistically robust, should be performed over, at least, two reporting cycles (12 years). For that reason, for the inter-period analysis, the years were grouped following the already performed HD 6-year cycle reports: 2008 to 2012 (5 years) compose the 1st period (corresponding to the third HD reporting cycle); 2013 to 2018 (6 years) compose the 2nd period (fourth HD reporting cycle); and 2019, 2021 and 2022 (3 years) compose the 3rd period (fifth HD reporting cycle). The visits and sightings of each period for each cell were the result of the sum of values previously obtained for the years composing the period.

Two different methodologies were applied, one referring to the observed occupancy and one referring to the modelled occupancy.

2.2.4.1. Observed occupancy analysis

Observed occupancy is considered the direct observation of the species’ presence/absence in the study area obtained from the aforementioned methods of data collection.

In order to minimize the bias resulting from different coverage, only cells that were visited at least once in all years composing the periods in question (common cells) were used. Every following calculation was performed for each species, period and grid resolution.

Foremost, detection/non-detection maps were assembled, where cells that had at least one sighting were defined as “detection”. Afterwards, the proportion of area occupied (PAO), also commonly referred to as naïve occupancy [63], was measured as a percentage by dividing the number of cells with detection per total number of common cells. Changes in distribution between periods were measured by calculating a shift index [43, Equation 4]. The values vary between 0 (complete shift in the cells) and 1 (no shift).

The cells with detection were considered for the encounter rate (ER), which was obtained by dividing the number of visits with presence per total number of visits. Given the differences in visits between cells, the values were weighted (number of visits by the maximum number of visits performed between the periods) and further normalized by the maximum value of ER obtained between the periods per species.

2.2.4.2. Modelled occupancy

Modelled occupancy considers not only observations but also imperfections in detection [63].

Likelihood-occupancy models use the method of maximum likelihood to produce estimates of the probability of occupancy (ψ; probability of a site being occupied by the species) and probability of detectability (p; probability of detecting the species given it is present at the site) based on the presence/absence data that was input in the model [44], [63]. This type of model finds the values of the parameters (ψ, p) that are most likely given the data, which will maximize the likelihood function ($\mathcal{L}(\Theta|x)$, where Θ is the parameters and x the data), called maximum likelihood estimators (MLEs). These can be obtained by the usage of software, such as *MARK* (marked individuals) [64] and *PRESENCE* (unmarked individuals) [65], or software packages, as *RPresence* [66] and *unmarked* [67] (both created for *R* software), where maximum likelihood techniques are employed. As denoted by the name, the *unmarked* package allows the performance of occupancy studies of unmarked individuals and encompasses a wide variety of occupancy models to be implemented based on the input data (i.e.: detection/non-detection; data with false positives; multi-species or multi-season studies). The package provides tools for data manipulation, and model fitting, as well as tests regarding goodness of fit, model comparison and selection, where differences in the functions occur depending on the type of model chosen.

In the case of the current study, given the interest in analysing the occupancy of a single species along one season (i.e., summer months), the model applied was a single-species, single-season occupancy model [55], [59]. In such a case, the presence/absence at unit i is defined as a random variable (Z_i) that follows a Bernoulli distribution (binary; 0 or 1), where ψ is the probability of presence ($Z_i \sim$ Bernoulli (ψ)). Being the detection/non-detection at survey j denoted as h_{ij}, which is also a Bernoulli random variable, the probability of detecting the species at unit i depends on Z_i ($h_{ij} \mid Z_i \sim$ Bernoulli($Z_i\, p_j$)). As referred by [63], probability refers to an *a priori* expected result regarding a site being occupied, whereas proportion is the actual outcome, therefore, in the case of the present work, the occupancy estimate may be interpreted as the proportion of area occupied. The main assumptions of the referred model are: i) within the survey season, the species is either always present or always absent (closure assumption); ii) the probability of a site being occupied, as well as the probability of detection, is constant across sites or modelled by covariates; iii) independency is verified for the detection of the species and its detection histories at each site; iv) no false detections when the species is absent (misidentification) [68]. Given that the species in question are very mobile, the assumption of closure may be violated, however [63] stated that, in the case the movements in and out of the sites occur randomly, the assumption may be relaxed by interpreting the occupancy estimates as proportion of sites "used" instead of proportion of sites "occupied". For that matter, hereinafter, instead of "proportion of area occupied", the term "proportion of area used" will be employed when referring to the modelled occupancy.

In the present study, an "intercept-only" model was performed, where ψ and p are held constant across units and surveys. To perform the occupancy model, a detection history needs to be assembled, which describes the detection or non-detection state of the species in each visited cell. Therefore, a detection history was obtained regarding each species and grid resolution for every visited cell. The cells received, for each year, a classification of "0" (non-detection) when no sighting occurred and "1" (detection) when, at least, one sighting within the year was noted. Missing observations (no detection state for site i at survey j) result in a probability of detection of zero and, therefore, do not contribute to the model likelihood. Based on the detection histories, the model likelihood [69] for the given data is created and the maximum likelihood estimates of the parameters may be retrieved.

In order to perform the occupancy model, the *R* package *unmarked*, which has in its origin the intention of providing models for data from spatial sampling studies of unmarked individuals [67] and therefore allows the estimation of site occupancy, was used. Firstly, the detection histories data provided needs to be formatted, hence, for a single-species single-season occupancy analysis, the function *unmarkedFrameOccu* was employed. The model was afterwards fitted, using the *occu()* function, defining ψ and p as constant, and estimates of occupancy and detection were obtained.

To assess if the target model represented the observed data, a goodness-of-fit test was performed by applying the function *mb.gof.test*, which simulated 5000 bootstrap data sets to be used to compute the Mackenzie-Bailey test statistic [69]. The overdispersion parameter ĉ was afterwards retrieved, with the model adequately fitting the observed data if the parameter is close to 1 (lower or higher values indicate less or more variation in the observed data than expected by the model, respectively), at an α of 5%. A large p-value suggests no evidence for the lack of fit of the model in question. The aforementioned process was first performed for the entire data set (14 years/visits) and subsequently reduced in years in order to test the limitations in terms of visits and grid resolution.

2.2.4.3. Summary of the workflow

The steps of the workflow are further summarized in Fig. 2.4.

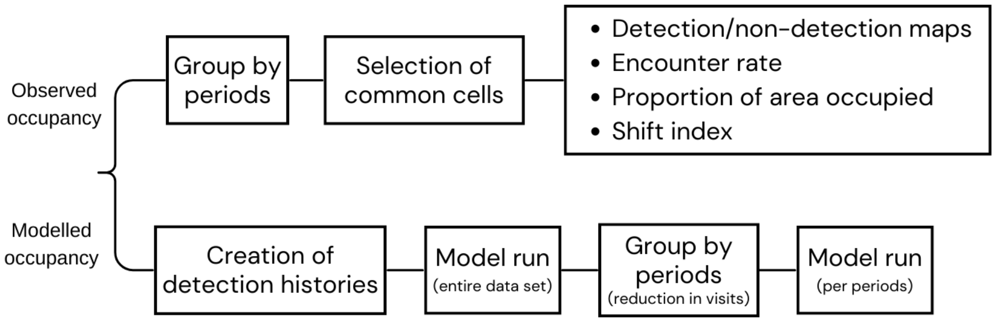

Fig. 2.4. Schematic representation of the workflow regarding data analysis.

2.3. Results

A total of 689 surveys were performed in the study area between 2008 and 2022 (except 2020). Each of the 4 species were encountered, at least, once every year, except the sperm whale for 2008, totalizing 4193 sightings for the entire study period. The general results are summarized in Table 2.1.

Table 2.1. Number of surveys and sightings per species and periods in the study area, from 2008 to 2022 (except 2020).

		1st period	2nd period	3rd period	Total
	# of surveys	280	310	99	689
Sightings	Striped dolphin	858	1077	416	2351
	Fin whale	513	720	294	1524
	Sperm whale	63	72	24	159
	Cuvier’s beaked whale	19	80	57	156

2.3.1. Observed occupancy analysis

For each grid resolution, the cells commonly visited between the periods were obtained, resulting in two distinct comparisons. The first was made between the three periods (hereafter referred to as “3-period comparison”), whereas the second one was made between the 2nd and 3rd periods (2-period comparison), given these had more cells in common, with each cell being visited, at least, 14 and 9 times, respectively to the comparisons. In both cases, it was assumed that the cells were well sampled given the ample number of times they were visited.

Within the study area, respecting the 50x50 km grid resolution, a total of 15 cells were visited, being the number of common cells between the periods 5 for the first comparison and 10 for the second (Fig. 2.5.). Regarding the 10x10 km grid, 187 cells were visited in total, with 27 in common between the three periods and 49 between the

2nd and 3rd periods (Fig. 2.6.). In respect to the finer resolution, a total of 557 cells were visited, with 54 common cells for the first comparison and 96 to the second (Fig. 2.7.).

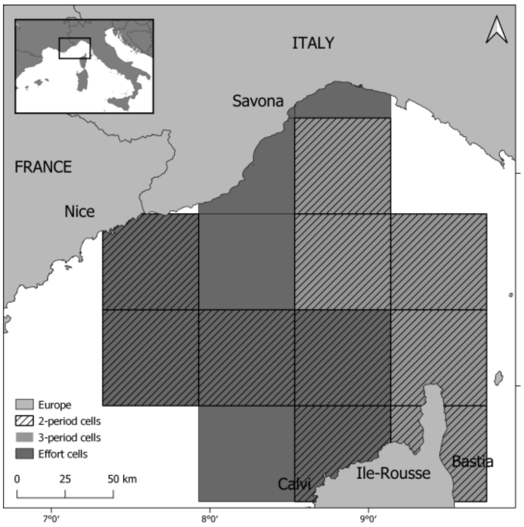

Fig. 2.5. 50x50 km grid: Total effort cells (dark grey), commonly visited cells between the three periods (light grey) and commonly visited cells between the 2nd and 3rd periods (striped) sampled in the study area from 2008 to 2022 (except 2020).

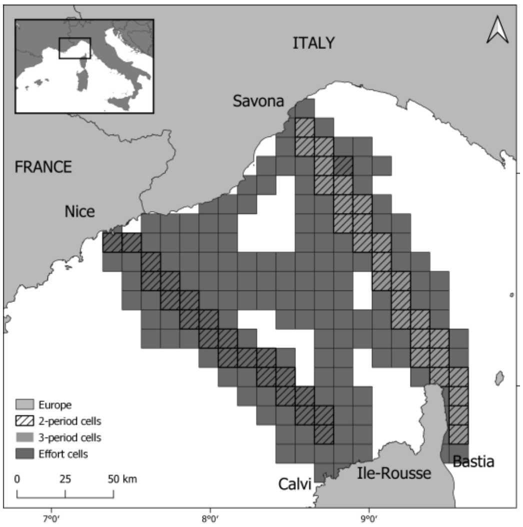

Fig. 2.6. 10x10 km grid: Total effort cells (dark grey), commonly visited cells between the three periods (light grey) and commonly visited cells between the 2nd and 3rd periods (striped) sampled in the study area from 2008 to 2022 (except 2020).

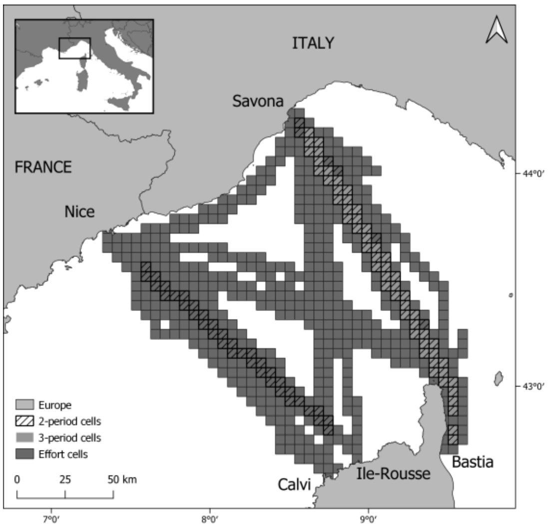

Fig. 2.7. 5x5 km grid: Total effort cells (dark grey), commonly visited cells between the three periods (light grey) and commonly visited cells between the 2nd and 3rd periods (striped) sampled in the study area from 2008 to 2022 (except 2020).

In order to visualize the occupancy obtained, detection/non-detection maps regarding each species and comparison between periods were assembled, with cells with, at least, one sighting being coloured (detection) and cells without sightings left blank (non-detection) (Fig. 2.8-2.13).

In respect to the 3-period comparison, regarding the 50x50 km resolution grid, the striped dolphin was detected in all 5 cells of the first comparison, except for one in the first period (Fig. 2.8). Both the fin whale and sperm whale maintained the same “detection” cells along the periods, being detected in all cells, except one for the second species, common to all periods. The Cuvier’s beaked whale was detected in 4 cells in the first two periods and all cells in the third period.

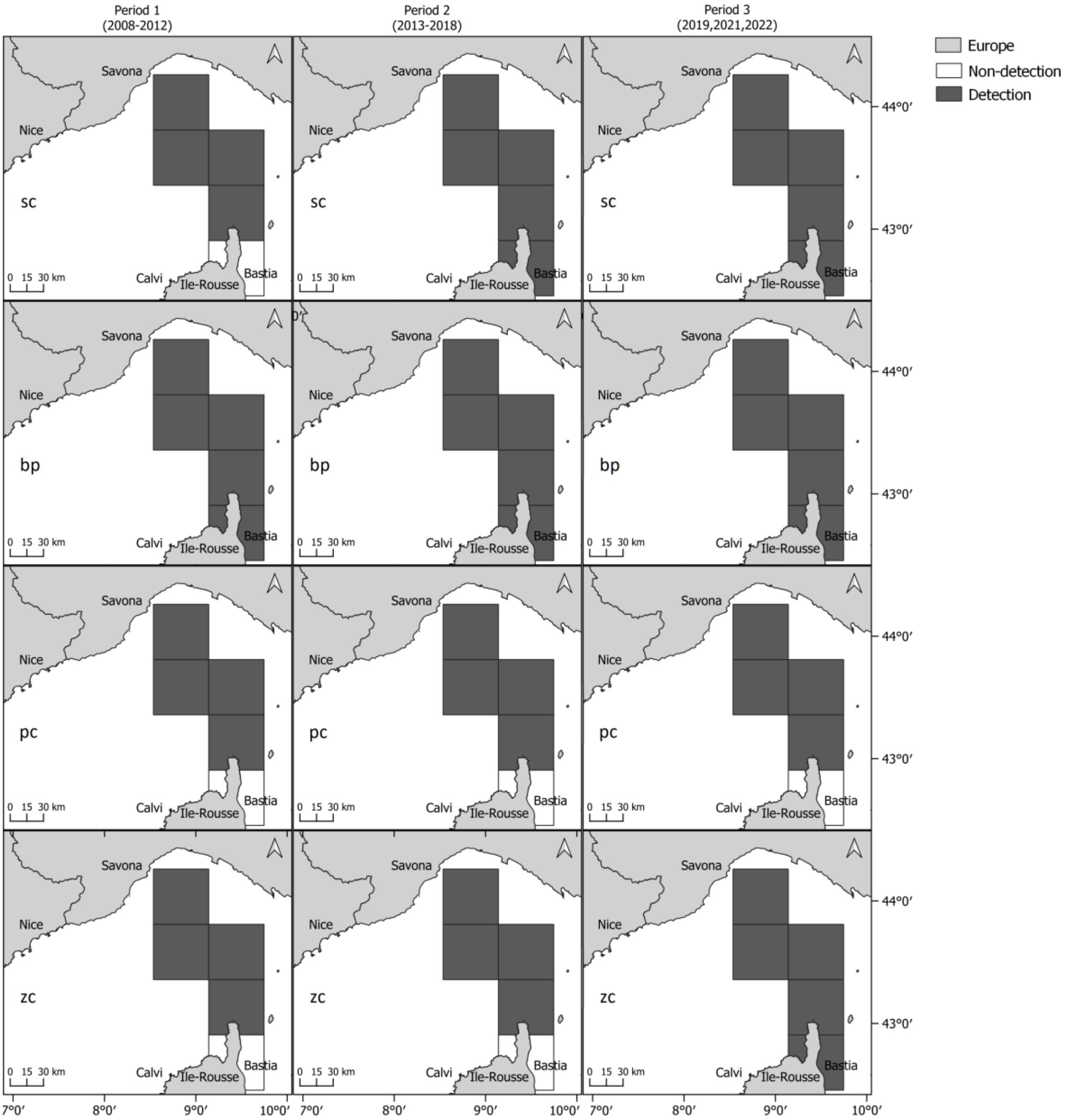

Fig. 2.8. Distribution of cells with detections of striped dolphin (sc), fin whale (bp), sperm whale (pc) and Cuvier’s beaked whale (zc) in the study area regarding the comparison between the three HD periods (50x50 km grid resolution).

When applying the 10x10 km resolution grid (Fig. 2.9), the striped dolphin was detected in 89, 96 and 93% of the 27 cells, whereas the fin whale was detected in 74, 81 and 81% of the total cells for the 1st, 2nd and 3rd periods, respectively. Sperm whale and Cuvier’s beaked whale were detected in fewer cells, with both species occurring mainly in the seamount and slope areas. An increase in number of occupied cells occurred regarding the latter, from the first to the last two periods.

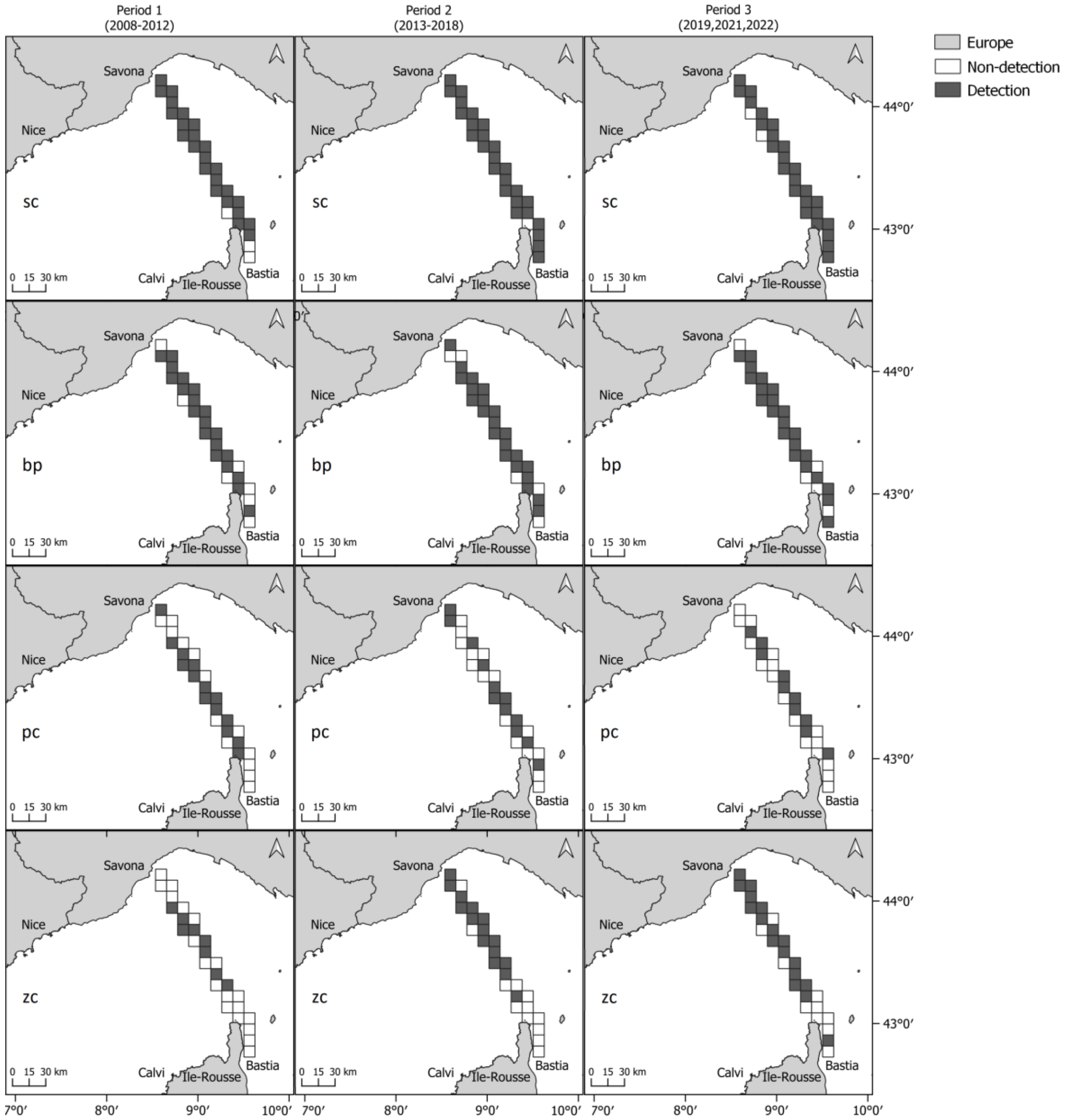

Fig. 2.9. Distribution of cells with detections of striped dolphin (sc), fin whale (bp), sperm whale (pc) and Cuvier’s beaked whale (zc) in the study area regarding the comparison between the three HD periods (10x10 km grid resolution).

Regarding the 5x5 km resolution grid (Fig. 2.10), once again, striped dolphin and fin whale were the species detected in the highest number of cells. The sperm whale showed a small decrease in the number of cells with detection throughout the periods (15, 1st period; 13, 2nd period; 12, 3rd period), whereas the Cuvier’s beaked whale presented an increase in the number of cells (9, 1st period; 24, 2nd period; 27, 3rd period).

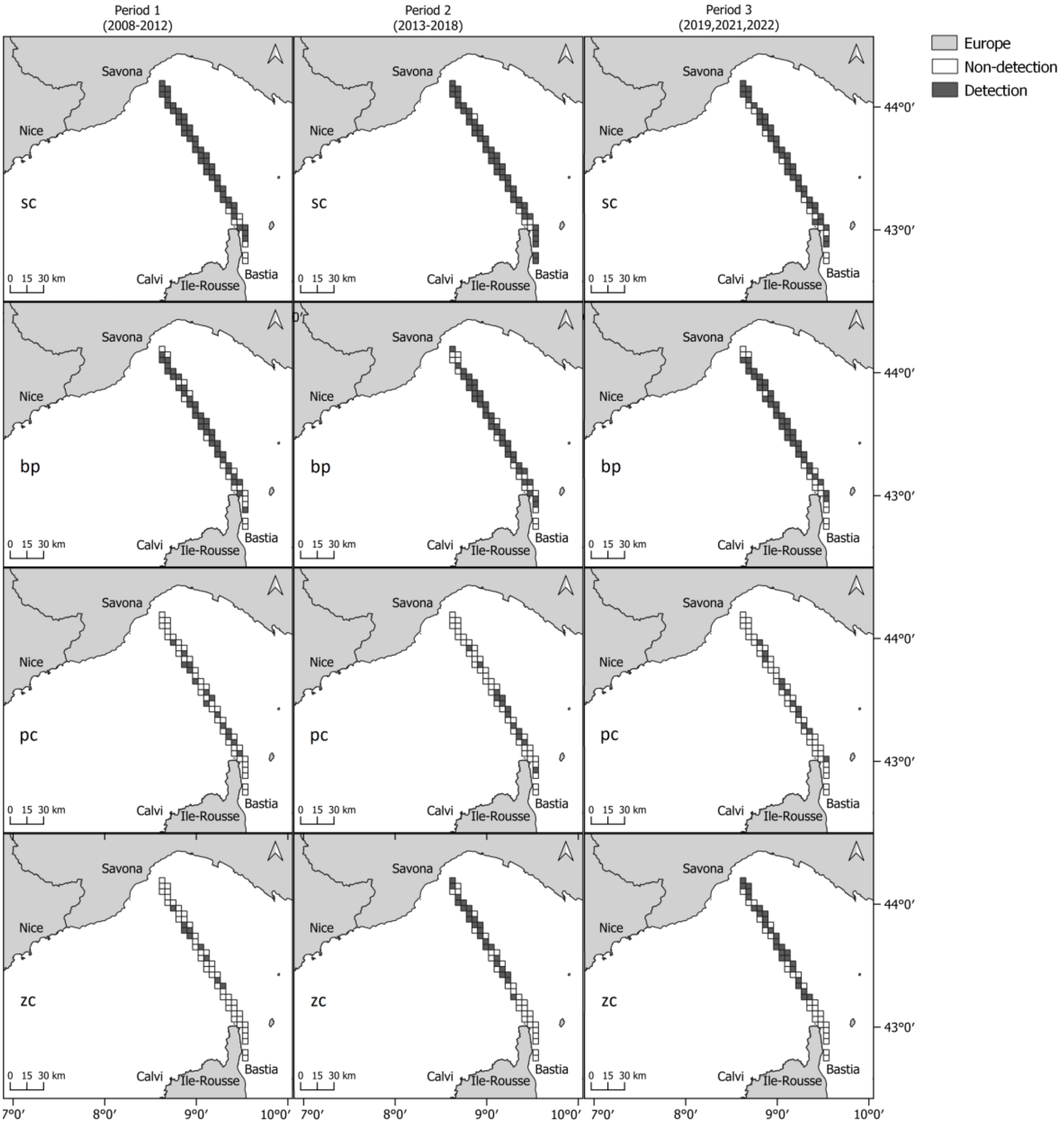

Fig. 2.10. Distribution of cells with detections of striped dolphin (sc), fin whale (bp), sperm whale (pc) and Cuvier's beaked whale (zc) in the study area regarding the comparison between the three HD periods (5x5 km grid resolution).

Focussing on the 2-period comparison, regarding the 50x50 km grid resolution (Fig. 2.11), both striped dolphin and fin whale were detected in the 10 cells. The sperm whale was found in 9 cells in the 2nd period and 5 in the 3rd period. The Cuvier's beaked whale was detected in 7 cells in both periods.

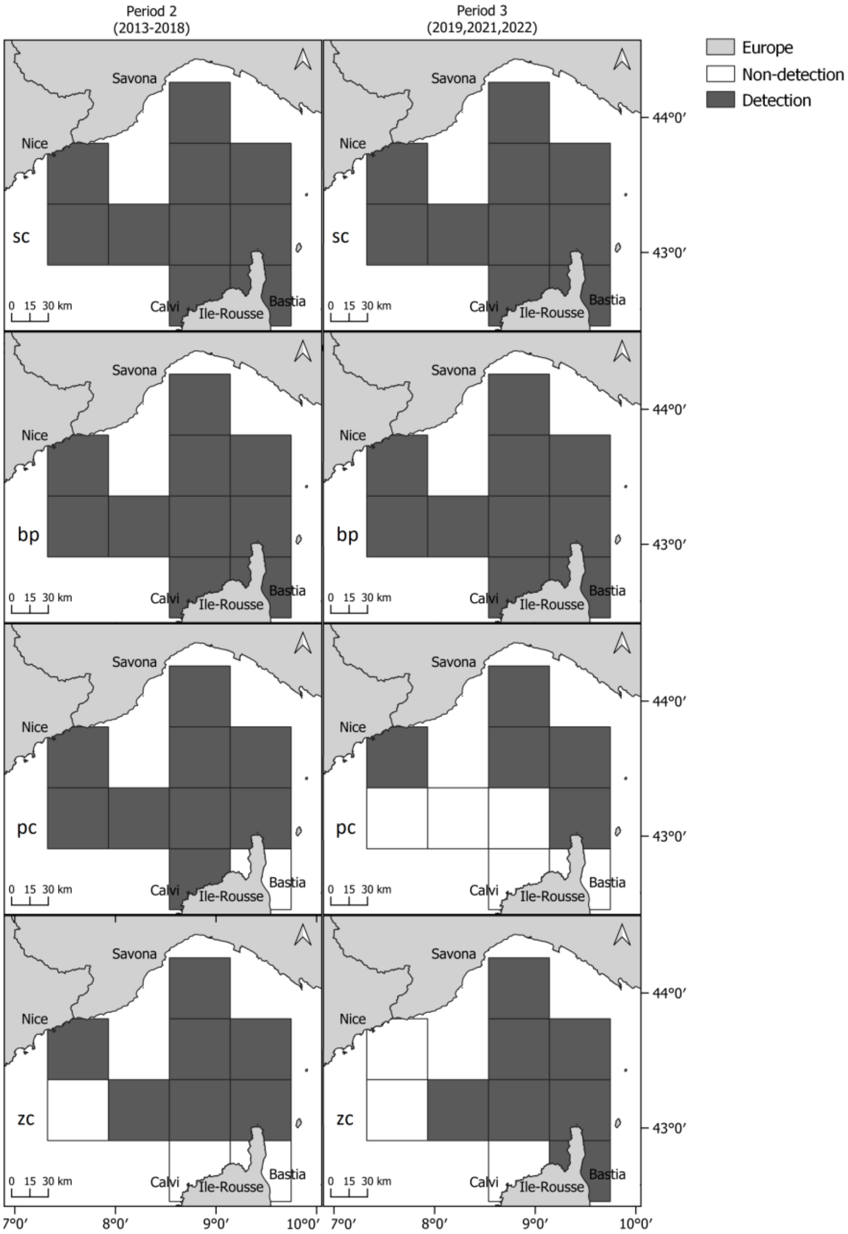

Fig. 2.11. Distribution of cells with detections of striped dolphin (sc), fin whale (bp), sperm whale (pc) and Cuvier’s beaked whale (zc) in the study area regarding the comparison between the 2nd and the 3rd HD periods (50x50 km grid resolution).

For the 10x10 km grid resolution (Fig. 2.12), the same image for the striped dolphin and fin whale can be retrieved. The sperm whale showed a decrease in detection in the offshore area, going from 24 “detection” cells to half in the last period. The Cuvier’s beaked whale was detected mostly in the route Savona-Bastia, being nearly absent from the abyssal plain cells.

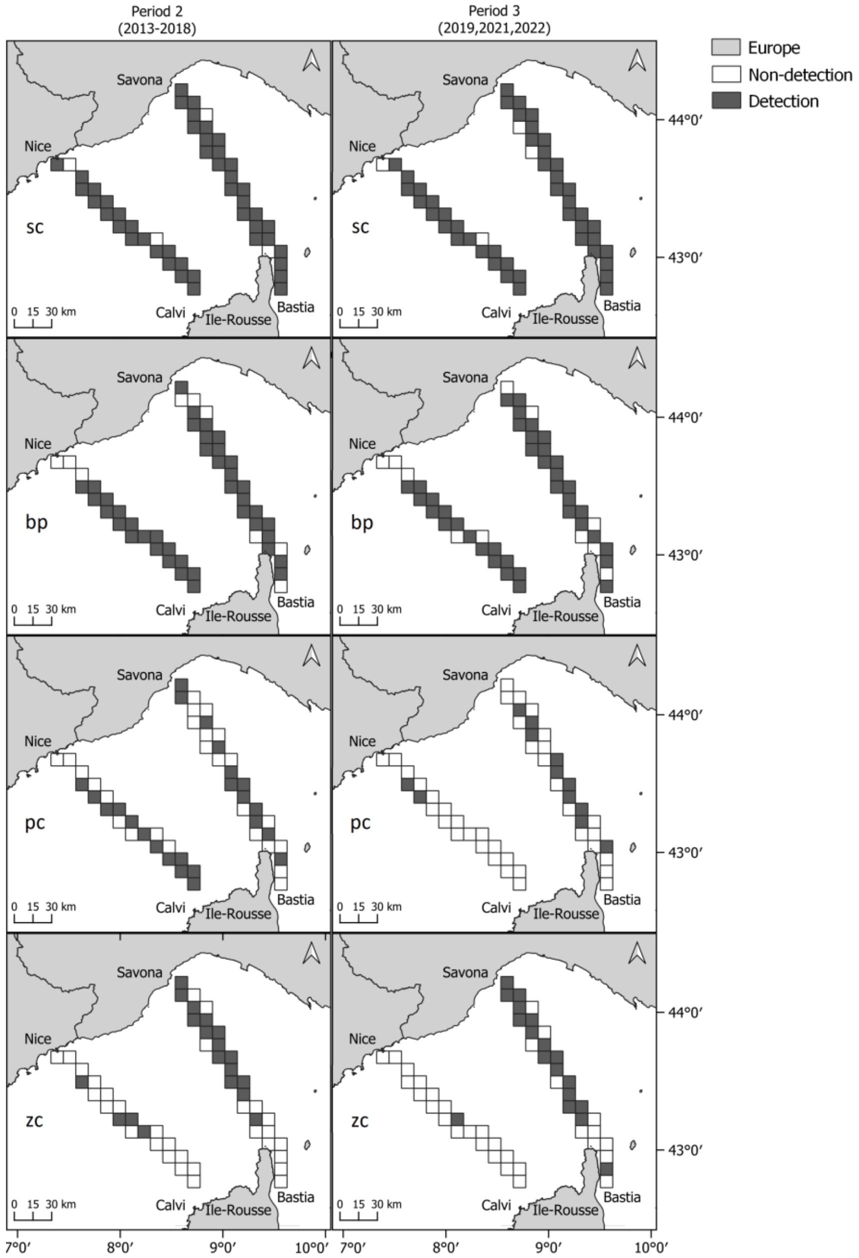

Fig. 2.12. Distribution of cells with detections of striped dolphin (sc), fin whale (bp), sperm whale (pc) and Cuvier's beaked whale (zc) in the study area regarding the comparison between the 2nd and the 3rd HD periods (10x10 km grid resolution).

As to the 5x5 km grid resolution (Fig. 2.13), striped dolphin and fin whale were encountered in almost all cells, whereas the sperm whale and Cuvier's beaked whale were detected in fewer cells, predominantly in cells characterized by slope and seamount habitats. Also, as described in the results from the previous resolution, the sperm whale showed a decrease in detection over offshore cells and the Cuvier's beaked whale was almost absent from the abyssal plain cells.

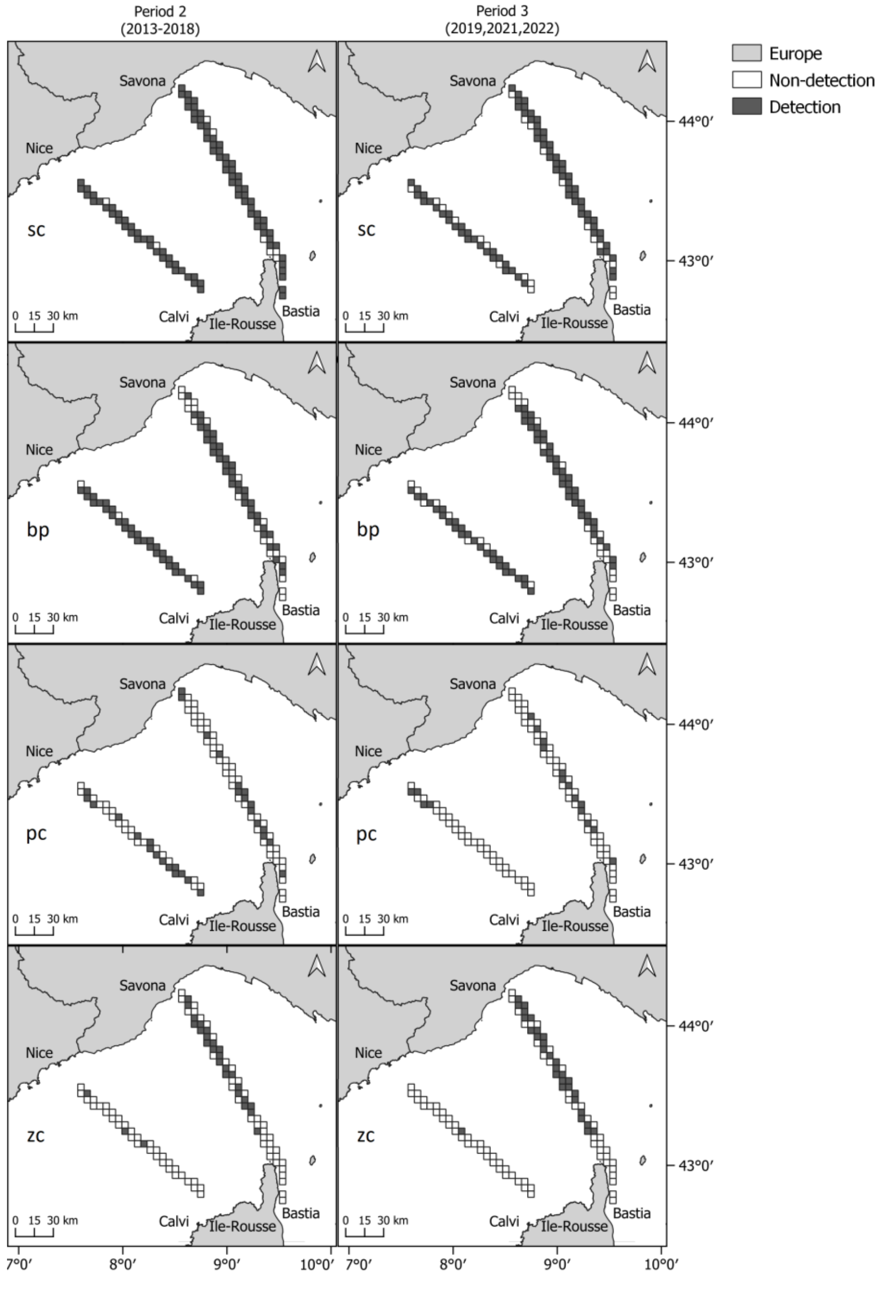

Fig. 2.13. Distribution of cells with detections of striped dolphin (sc), fin whale (bp), sperm whale (pc) and Cuvier's beaked whale (zc) in the study area regarding the comparison between the 2nd and the 3rd HD periods (5x5 km grid resolution).

The PAO was calculated for each grid resolution regarding both comparisons for the four species (Table 2.2). Focussing on the 3-period comparison, for the 50x50 km grid resolution, all species were detected in, at least, 80% of the selected cells for the three periods. Whereas, a bigger variation occurred for the 10x10 and the 5x5km grid resolutions, with values ranging from 29.6 to 96.3% and 16.7 to 90.7%, respectively. Regarding the intermedium grid resolution, the striped dolphin showed an increase from the 1st period to the 2nd period, followed by a slight decrease in the 3rd period. The fin whale described an increase from the 1st to the 2nd period, followed by a stabilization, whereas the Cuvier's beaked whale described an increase in PAO along the three periods, being more than 33% from the 1st period to the 3rd period. The sperm whale showed an opposite pattern, with decreasing values of PAO along the periods. Concerning the finer resolution, the same image from the previous grid resolution can be retrieved.

Table 2.2. Number of selected cells, sightings, cells with presence and PAO in the study area for each species and grid resolution regarding the 3-period comparison.

Grid	# cells	Species	Period	# sightings	# cells with presence	PAO (%)	ΔPAO (%)
50	5	Striped dolphin	1	370	4	80.0	+20 0
			2	603	5	100.0	
			3	288	5	100.0	
		Fin whale	1	129	5	100.0	0 0
			2	264	5	100.0	
			3	138	5	100.0	
		Sperm whale	1	26	4	80.0	0 0
			2	28	4	80.0	
			3	18	4	80.0	
		Cuvier's beaked whale	1	14	4	80.0	0 +20
			2	68	4	80.0	
			3	50	5	100.0	
10	27	Striped dolphin	1	361	24	88.9	+7.4 -3.7
			2	443	26	96.3	
			3	270	25	92.6	
		Fin whale	1	119	20	74.1	+7.4 0
			2	152	22	81.5	
			3	129	22	81.5	

		Sperm whale	1	24	14	51.9	-7.5
			2	22	12	44.4	-7.4
			3	17	10	37.0	
		Cuvier’s beaked whale	1	12	8	29.6	+26
			2	48	15	55.6	+7.4
			3	50	17	63.0	
5	54	Striped dolphin	1	332	47	87.0	+3.7
			2	411	49	90.7	-11.1
			3	241	43	79.6	
		Fin whale	1	109	37	68.5	+1.9
			2	146	38	70.4	+1.8
			3	122	39	72.2	
		Sperm whale	1	21	15	27.8	-3.7
			2	19	13	24.1	-1.9
			3	16	12	22.2	
		Cuvier’s beaked whale	1	11	9	16.7	+27.7
			2	47	24	44.4	+5.6
			3	46	27	50.0	

Regarding the 2-period comparison (Table 2.3), for the 50x50 km grid resolution, the PAO was equal between periods for all species but the sperm whale, which showed a decrease from 90% in the 2nd period to 50% in the 3rd period. For the 10x10 km grid resolution, the striped dolphin had no differences, while the 3 remaining species described a decrease in PAO along the periods, with the sperm whale being detected in half of the cells in the last period. When looking at the finer resolution, a general decrease in PAO can be seen for all species, except Cuvier’s beaked whale which had an increase from the 2nd to the 3rd period.

Table 2.3. Number of selected cells, sightings, cells with presence and PAO in the study area for each species and grid resolution regarding the 2-period comparison.

Grid	# cells	Species	Period	# sightings	# cells with presence	PAO (%)	ΔPAO (%)
50	10	Striped dolphin	2	1057	10	100.0	0
			3	396	10	100.0	
		Fin whale	2	690	10	100.0	0
			3	249	10	100.0	
		Sperm whale	2	70	9	90.0	-40
			3	23	5	50.0	
		Cuvier's beaked whale	2	79	7	70.0	0
			3	57	7	70.0	
10	49	Striped dolphin	2	726	45	91.8	0
			3	340	45	91.8	
		Fin whale	2	406	40	81.6	-4
			3	199	38	77.6	
		Sperm whale	2	49	24	49.0	-24.5
			3	22	12	24.5	
		Cuvier's beaked whale	2	52	19	38.8	-2.1
			3	51	18	36.7	
5	96	Striped dolphin	2	646	88	91.7	-14.6
			3	313	74	77.1	
		Fin whale	2	360	73	76.0	-5.2
			3	195	68	70.8	
		Sperm whale	2	44	30	31.3	-13.6
			3	22	17	17.7	
		Cuvier's beaked whale	2	50	27	28.1	+2.1
			3	50	29	30.2	

The shift index was calculated between all the periods (Tables 2.4 and 2.5), showing the differences in the number of shared cells with detection between the periods in question, being 0 for a complete shift and 1 when the periods share the same cells with detection. The results were coloured for a quicker understanding depending on the intensity of the shift following a gradient: red (0-0.40; considerable shift), orange (0.40-0.60; moderate shift), yellow (0.60-0.80; small shift) and green (0.80-1.0; no shift).

Concerning the 3-period comparison (Table 2.4) and regarding the 50x50 grid resolution, nearly no shift occurred between the periods for all the species. For the intermedium grid resolution, the striped dolphin and fin whale remained with almost no shift between periods, whereas the sperm whale had a considerable shift between the first two periods (0.69) and shared half of the cells between the 1st and 3rd periods, and the 2nd and 3rd periods. Cuvier's beaked whale showed bigger differences when including the 1st period in the equation, but close to no shift between the last two periods. These shift values were enlarged in the finer resolution, mainly for sperm whale and Cuvier's beaked whale, where the largest shifts overall were obtained when calculated between the 1st and 3rd periods (0.37 and 0.33, concerning the species).

Table 2.4. Shift index calculated between the periods, for each species, regarding the 3-period comparison.

		Shift index		
		Grid (km)		
Periods	**Species**	**50**	**10**	**5**
1 and 2	Striped dolphin	0.88	0.92	0.94
	Fin whale	1.00	0.86	0.80
	Sperm whale	1.00	0.69	0.57
	Cuvier's beaked whale	1.00	0.52	0.42
2 and 3	Striped dolphin	1.00	0.94	0.87
	Fin whale	1.00	0.82	0.83
	Sperm whale	1.00	0.55	0.48
	Cuvier's beaked whale	1.00	0.81	0.63
1 and 3	Striped dolphin	0.88	0.90	0.87
	Fin whale	1.00	0.82	0.84
	Sperm whale	1.00	0.50	0.37
	Cuvier's beaked whale	0.88	0.56	0.33

When looking at the 2-period comparison (Tables 2.5), for all grid resolutions, striped dolphin and fin whale described almost or no shift (>0.80). Nevertheless, with a decrease in cell size, slight differences can be observed. All species showed an increase in the shift between the periods with a decrease in cell size, being more evident for the sperm whale and Cuvier's beaked whale.

Table 2.5. Shift index calculated between the periods, for each species, regarding the 2-period comparison.

		Shift index		
		Grid (km)		
Periods	**Species**	**50**	**10**	**5**
2 and 3	Striped dolphin	1.00	0.93	0.86
	Fin whale	1.00	0.87	0.84
	Sperm whale	0.71	0.44	0.34
	Cuvier's beaked whale	0.86	0.76	0.57

ER was obtained for all comparisons, species and grid resolutions and illustrated as shown in the maps below (Fig. 2.14-2.19). The values were classified according to classes: 0, absent; 0-0.2, rare; 0.2-0.5, present; 0.5-1, regular.

Concerning the 3-period comparison, from the coarsest resolution (Fig. 2.14), it is possible to notice that 3 of the 4 species had a general increase in ER from the 1st to the 2nd periods, except for the sperm whale, followed by a decrease in the 3rd period for all species. As seen in the intermediate resolution (Fig. 2.15), the species with the widest distribution are striped dolphin and fin whale, occurring nearly in the entire study area, with the latter being more regular in open sea. For both species, the ER increased and afterwards decreased. The sperm whale showed preference for slope and seamount areas, with a decreasing ER along the periods. The Cuvier's beaked whaled described a considerable increase from the 1st to the 2nd periods, expanding its occurrence along the slopes as well as to more coastal zones. Regarding the finest resolution (Fig. 2.16), the same pattern from the previous can be observed for the striped dolphin and fin whale, and the preferences of the sperm whale and Cuvier's beaked whale for areas of slope and seamounts can be denoted at a greater extent.

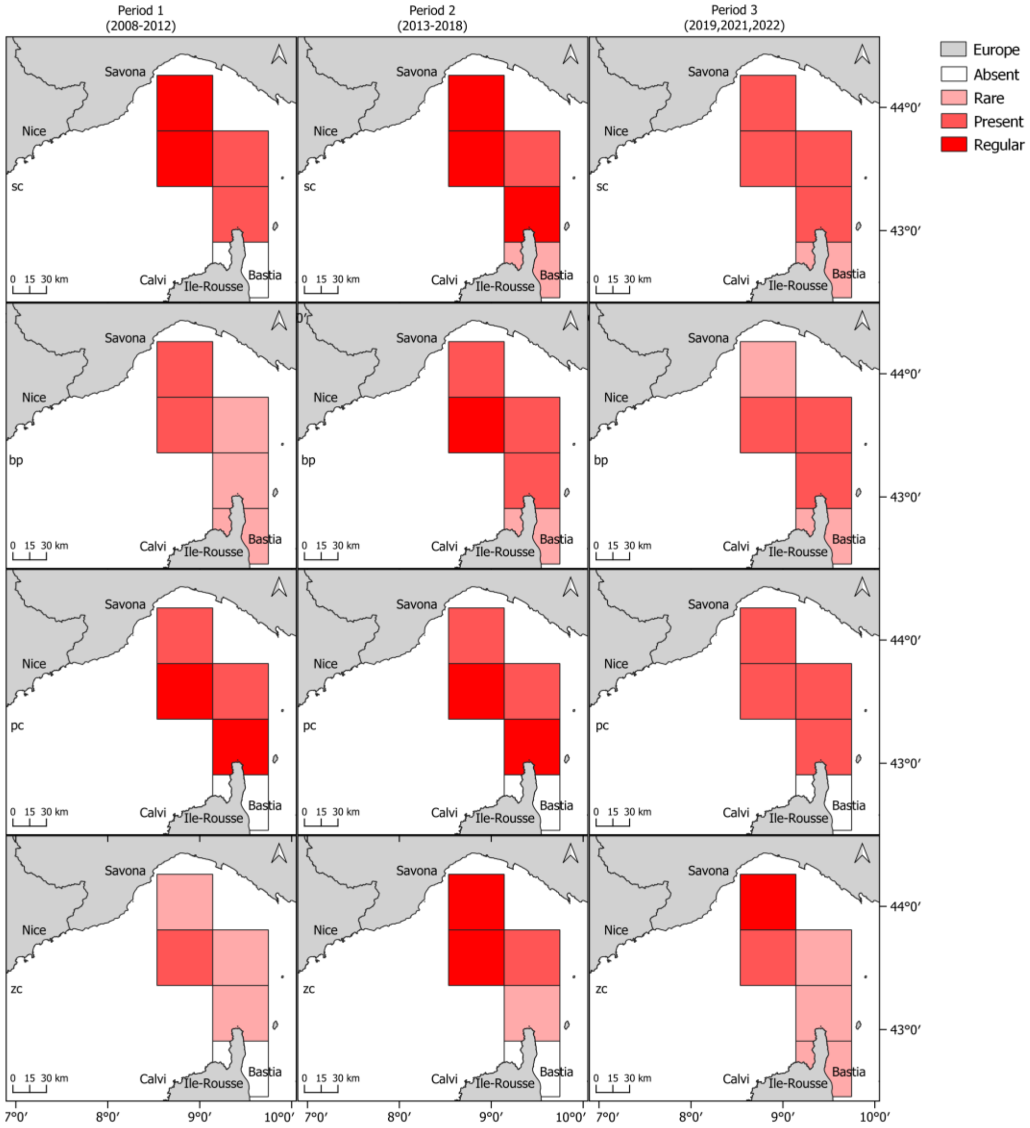

Fig. 2.14. Encounter rate of striped dolphin (sc), fin whale (bp), sperm whale (pc) and Cuvier's beaked whale (zc) in the study area regarding the comparison between the three periods (50x50 km grid resolution).

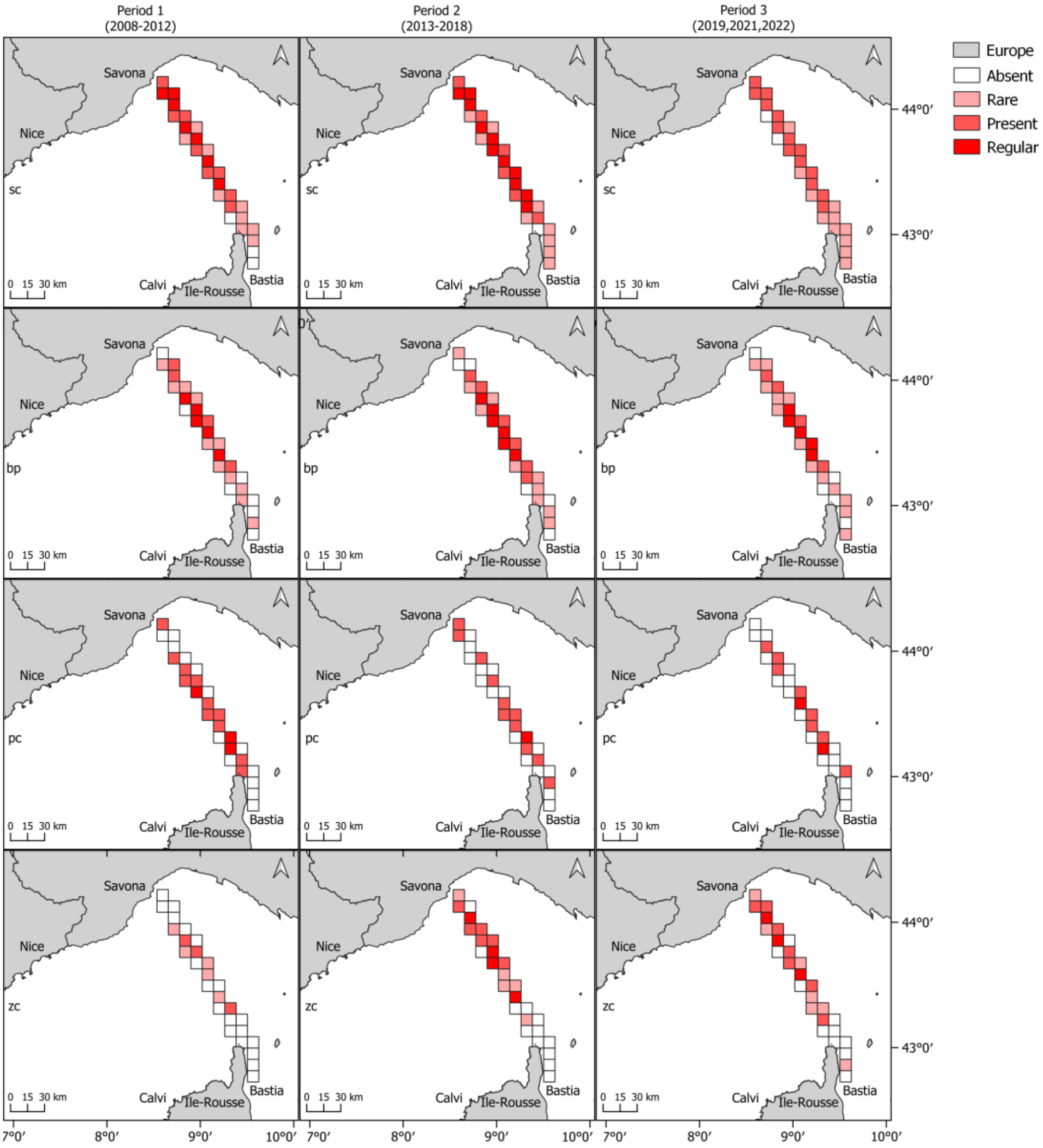

Fig. 2.15. Encounter rate of striped dolphin (sc), fin whale (bp), sperm whale (pc) and Cuvier's beaked whale (zc) in the study area regarding the comparison between the three periods (10x10 km grid resolution).

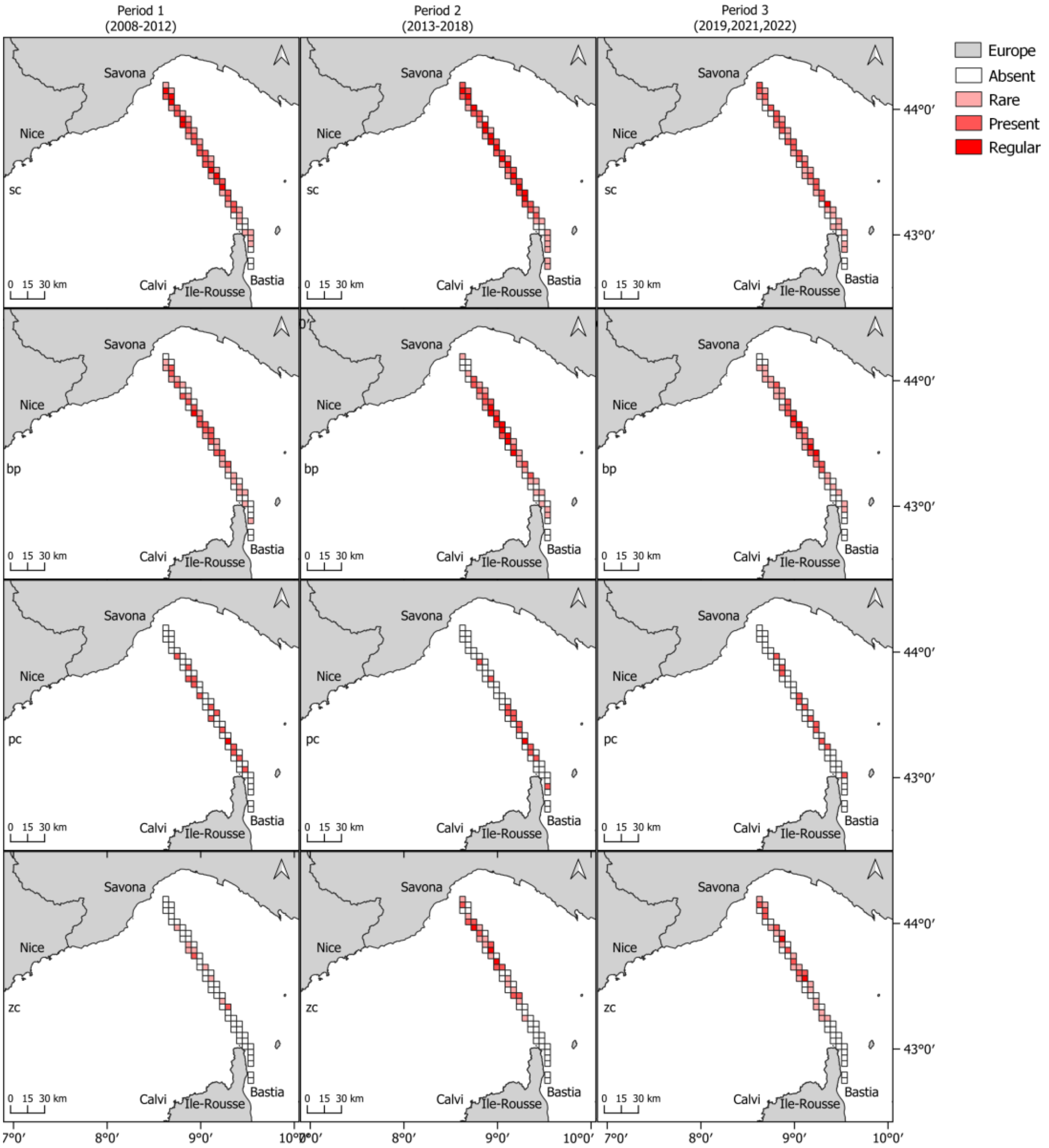

Fig. 2.16. Encounter rate of striped dolphin (sc), fin whale (bp), sperm whale (pc) and Cuvier's beaked whale (zc) in the study area regarding the comparison between the three periods (5x5 km grid resolution).

Regarding the 2-period comparison, a general decrease in ER can be denoted for all species and grid resolutions (Fig. 2.17-2.19), being stronger for the striped dolphin, fin whale and sperm whale and preeminent in the western part of the study area. Furthermore, a complete non-detection of sperm whale occurred in offshore areas following the route Nice-Ile Rousse.

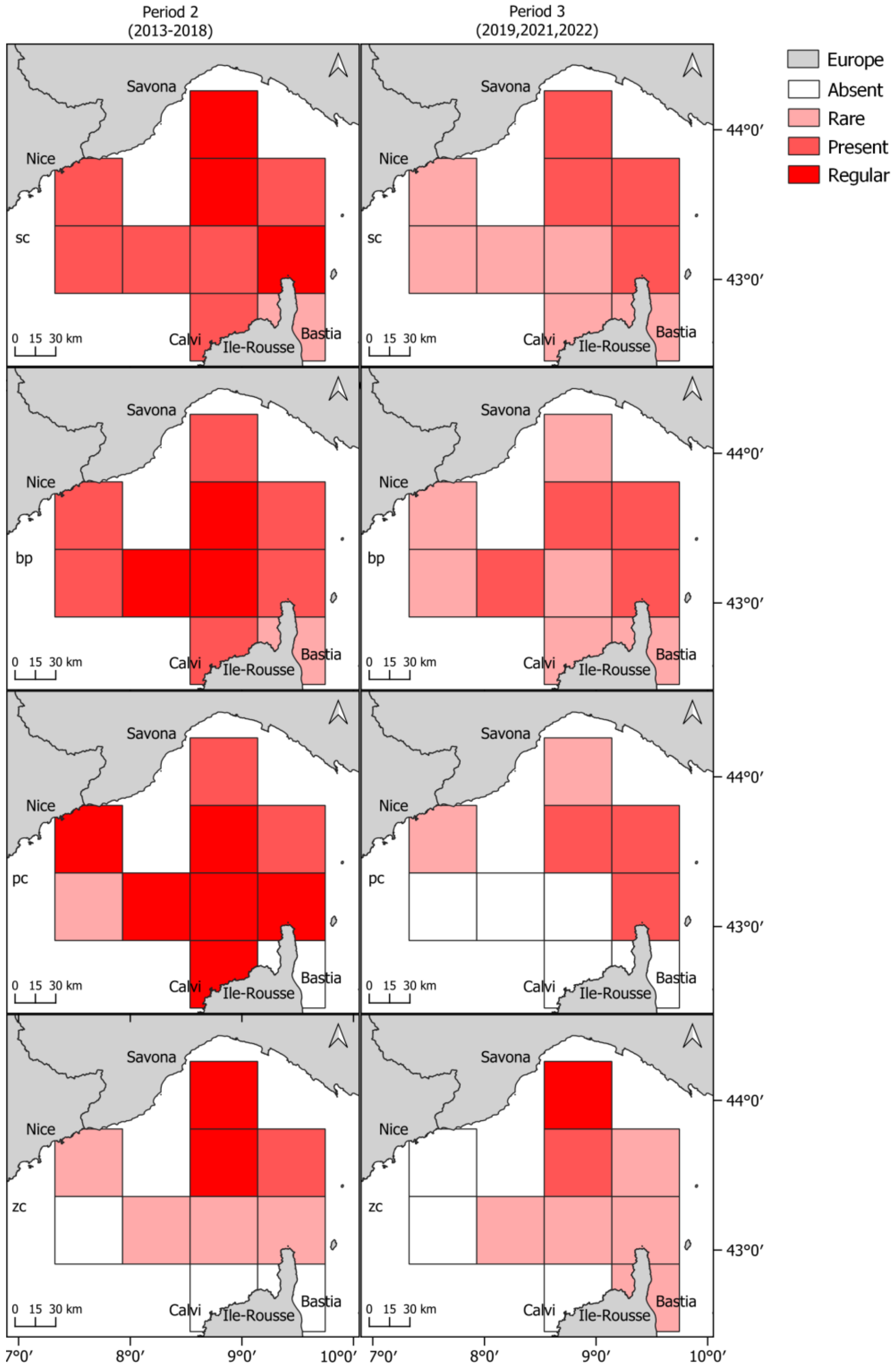

Fig. 2.17. Encounter rate of striped dolphin (sc), fin whale (bp), sperm whale (pc) and Cuvier's beaked whale (zc) in the study area regarding the comparison between 2nd and 3rd periods (50x50 km grid resolution).

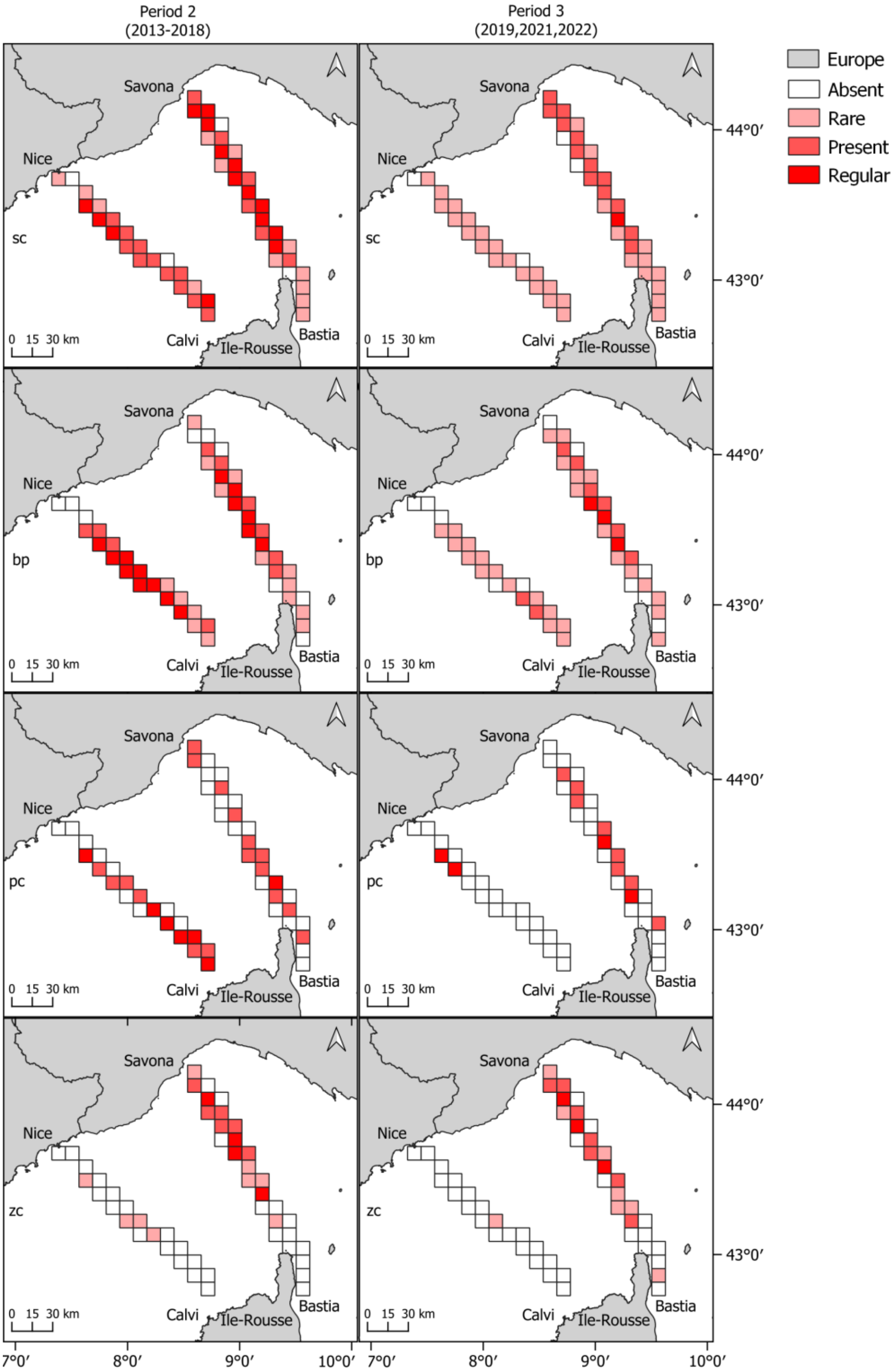

Fig. 2.18. Encounter rate of striped dolphin (sc), fin whale (bp), sperm whale (pc) and Cuvier’s beaked whale (zc) in the study area regarding the comparison between 2nd and 3rd periods (10x10 km grid resolution).

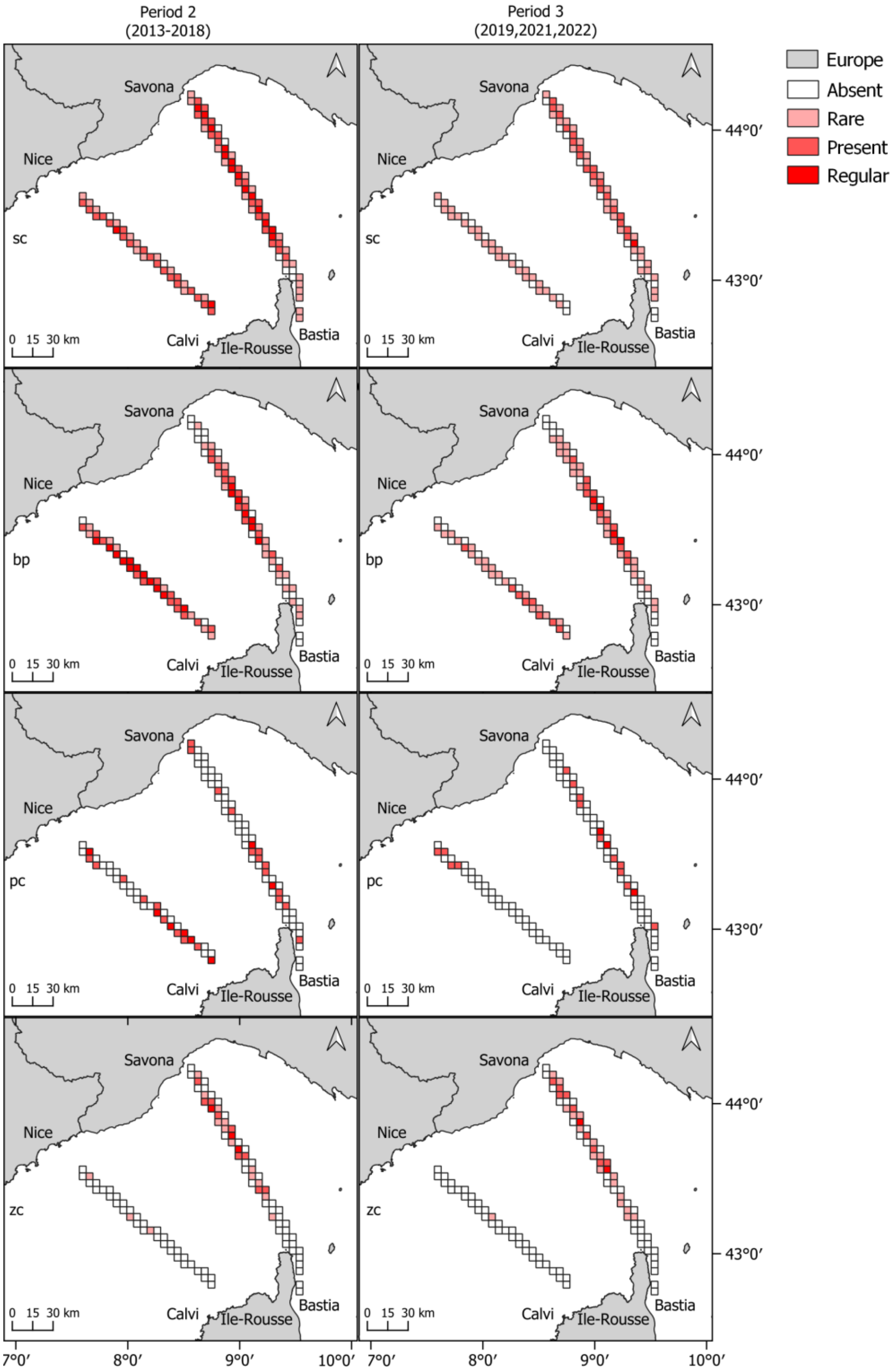

Fig. 2.19. Encounter rate of striped dolphin (sc), fin whale (bp), sperm whale (pc) and Cuvier's beaked whale (zc) in the study area regarding the comparison between 2nd and 3rd periods (5x5 km grid resolution).

The percentage of "present" and "regular" cells were obtained for each comparison and are summarized in Tables 2.6 and 2.7. Given that "rare" cells describe the low ER of the species, this category was assumed to not be useful to understand distribution shifts and was therefore not taken into account.

Table 2.6. Percentage (%) of “present” and “regular” cells regarding each grid resolution (km), species and period for the 3-period comparison. The tendency is described as increase (↗), stable (=) or decrease (↘).

		3-period comparison							
		“Present” cells (%)				“Regular” cells (%)			
Grid	Species	1st	2nd	3rd	Tendency	1st	2nd	3rd	Tendency
50	Striped dolphin	40	20	80	↘↗	40	60	0	↗↘
	Fin whale	40	60	60	↗ =	0	20	0	↗↘
	Sperm whale	40	40	80	= ↗	40	40	0	= ↘
	Cuvier's beaked whale	20	20	20	=	0	40	20	↗↘
10	Striped dolphin	22	22	52	= ↗	26	41	0	↗↘
	Fin whale	15	22	15	↗↘	19	22	19	↗↘
	Sperm whale	41	41	30	= ↘	11	4	7	↘↗
	Cuvier's beaked whale	11	22	26	↗	0	15	11	↗↘
5	Striped dolphin	35	35	35	=	15	26	2	↗↘
	Fin whale	30	22	24	↘↗	2	15	7	↗↘
	Sperm whale	26	22	22	↘ =	2	2	0	= ↘
	Cuvier's beaked whale	4	17	15	↗↘	0	6	4	↗↘

Table 2.7. Percentage (%) of “present” and “regular” cells regarding each grid resolution (km), species and period for the 2-period comparison. The tendency is described as increase (↗), stable (=) or decrease (↘).

		2-period comparison					
		“Present” cells (%)			“Regular” cells (%)		
Grid	Species	2nd	3rd	Tendency	2nd	3rd	Tendency
50	Striped dolphin	60	40	↘	30	0	↘
	Fin whale	60	40	↘	30	0	↘
	Sperm whale	20	30	↗	60	0	↘
	Cuvier's beaked whale	10	10	=	20	10	↘
10	Striped dolphin	35	27	↘	31	2	↘
	Fin whale	20	20	=	31	6	↘
	Sperm whale	35	16	↘	14	8	↘
	Cuvier's beaked whale	12	14	↗	8	6	↘
5	Striped dolphin	41	20	↘	17	1	↘
	Fin whale	27	22	↘	20	4	↘
	Sperm whale	23	15	↘	8	3	↘
	Cuvier's beaked whale	9	9	=	3	2	↘

2.3.2. Modelled occupancy

Parameters estimates of use (ψ) and of detection (p) taking into account imperfection in detection were obtained from the occupancy model applied and the results tested for goodness-of-fit. The cases where the model showed no evidence for lack of fit (p-value > 0.05) are presented in Tables 2.18-2.10. The model fitted with higher success when the data set given was composed of 14 visits (Table 2.8) when compared to 6 (Table 2.9) and 5 (Table 2.10) visits. Regarding grid resolution, both the maximum and intermediate resolution grids performed relatively worse than the finest resolution. Focusing on the latter, the model showed that the striped dolphin was the species with the highest use of the area (>80 %), followed by the fin whale and sperm whale with intermediate values (≈60 %), and lastly by the Cuvier's beaked whale with a lower use (≈40 %). Concerning detection, the model describes the striped dolphin as the species with the highest detection probability, being detected almost 50 % of the time when present. The fin whale was detected ≈30 % of the time, whereas the sperm whale and Cuvier's beaked whale share a very low probability of detection (≈10 %). In all cases, the highest standard errors obtained were for the sperm whale for both parameters estimates.

Table 2.8. Parameters estimates (probability of area used ψ and probability of detection p) and respective standard errors (SE) retrieved from the occupancy model performed with 14 repetitions (entire data set). P-value and estimated overdispersion parameter ($\hat{c}$) obtained from the goodness-of-fit test statistic based upon 5 000 bootstrap simulations.

Grid	**Species**	**ψ**	**SE (ψ)**	**p**	**SE (p)**	**p-value**	**$\hat{c}$**
50	Striped dolphin	-	-	-	-	-	-
	Fin whale	-	-	-	-	-	-
	Sperm whale	0.89	0.10	0.51	0.04	0.45	1.01
	Cuvier's beaked whale	-	-	-	-	-	-
10	Striped dolphin	-	-	-	-	-	-
	Fin whale	0.79	0.04	0.45	0.02	0.51	1.00
	Sperm whale	-	-	-	-	-	-
	Cuvier's beaked whale	-	-	-	-	-	-

5	Striped dolphin	0.87	0.02	0.47	0.01	0.42	1.02
	Fin whale	0.65	0.03	0.33	0.01	0.58	0.86
	Sperm whale	0.64	0.07	0.10	0.01	0.10	0.41
	Cuvier's beaked whale	0.39	0.05	0.11	0.01	0.10	1.06

Table 2.9. Parameters estimates (probability of area used ψ and probability of detection p) and respective standard errors (SE) retrieved from the occupancy model performed with 6 repetitions (2nd period). P-value and estimated overdispersion parameter ($\hat{c}$) obtained from the goodness-of-fit test statistic based upon 5 000 bootstrap simulations.

Grid	**Species**	**ψ**	**SE (ψ)**	**p**	**SE (p)**	**p-value**	**$\hat{c}$**
50	Striped dolphin	-	-	-	-	-	-
	Fin whale	-	-	-	-	-	-
	Sperm whale	0.76	0.13	0.54	0.07	0.73	0.92
	Cuvier's beaked whale	-	-	-	-	-	-
10	Striped dolphin	-	-	-	-	-	-
	Fin whale	0.72	0.05	0.49	0.03	0.61	0.97
	Sperm whale	-	-	-	-	-	-
	Cuvier's beaked whale	-	-	-	-	-	-
5	Striped dolphin	-	-	-	-	-	-
	Fin whale	0.71	0.04	0.38	0.02	0.17	1.15
	Sperm whale	0.52	0.13	0.12	0.03	0.72	0.51
	Cuvier's beaked whale	0.42	0.08	0.15	0.03	0.69	0.55

Table 2.10. Parameters estimates (probability of area used ψ and probability of detection p) and respective standard errors (SE) retrieved from the occupancy model performed with 5 repetitions (1st period). P-value and estimated overdispersion parameter ($\hat{c}$) obtained from the goodness-of-fit test statistic based upon 5 000 bootstrap simulations.

Grid	**Species**	**ψ**	**SE (ψ)**	**p**	**SE (p)**	**p-value**	**$\hat{c}$**
50	Striped dolphin	-	-	-	-	-	-
	Fin whale	-	-	-	-	-	-
	Sperm whale	0.84	0.15	0.55	0.08	0.34	1.03
	Cuvier's beaked whale	-	-	-	-	-	-
10	Striped dolphin	-	-	-	-	-	-
	Fin whale	-	-	-	-	-	-
	Sperm whale	-	-	-	-	-	-
	Cuvier's beaked whale	-	-	-	-	-	-
5	Striped dolphin	-	-	-	-	-	-
	Fin whale	-	-	-	-	-	-
	Sperm whale	0.63	0.18	0.10	0.03	0.20	1.19
	Cuvier's beaked whale	-	-	-	-	-	-

2.4. Discussion

The Ligurian Sea has been described by several authors to be a hotspot of cetaceans in the Mediterranean Sea [16], [18], [21], [25], [70], given the complex physiography that occurs in the area. It is, therefore, of special importance to study the presence of these species, in order to understand their past, current and future distribution dynamics and detect possible changes. Given the lack of knowledge in this field of study and the requirement of grid-based monitoring by the EU Directives, the present work adds a perspective regarding suitable scale resolution and methods of analysis to be employed in cetacean distribution studies.

The detection/non-detection maps, as well as the ER analysis, showed overall use of the study area by the striped dolphin and, with a slight preference for the pelagic domain, fin whale, whereas a higher level of preference could be distinguished for the Cuvier's beaked whale and sperm whale in slope, submarine canyon and seamount areas, although, the latter was further encountered in open sea. Such observations are in accordance with [70], which, also using ferries as platforms of opportunity in the study area in 2008 and 2009, described the same distribution pattern, except for the non-detection of fin whale in the route Savona-Bastia, contrary to the present study. Furthermore, research performed in minor parts of the study area with the use of sailing vessels confirms the preferences of the species for the different types of physiographic characteristics [25], [71], [72]. Lastly, a recent extensive study [73] joining the data collected from 2004 to 2018 by 32 research groups summarizes and supports the distribution patterns observed in the 1st and 2nd periods in the study area described in the present study.

However, when looking into detail, the data collected in the last period (from 2019 to 2022) showed a complete non-detection of sperm whale in the open sea area closer to

Ile-Rousse, which, given that the monitoring methods were kept unchanged over the years, suggests a recent acute decrease in presence of the species in this area. No other studies noting such changes in the distribution of the species throughout the referred area have been published in the meantime, to the best of our knowledge. On another note, an increase in number of cells with detection of Cuvier's beaked whale occurred from the 1st period to the following, indicating an expansion of the movement of the species. [72], with data analysis performed over 10 years, from 2004 to 2014, suggested that changes in occurrence in the study area were possibly related to a decreasing trend in primary productivity and, consequently, in fisheries, with striped dolphin, sperm whale and Cuvier's beaked whale increasing its presence, on the contrary of fin whale, which had its occurrence decreased. Except for Cuvier's beaked whale increase in occurrence, disparate changes in distribution were found between the studies, mainly for the sperm whale and fin whale. However, given that only six years are in common between the studies (2008 to 2014), possible comparisons need to be done cautiously. Regarding the movement expansion of Cuvier's beaked whale, similar results were found by [74], where an expansion of the Observed Distributional Range (ODS) of the species was denoted between the third and fourth HD cycle reports (here, 1st and 2nd periods).

From the calculation of the PAO, the same image can be retrieved, where the striped dolphin and fin whale presented high percentages of cells with detection in the study area (≥70%). The sperm whale showed, in finer resolutions, a decrease of "detection" cells along the periods, with a difference in PAO in the 2-period comparison of 24.5 and 22.9% for the 10 and 5 km grid resolutions, respectively, resulting from the aforementioned decrease in presence. On the contrary, Cuvier's beaked whale described an increase, being the difference of ≈33.3% between the 1st and 3rd periods in the 3-period comparison, supporting the idea of movement expansion.

Looking from a spatial point of view, the shift index showed that the striped dolphin, as well as the fin whale, shares, along the periods, a high percentage of cells (≥80%) in all grid resolutions, meaning that the distribution did not suffer substantial changes. For the remaining species, given the relatively smaller distribution compared to the formers, the spatial occupancy of the cells varied more easily, with sperm whale having the largest differences between the 2nd and 3rd periods (only sharing 34% of occupied cells on the finer grid) and the Cuvier's beaked whale between the 1st and following periods, where only 42% and 33% of the cells were commonly occupied also in the 2nd and 3rd periods, respectively. Nevertheless, the previously mentioned expansion of movement of the species is maintained in areas of slope and submarine canyons, not increasing its presence in open sea areas.

The ER enables not only the understanding of distribution from a qualitative point of view (if the species is there or not), but also from a quantitative one (how often the species is detected), which allows the denotation of cells with higher and lower importance for the species and, between periods, changes in the number of detections. In general, the ER maps showed an increase in detection from the 1st period to the 2nd, followed by a decrease in the 3rd period for the 4 species, which could describe an overall decrease in presence. It can also be denoted, from the 2-period comparison, that the referred decrease was visually preeminent in the western part of the study area, where the striped dolphin and fin whale went from being regularly detected to being rare. A spatial analysis on ER is necessary to draw further conclusions. By obtaining the percentage of “present” and “regular” cells, an even more detailed analysis of the intensity of distribution changes can be performed. The category of “regular” cells showed clearly the tendencies described by the species, being its use recommended to the detriment of the “present” cells category.

Based on the estimates retrieved from the occupancy model applied to 14 years (visits) with the finest resolution, the striped dolphin was the species with the highest values of the proportion of area used, being present in 87% of the cells, and of detection probability (47%), expected results given the commonness and conspicuity of the species. Fin whale and sperm whale were described to have a similar proportion of use of the area (65 and 64%, respectively), although the detection probability of the first was higher at 23% (33 and 10%, respectively). Cuvier's beaked whale was predicted to use 39% of the cells, having similar probabilities of detection (11%) to the sperm whale, attributed to the elusive behaviour of both species. When comparing to the PAO obtained in the observed occupancy section (see section 4.1. Observed occupancy analysis), considering the differences in number of cells employed for each method, as well as the number of visits (periods *versus* years), direct comparisons are not recommended, but general ideas can be retrieved and seen as a rough overview. From the observed PAO, higher values were obtained for the striped dolphin and fin whale when compared to the modelled estimates, although, due to the fact that the model takes into account imperfections in detection, one would expect opposite results. However, as aforementioned, the model was performed for a substantially higher number of cells and possibly included more cells where the species was not detected. Nevertheless, both results suggest the striped dolphin as the most common species in the entire study area. The observed PAO showed bigger differences between the fin whale and sperm whale, not describing the similarity denoted by the model. Regarding the most inconspicuous species, the model described higher values of area used than the observed PAO, which may be attributed to a higher number of cells where the species was detected given to the model or/and the pertinence of taking into account imperfections in detectability in order to not underestimate the area used by the species.

On another note, model limitations regarding grid resolution and number of visits can be noted: the capability of the model to describe the variability of the given data decreased with a decrease both in grid resolution and in the number of visits performed in each cell. The chosen model fitted with distinct success for the finer resolution (5x5 km), whereas coarse resolutions were mostly found poor fit. Such results may be attributed to the effect of having a large number of visits (>10) or a considerable amount of missing values in the detection histories [75], although such cases did not seem to affect when the finer resolution was the one in question. [69] and [76] also described a poor fit being caused by a substantial number of cells where the counts were either always present or always absent for the visits performed in the same cell, which we find to be the reason in the case of the present study, showing that coarse resolutions do not show much variation. Regarding the number of visits, [69] recommends a minimum number of 5 visits per cell, with the power of the goodness-of-fit test being low for small datasets. As shown in the present work, when decreasing the number of visits given to the model from 14 to 6 and even 5 visits the model performed relatively worse, which may have affected as well the precision of the occupancy estimate and accuracy when the probability of detection is low [44]. Moreover, it is interesting to notice the capability of the model to perform well even for species whose detection is lower given its elusive and cryptic behaviour, such as the sperm whale and Cuvier’s beaked whale.

As outlined throughout the present study, the changes in spatial distribution could be observed when applying the 10x10 km grid resolution and even to a greater extent with the finer resolution of 5x5 km, whilst the 50x50 km was not as successful (except for the ER maps), unless extreme events occur, such as the non-detection of sperm whale in any cells of open sea closer to Ile-Rousse. Despite the fact that the EU Directives commonly request coarse resolutions, the complexity of the dynamic systems encountered in areas such as the Ligurian Sea requires the use of finer-scale resolutions when performing studies where the local environmental heterogeneity needs to be considered, in order to appropriately describe the variation composing the system, as the case in distribution studies [77], [78]. In accordance, research conducted in the study area regarding grid-based distribution of cetacean species typically uses between 1x1 and 10x10 km cell size [20], [25], [32], [42], [71], [79]–[82]. With this in mind, the present study suggests a downgrading of the recommended resolution by the EU Directives, from 50x50 to 10x10 km in the case of the MSFD and even 5x5 km for both Directives, when requesting the report of the spatial distribution of cetaceans, in order to appropriately describe the system in study.

The present study showed that, although no substantial changes in the cells used occurred for 2 of the 4 studied species, a general decrease in the number of detections within those cells can be denoted for all species, which may be interpreted as a decrease in their presence. The striped dolphin and fin whale showed non-considerable changes in the cells used, whereas the sperm whale had a decrease in the areas close to Ile-Rousse. On the other hand, the Cuvier’s beaked whale described an increased use of the area in the route Savona-Bastia while maintaining its habitat preferences. The PAO and shift indexes were, therefore, proven to be useful measures of distribution, allowing the identification of changes between times series, being a viable and simple method to answer the EU Directives requests. However, more precise conclusions and the entirety of the distribution image can be retrieved when the results of all methods (detection/non-detection and ER maps) are joined together. The shift, for example, shows the changes in the use of cells but does not describe if the change was towards the increase or decrease of used cells. In the present study, such an index described a big shift for Cuvier’s beaked whale, but without other methods, it would not be possible to denote the expansion of movement of the species. It is furthermore recommended to take advantage of occupancy models, which have shown to be suitable to study cetaceans taking into account imperfections in detection, while keeping the process simple but effective to be employed by different groups (i.e., researchers and politicians), and where a higher number of cells can be used for the analysis. Lastly, the study supports the downgrading for finer grid resolutions when reporting to the Directives. As a next step, it would be interesting to perform a spatial analysis on the ER to understand where the main changes in the number of detections occurred (western vs. central part). Additionally, it would also be advantageous to test the appliance of the presented methods to study the distribution of other species that occur in the Mediterranean Sea, such as the bottlenose dolphin, Risso's dolphin, long-finned pilot whale and common dolphin.

As a last remark, future research projects should focus on the use of grid resolutions of 10x10 km for the striped dolphin and fin whale and 5x5 km for the sperm whale and Cuvier's beaked whale. To study distribution trends, the comparison between periods should be only performed for cells that were visited at least once every year that compose the periods being compared. Finally, when performing occupancy models, the detection histories given to the model should be composed of a minimum number of years equivalent to two reporting periods.

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