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Movements of Hatchery-Reared Dusky Groupers Released in a Northeast Atlantic Coastal Marine Protected Area

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Abstract: No-take areas are key instruments to promote the effectiveness of Marine Protected Areas (MPAs), particularly concerning the protection of endangered species such as the dusky grouper (*Epinephelus marginatus*). However, despite the establishment of no-take areas and the prohibition of catching this species in a southwestern Portuguese MPA (SACVMP—'Sudoeste Alentejano' and 'Costa Vicentina' Marine Park) since 2011, there is still no evidence of population recovery. By using acoustic biotelemetry, this work aimed to evaluate the feasibility of restocking hatchery-reared adult dusky groupers in two no-take areas within the SACVMP. In 2019 and 2021, thirty groupers were tagged with acoustic transmitters and the site attachment and movements of the groupers were assessed in the releasing sites (no-take areas). None of the tagged fish settled down in either of the areas, leaving the no-take areas mainly at dusk and night. Some individuals displayed extended movements of more than a hundred kilometers along the Portuguese coast which was rarely reported for this species. At least in some coastal stretches, those movements were performed close to the shore, which may evidence the importance of coastal MPAs to protect and promote the connectivity of species more associated with rocky reef habitats. Following studies should focus on the conditions that promote site attachment and fidelity by hatchery-reared dusky groupers so that future large-scale restocking programs can be successful in MPAs with appropriate habitats.

Keywords: *Epinephelus marginatus*; acoustic biotelemetry; tracking; ranging behavior; MPA; restocking trials

1. Introduction

Marine protected areas (MPAs) have been widely implemented as valuable tools to protect endangered habitats and species and promote the recovery of overexploited fishery resources. MPAs commonly comprise areas with different levels of protection and no-take areas are the most restrictive ones, as no fishing or other extractive activities are allowed [1–3]. These areas are particularly crucial to the MPAs' effectiveness, and their size is quite relevant as the 'reserve effect' depends on the relation between the size of the protected area and the ecology of the species protected [4–7]. No-take areas must encompass most of the area of those species' home ranges, at least during the most vulnerable periods of their life cycle [4,5]. Yet, if aiming to contribute to surrounding fisheries through the spillover effect of commercial species, these areas should not be too large, otherwise, the net emigration of individuals can be hindered, especially when home ranges are smaller [4,6,7]. Small no-take areas are known to be more effective at protecting sedentary and territorial species [4]. This is the case for the dusky grouper, *Epinephelus marginatus* (Lowe, 1834), one of the most targeted and vulnerable serranid species throughout its distribution range [8–11]. Due to its stately figure and behavior, dusky groupers are one of the most wanted fish in spearfishing and scuba diving, making it a highly economically valuable species [12,13].

Dusky groupers are slow-growing fish, with a complex life cycle, involving protogynous hermaphroditism [14,15], and complex distribution patterns, social structures and sexual behaviors, reproductive small-scale migrations, and spawning aggregations [16–18], all contributing to their becoming highly vulnerable to human pressures, especially to fishing. Globally classified with the threat status of "Vulnerable" [8], the dusky grouper has already been classified as an "Endangered" species in Europe (the Mediterranean Sea and northeastern Atlantic) [9–11]. Thus, it has been strongly recommended to reduce the fishing effort directed at this species, protect it through no-take area designations, and increase the scientific knowledge of the species, including its life history and ecology [8,13,19,20].

Considering these recommendations, efforts have been made in the last decades to preserve the species through the implementation of MPAs, most intensively in the northwest Mediterranean. From Cabo de Palos in Murcia (Spain) to Port-Cros in Marseille (France), including the Balearic (Spain), Corsica (France), Sardinia, and Ustica (Italy) islands, there is much evidence of the positive reserve effect in terms of increasing the abundance, size, and biomass of dusky grouper [21–26]. In the northeast Atlantic, particularly on the southwestern Portuguese mainland coast, no such studies exist. Despite the establishment of thirteen small highly protected areas (nine small islets fully protected and four partially protected areas where most fishing activities are prohibited, including the capture of fish), and the prohibition of catching this species since 2011 in all the 'Sudoeste Alentejano' and 'Costa Vicentina' Marine Park (SACVMP), there was an apparent decreasing trend in catches since 2007 in the region [27] and fishers stated that catches are increasingly rare and limited to very few groupers per year in the area [27,28].

The persistent low number of dusky groupers in the region and the strong site fidelity when conditions are ideal (e.g., complex rocky habitat, between 15 to 40 m of depth) [29–34] can make restocking programs with hatchery-reared individuals a solution to local species recovery, though few studies have addressed this possibility [35,36]. The present work aimed to evaluate the feasibility of hatchery-reared young adult dusky grouper restocking in two small coastal no-take areas within SACVMP, specifically analyzing site attachment, activity patterns, and movements in the study areas using acoustic biotelemetry. Additional data also provided the opportunity to analyze the long-distance-ranging movements of the tagged fish beyond the study areas.

2. Materials and Methods

2.1. Study Area

The study was conducted in SACVMP, a coastal MPA that includes a marine stretch 2 km wide along 130 km of the southwestern coast of mainland Portugal (Figure 1), the most extensive national coastal MPA.

This MPA was designated in 1995 as part of a natural park ('Sudoeste Alentejano' and 'Costa Vicentina' Natural Park), with poor marine regulation and lack of enforcement. In 2011, the marine area became a marine park, redesigned according to a zoning plan: nine small islets and their vicinities were fully protected (as 'no-go' zones), and four partially protected areas were established. Almost all extractive activities were prohibited in these partially protected areas, including recreational and professional fishing (except for commercial harvesting of stalked barnacle *Pollicipes pollicipes* (Gmelin, 1790) on the mainland coast). Thus, in this study, and for simplicity, these areas will be referred to as 'no-take' areas. Additionally, in the remaining areas of the SACVMP—'buffer' areas—commercial fishers need a valid license to operate, with restrictions to the operation of specific fishing gears regulated by the national fishing law. The catch and the detention of dusky groupers have also been prohibited inside this marine park since 2011.

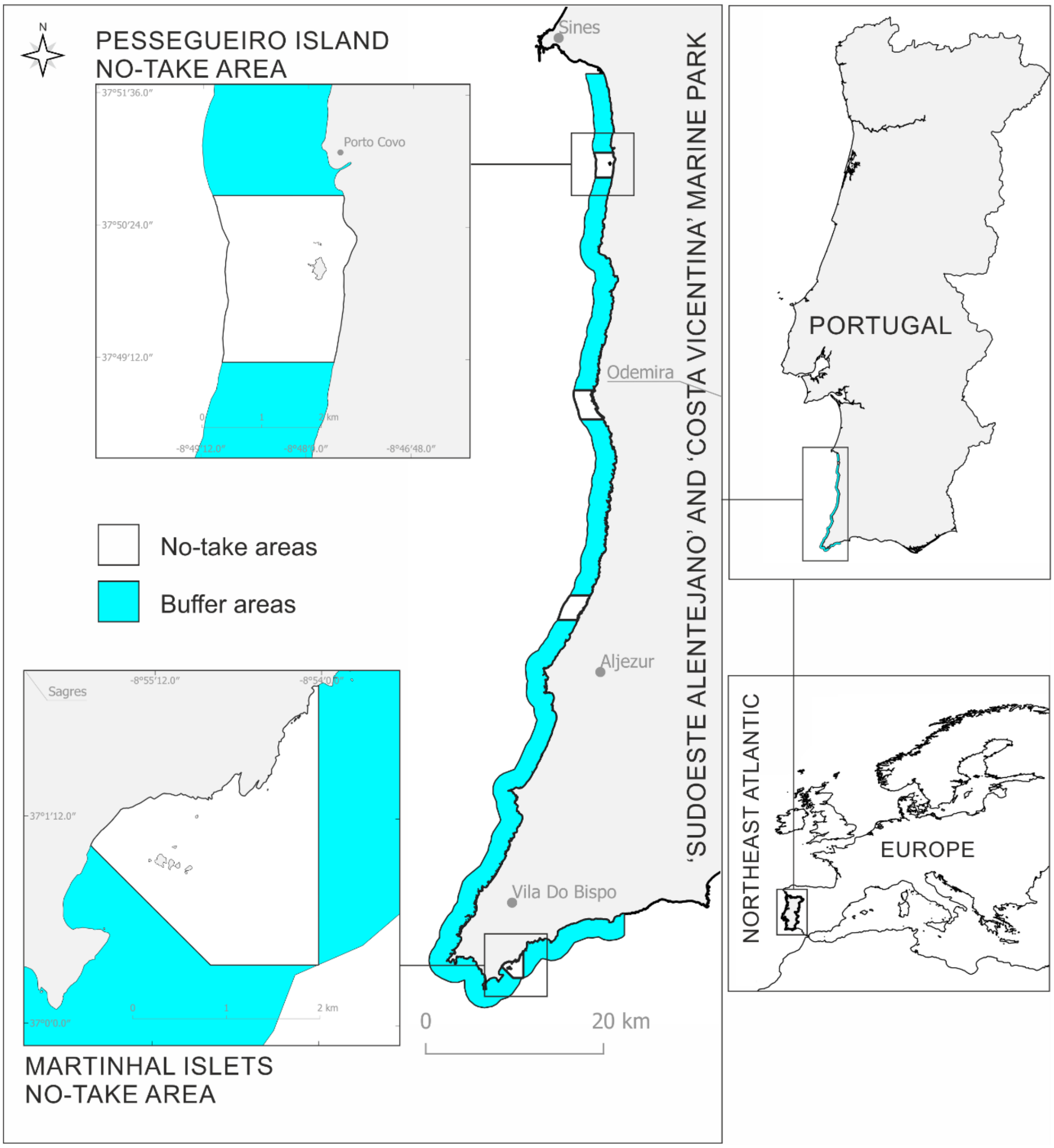


Figure 1. Location of the 'Sudoeste Alentejano' and 'Costa Vicentina' Marine Park and the two no-take areas—Pessegueiro island and Martinhal islets no-take areas.

Last reviews and surveys on the SACVMP biodiversity listed the occurrence of more than 230 fish species inside this MPA, including the dusky grouper [37], one of the most priced commercial species in the markets of the region [27]. As a tropical and sub-tropical species, as well as a thermophilic one [10,38], the distribution range of dusky grouper in the northeast Atlantic is limited. Thus, along with the Azores, the southwestern Portuguese mainland coast, specifically, the SACVMP, is the northernmost area with relevance to this population [37,39,40].

In the present study, two trials were conducted: the first trial was conducted in the Pessegueiro island no-take area in May 2019 and the second trial was conducted in the Martinhal islets no-take area in April 2021. These small no-take areas (4–6 km^2) extend from the coastline up to 2 km offshore and are composed of complex rocky reefs associated with the islands, between 5–25 m deep. The subtidal bottom of Pessegueiro island no-take area is composed of rocky (35% of its total area) and sandy substrate (65%), the reefs near the island form several underwater caves, and the area is highly hydrodynamic, due to the direct exposure to northwest swell, with severe winter ocean conditions [41]. In the subtidal bottom of the Martinhal islets no-take area, the rocky substrate (20%) is restricted to the islets and the shoreline with underwater caves, and the area is protected from the dominant northwest swell due to its location (southern coast of Algarve) [41]. Thus, these areas provide shelter to cryptic and territorial median/big size fish species, such as conger eels *Conger conger* (Linnaeus, 1758) and morays *Muraena helena* Linnaeus, 1758, and are highly biodiverse and abundant in possible dusky grouper preys [41–45].

2.2. Tagging and Tracking

Dusky groupers were reared in the Aquaculture Research Station of Olhão (EPPO), a governmental infrastructure run by the Portuguese Institute for Sea and Atmosphere (IPMA) and used only for scientific purposes. Wild breeders with geographical origin on the Portuguese shore were adapted and reproduced in EPPO breeders' tanks using the same methodology already described [46]. The offspring used for this work were born in EPPO facilities in 2012 and 2013. Larval rearing was performed with a mesocosm approach [47] and growing juvenile and young adult phases were maintained in low-density cultivation in 750 m^3 ponds among some other cultivated Sparidae fish. Therefore, although inert feeds were provided, these groupers were also familiarized with feeding on benthonic fauna that naturally colonizes the bottom and walls of the ponds. To prepare the group for transportation and release, and to facilitate assessing their condition, fish were transferred to outdoor fiberglass 18 m^3 tanks with a 2 m depth in an open circuit with water from the Ria Formosa Lagoon. During this adaptation period, fish were fed a rich diet that included frozen squids, mackerels, and sardines supplemented with a top-quality semi-moist feed for brooders, to ensure that the fish were at their best fit conditions, being progressively transferred to an integral live prey that included fresh mackerels and also some cultivated seabass or seabream of a suitable size. The fish release was only considered acceptable when all the fish in the group presented predation behavior and were, therefore, able to feed in the wild.

A total of thirty young adult groupers were tagged and released, 20 in 2019 and 10 in 2021. The tagging procedures were carried out in EPPO facilities. After prior light anesthesia in the holding fiberglass tank (18 m^3), groupers were individually placed in a 250 L well-oxygenated water tank and anesthetized with a 2-phenoxyethanol solution (0.6 $mL.dm^{-3}$). After anesthesia induction, the tagging procedure took 5 to 8 min. Each fish was measured (total length, L_T, and total weight, W_T), surgically implanted with an ultrasonic transmitter in the peritoneal cavity, and externally tagged at the base of the dorsal fin with a colored plastic tipped dart tag printed with a serial number (Hallprint, Australia). The transmitter was surgically implanted through a 1.5 to 2 cm incision at the ventral midline, which was sutured with synthetic, braided non-absorbable polyamide monofilament (B Braun Dafilon, USP 2/0 DS 24). In 2019, 10 groupers were tagged with an acoustic transmitter model V16-6H (Vemco Ltd., Halifax, Nova Scotia), while the other 10 individuals were tagged with a V13-1L model. These transmitters emit a 69 kHz acoustic coded signal every 40–80 s and have an expected battery life of 2538 days and 817 days, respectively. In 2021, groupers were tagged with V9-2L (60–120 s transmission rate; 651 days of expected life battery) (Table 1). After the tagging procedure, groupers remained for 22 days in the holding tank to recover. During this period, their diet was entirely based on hatchery-reared live prey of suitable size.

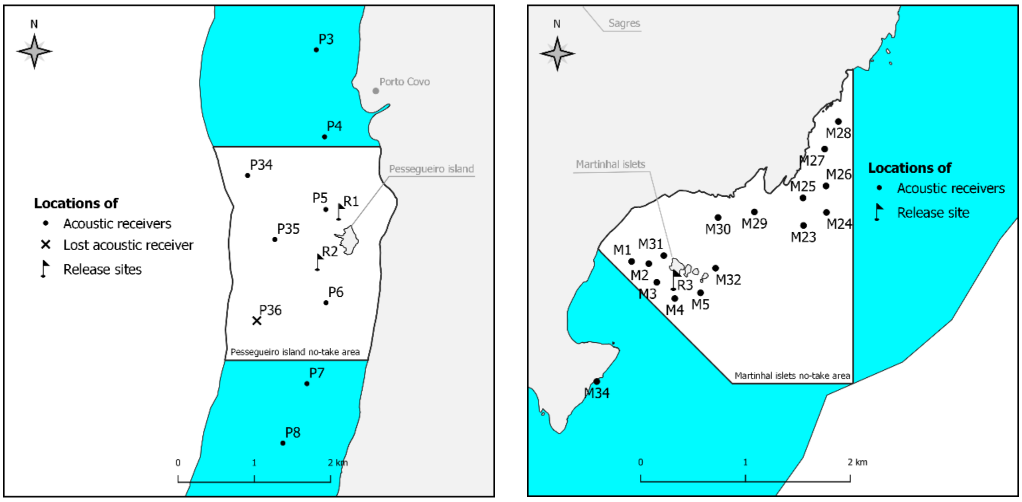
Table 1. Summary data of the tagged and tracked dusky groupers.

Fish ID	Total Length (mm)	Weight (g)	Transmitter ID/Model [1]	TBWR (%)	Release Site	Release Date/Time (UTC)
G1	542	2995	8041/V16-6H	0.5	R2	22 May 2019 12:00
G2	538	2802	8040/V16-6H	0.5	R2	22 May 2019 12:00
G3	515	2325	8039/V16-6H	0.6	R2	22 May 2019 12:00
G4	508	2814	8038/V16-6H	0.5	R2	22 May 2019 12:00
G5	500	2480	8037/V16-6H	0.6	R1	22 May 2019 14:00
G6	518	2481	8036/V16-6H	0.6	R1	22 May 2019 14:00
G7	495	2217	8035/V16-6H	0.7	R2	22 May 2019 12:00
G8	517	2434	8034/V16-6H	0.6	R1	22 May 2019 14:00
G9	517	2666	8042/V16-6H	0.6	R2	22 May 2019 12:00
G10	488	2362	8043/V16-6H	0.6	R1	22 May 2019 14:00
G11	515	2609	22984/V13-1L	0.2	R2	22 May 2019 12:00
G12	489	1925	22983/V13-1L	0.3	R1	22 May 2019 14:00
G13	502	2481	22982/V13-1L	0.3	R1	22 May 2019 14:00
G14	500	2470	22976/V13-1L	0.3	R1	22 May 2019 14:00
G15	530	2700	22977/V13-1L	0.2	R1	22 May 2019 14:00
G16	513	2513	22978/V13-1L	0.3	R1	22 May 2019 14:00
G17	486	2083	22979/V13-1L	0.3	R2	22 May 2019 12:00
G18	512	2678	22980/V13-1L	0.2	R2	22 May 2019 12:00
G19	486	2137	22970/V13-1L	0.3	R2	22 May 2019 12:00
G20	495	2078	22981/V13-1L	0.3	R2	22 May 2019 12:00
G21	650	5200	11418/V9-2L	<0.1	R3	19 April 2021 12:00
G22	650	5150	11429/V9-2L	<0.1	R3	19 April 2021 12:00
G23	620	4953	11428/V9-2L	<0.1	R3	19 April 2021 12:00
G24	615	5232	11430/V9-2L	<0.1	R3	19 April 2021 12:00
G25	650	5738	11431/V9-2L	<0.1	R3	19 April 2021 12:00
G26	640	6480	11432/V9-2L	<0.1	R3	19 April 2021 12:00
G27	610	5220	11433/V9-2L	<0.1	R3	19 April 2021 12:00
G28	560	3430	11424/V9-2L	<0.1	R3	19 April 2021 12:00
G29	590	3690	11425/V9-2L	<0.1	R3	19 April 2021 12:00
G30	580	4250	11426/V9-2L	<0.1	R3	19 April 2021 12:00

Fish ID: G—grouper; TBWR: tag-to-body-weight ratio; Release sites: R1—Pessegueiro island no-take area site 1, R2—Pessegueiro island no-take area site 2, R3—Martinhal islets no-take area. [1] Transmission frequency = 69 kHz

After recovering, groupers were translocated to the releasing sites inside individual plastic bags filled with 45 L of holding tank saltwater with a low sedative concentration (2-phenoxyethanol, 3×10^{-3} mL.dm^{-3}) and 120 L of oxygen. Onboard a vessel, each group of ten groupers was translocated to a modified fish trap placed on the sea surface, in the case of the Pessegueiro island no-take area. In the Martinhal islets no-take area, the groupers were individually placed on the sea surface inside their respective plastic bag, which was then perforated. In both cases, groupers were later placed on the rocky bottom, in areas with abundant caves and crevices that could serve as refuges. After the careful selection of the releasing sites, the fish trap and plastic bags were opened and the groupers were released. These operations were assisted by scuba divers, including the observation of the groupers' behavior immediately after the release. In 2019, the two sets of ten groupers were released in two slightly different locations inside the Pessegueiro island no-take area (R1 and R2, Figure 2a), between 8 to 10 m deep, while in 2021, all the groupers were released at the same location, i.e., the west side of the Martinhal islets, between 10 and 15 m deep (R3, Figure 2b).

The tagged groupers were tracked through a fixed biotelemetry passive acoustic receivers' array (VEMCO, models VR2W, VR2Tx, 69 kHz listening frequency), that logged the code and respective date-time of each ID signal detected. In the Pessegueiro island area, nine receivers were deployed: five receivers inside the no-take area (including, two VR2Tx receivers: R34 and R6, Figure 2a) and four receivers beyond its limits, two to the north and two to the south (Figure 2a). Except for receivers R35 and R36, which were fixed to a heavy cement block on the sandy bottom (ca. 100 kg), all other receivers were directly fixed to the rocky substrate. Receivers were deployed between 10 to 25 m deep, fixed through a cable and a buoy, keeping a distance of ca. 3 m from the bottom. In the Martinhal islets no-take area, sixteen receivers were deployed: seven around the islets, eight in the northeast nearshore rocky bottom, and one outside of it, southwest from the no-take area on a rocky reef close to the shore (Figure 2b). Each receiver was fixed to a heavy cement block placed at the sea bottom, between 5 to 25 m deep.



(**a**) (**b**)

Figure 2. Acoustic biotelemetry receivers' arrays used to track the dusky groupers in the (**a**) Pessegueiro island no-take area (total of 9 receivers) and (**b**) Martinhal islets no-take area (total of 16 receivers). Each acoustic receiver was identified with a letter (P—Pessegueiro island no-take area, M—Martinhal islets no-take area) and a number. Numbers assigned to the receivers are not necessarily in sequence and were independently attributed in the two arrays. Release sites were identified with the letter R and a number (R1, R2 in Pessegueiro island no-take area, and R3 in Martinhal islets no-take area).

The first retrieval of detection data logged by the biotelemetry receivers took place after two months of continuous tracking in each study area. Additionally, four manual tracking sessions were performed to complement the passive tracking in terms of spatial area covered: inside Pessegueiro island no-take area in July 2019; in the Cape of Sines in September 2019 and January 2020; and a one-day continuous active tracking for more than 80 km, i.e., 40 km along the coast in a round trip from the north of the Cape of Sines (38.00° N) to the Cape Sardão no-take area (37.57° N), by sailboat, at approximately 1 km off the coast in the south direction and ca. 2 km offshore northwards in August 2019. A manual acoustic receiver (VEMCO, model VR100) with an omnidirectional hydrophone, and two passive receivers attached to a submerged trawled structure, with the respective hydrophones pointing in opposite directions, were used in those campaigns.

2.3. Data Analysis

Collected data was analyzed in terms of (1) site attachment in the no-take areas, (2) activity patterns and movements in the no-take areas (short-distance ranging movements), and (3) movements after the departure of the dusky groupers from these areas (long-distance ranging movements).

Ranging movements consisted of exploratory movements of the individuals over a habitat, potentially searching for a suitable home range, i.e., an area to which an organism regularly restricts its presence, as it provides the necessary resources for the basic life functions [48,49]. The amplitude of ranging movements varied along a wide spectrum of distance scales, from short-distance to long-distance movements, depending on the encounter of suitable areas. Ranging movements likely cease or substantially diminish as soon as a suitable home range area is encountered [48]. This is followed by a process of site attachment and the establishment of a home range, to which the movements become limited.

Before the analysis, signals of the same transmitter, which were logged in different receivers with an interval of less than the minimum transmission delay, were filtered out and only the first timestamp was considered since the following was assigned to the record of the exact same emitted signal within the range of those multiple receivers.

1. The site attachment in the no-take areas, i.e., the selection of an area to settle [50], was assessed by analyzing the presence of each tagged fish in the study areas and how persistent was that presence.
2. The activity patterns and the movements of the dusky groupers inside the study areas (short-distance ranging movements) were also analyzed. Each fish was considered to be present in the study area while the interval between consecutive detections was less than 24 h. When the detections ceased permanently after a period of continuous presence, or whenever two consecutive detections were 24 h (or more) apart, it was assumed that the fish left the study area. Thus, for this analysis, only the set of detections between the releasing and the departure of the fish from the study area was considered. The activity and the movement patterns of the dusky groupers were analyzed in terms of the daily variation of the proportion of time in continuous activity and the number of different receivers with detections. The latter was evaluated as a proxy for the type of activity: stationary activity (detections in the same receiver) vs. movements (detections in multiple receivers) and as a proxy for the amplitude of those movements. The more receivers with detections, the wider the movements. Groupers were considered to be continuously active when intervals between consecutive detections were less than 10 min. Above this interval, individuals were assumed to be rarely and shortly active and hidden in refuges [44]. For each day in the study area, an activity index (I_A) was calculated for each period of the day—Dawn, Daytime, Dusk, and Night (classified according to the national entity of Astronomy data [51]). The I_A was calculated as the proportion of time of each period of the day during which each grouper was continuously active:

$$I_A = \frac{\sum_{i=1}^{n} \Delta a_i}{\Delta T}$$

where Δa are the multiple (n) intervals in continuous activity and ΔT is the total duration of each period of the day. The I_A values were then statistically compared to detect the daily variation of the fish activity. The number of receivers with detections at the four different periods of the day was weighted by the total duration of each period (N_R) and the daily variation of this metric was also tested. All tests were performed through univariate Permutational analysis of variance (PERMANOVA) with Primer 6 and PERMANOVA+ [52], considering the 'day period' as a fixed factor with four levels—Dawn, Daytime, Dusk, and Night. Boxplots were made in IBM SPSS Statistics 27.

The general routes used by the dusky groupers within the no-take areas and when exiting them were also tracked. These were computed through Refined Shortest Paths (RSP), an R package of tools that estimates the shortest distances traveled by monitored animals between pairs of detections using a least-cost path analysis by accounting for the presence of topographic features and constraints [53]. For consecutive detections in different acoustic receivers at least 250 m apart and with a10 min interval (minimum), intermediate positions of the tagged individuals were interpolated by RSP. Compared to the single use of the raw tracking data, more realistic movements of tracked animals were able to be estimated with this analysis tool. Each set of detections before a 24-h interval with no detections was treated as a distinct track, and distances traveled per track were computed in the R environment (RStudio v1.4.1717, RStudio, PBC) [53]. According to these calculations, the minimum distances traveled by each dusky grouper during the short-distance ranging movements in the study areas were estimated. The general routes of the movements were also visually evaluated by mapping the detections based on a Network Analysis, which evaluates the type and degree of interactions (edges) between activity centers (nodes) [54]. In this case, a spatial network, nodes were considered to represent acoustic receivers connected by edges, i.e., fish movements [44,55]. In this visual analysis, nodes were dimensioned according to the number of detections logged in the respective receivers and edge thickness according to the number of movements between two receivers [44,55]. The balance between the number of movements in each direction was represented by arrows. The proportion of fish detected for the last time was calculated and represented at the receivers where those detections occurred. These maps were prepared in QGis v3.16.8-Hannover, QGIS Development Team. Due to the distinct behavior of the grouper G29, i.e., a higher residency period inside the Martinhal islets no-take area, data analysis on this grouper was performed separately and was restricted to a visual graphical analysis (R packages 'circular' v0.4_93 and 'circlize' v0.4.13, RStudio v1.4.1717, RStudio, PBC) and mapping (QGis 3.16.8-Hannover, QGIS Development Team). Hourly circular graphs were built to visually analyze the daily variation of the total activity time and the number of receivers with detections, which were the same metrics statistically evaluated for the rest of the groupers. As for the other groupers, the general routes of the short-distance ranging movements of this fish were mapped.

3. The ranging movements outside the study areas (long-distance ranging movements) were evaluated according to multiple reported events, such as detections in other biotelemetry acoustic receivers' arrays deployed on the Portuguese coast in the framework of other projects and CoastNet infrastructure [56], recaptures by professional and recreational fishers, and researcher underwater sightings. Based on the date-timestamp of each reported event, and considering continuous and unidirectional movements at constant depths between 15 to 25 m, minimum distances and maximum time traveled were assigned, and ground speeds were estimated whenever possible.

3. Results

The behavior of the dusky groupers during the release was considered normal, immediately seeking shelter in nearby crevices and caves of rocky reefs. Such behavior was followed by a stationary period frequently spent in groups or maintaining positions very close to each other in those shelters, with no obvious signs of stress.

In the Pessegueiro island no-take area, a total of 1034 detections of different acoustic signals were logged in the eight receivers. The total number of detections of each fish varied between 4 and 271. In the Martinhal islets, 934 acoustic signals were detected, almost half of them from the same grouper (G29; N = 421). The other nine groupers were detected between 20 to 117 times, exclusively in the western area, around the islets, and in the receiver adjacent to the no-take area. Regarding the fish released in the Pessegueiro island and the Martinhal islets, an additional 1114 and 14 detections (from five and two individuals), respectively, were recorded outside the no-take arrays.

3.1. Site Attachment in the No-Take Areas

Detection data from both trials indicate that the dusky groupers did not settle in any of the no-take areas, i.e., a site attachment process was not observed. In the Pessegueiro island, all groupers left the area covered by the receivers within the first 34 h after being released, while in the Martinhal islets all but one (G29) left the no-take area within the first 31 h after the release. G29 took 25 days to leave the study area and was detected on 20 of those days. Ultimately, all the fish left the study areas, and for that reason, data analysis focused on the activity patterns and the short-distance ranging movements instead of residency.

3.2. Activity Patterns and Short-Distance Ranging Movements (Inside the Study Areas)

A significant circadian pattern was detected in the activity of the groupers in both study areas (Table 2). Despite the higher number of overall detections during the daytime (Table 3), groupers were more active during dusk (I_A = 22.6%) and night (I_A = 5.2%) (Table 3 and Figure 3). The activity index varied slightly between the study areas, but was predominantly nocturnal, as groupers were more active during the dusk on the Pessegueiro island and the night in the Martinhal islets (Figure 3). Conversely, levels of activity were consistently lower during diurnal periods in both areas, particularly during dawn (Table 4 and Figure 3).

Table 2. Results of univariate PERMANOVAs main tests regarding the daily variation of the dusky groupers activity index (I_A) and the number of receivers with detections per hour (N_R) in the Pessegueiro island and Martinhal Islets study areas.

Variable	Factor	Df	SS	MS	Pseudo-F	*p*	Perm
I_A	Day period	3	22,362	7454	3.0708	0.010	997
N_R		3	35,331	11,777	5.7491	0.001	997

Df—degrees of freedom, SS—sum of squares, MS—mean sum of squares, Pseudo-F—test statistics, *p*—calculated probability to significance level α = 0.05, Perm—number of permutations.

Table 3. General data on the activity and short-distance ranging movements of dusky groupers in the Pessegueiro island and Martinhal Islets study areas.

Day Period	Total nr. Detections	Time per Day (min) [1]	I_A (%) [2]	N_R [2]
Dawn	26	75	0	0
Daytime	799	900	2.90	0.14
Dusk	258	75	22.59	1.45
Night	298	390	5.20	0.56

[1] daily average duration of each period of the day; [2] medians.

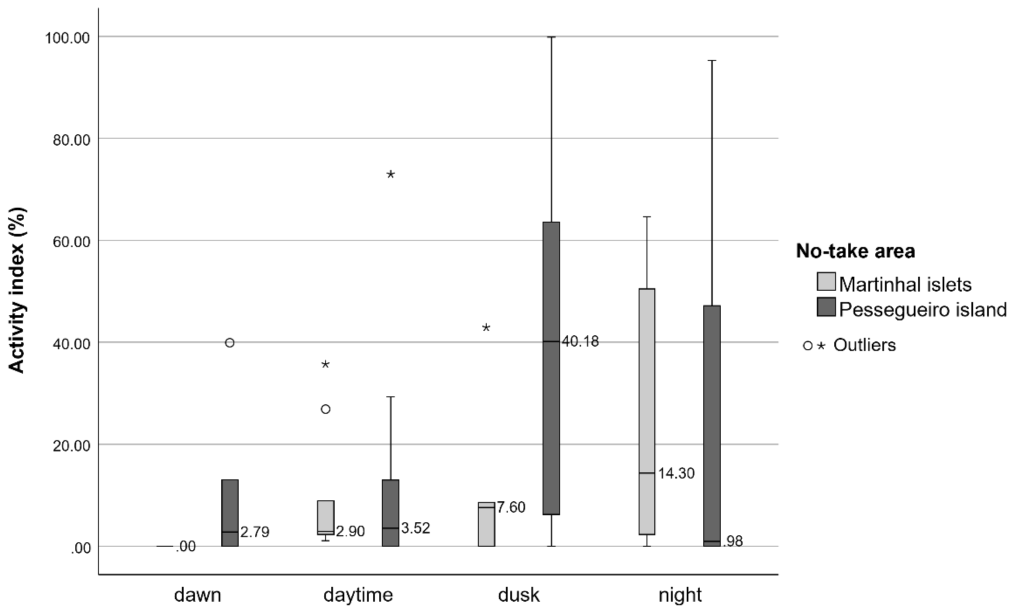


Figure 3. Boxplot of daily variation of dusky groupers' activity levels, i.e., activity index (I_A) calculated from individual fish released in the Pessegueiro island and Martinhal Islets no-take areas.

Similarly, a significant circadian pattern in the amplitude of the short-distance ranging movements (N_R) was also found. Groupers performed wider movements during dusk and night periods, with significantly higher N_R in these periods (Tables 2–4, and Figure 4).

Table 4. Results of univariate PERMANOVAs pairwise tests (p [1]) regarding the daily variation of the dusky groupers activity index (I_A) and the number of receivers with detections per hour (N_R) in the Pessegueiro island and Martinhal Islets study areas.

Variable	Factor Levels	Dawn	Daytime	Dusk	Night
I_A	Dawn	-	0.010	0.024	0.211
	Daytime	-	-	0.026	0.151
	Dusk	-	-	-	0.509
	Night	-	-	-	-
N_R	Dawn	-	0.004	0.020	0.013
	Daytime	-	-	0.001	0.038
	Dusk	-	-	-	0.197
	Night	-	-	-	-

[1] p—calculated probability to significance level $\alpha = 0.05$.

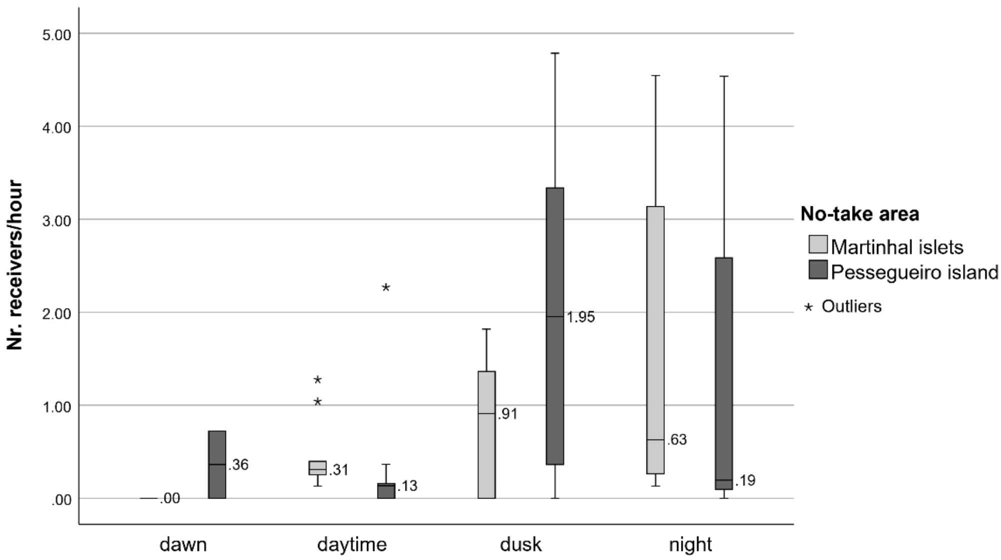


Figure 4. Boxplot of daily variation of the amplitude of movements, i.e., the number of receivers with detections per hour (N_R) calculated from individual fish released in the Pessegueiro island and Martinhal Islets no-take areas.

On average, groupers moved for about 12 h before leaving the study areas, moving ca. 2.5 km, with a mean ranging speed of 590 m.h^{-1} (Table 5).

In the Pessegueiro island no-take area, 60% (N = 601) of the detections were recorded in the receiver closer to the northernmost release site (P5), and the most frequent movements occurred between this receiver and the most central receiver (P35), followed by the movements between P35 and P34 receivers in the northwestern tip of the no-take area. The higher percentages of last detections were logged in these two receivers (80%), reflecting the exiting route followed by the large majority of the groupers (N = 16) (Figures 2a and 5).

About four to seven days after leaving the Pessegueiro island no-take area, three groupers (G7, G10, and G15; 15%) were detected again by the acoustic array, but only for a brief period of a few minutes to a maximum of two hours, possibly in transit.

In the Martinhal islets no-take area, nearly 50% (N = 184) of detections of the nine groupers (all except G29) were logged in the M4 receiver, close to the location where they were released (Figures 2b and 6), which was also the receiver where the highest percentage of last detections occurred (33%), totaling more than 50% of the last detections along with M5 (Figures 2b and 6). Two groupers were detected in the M34 receiver (Figures 2b and 6), outside adjacent and southwest of this no-take area. This means that most groupers probably left the no-take area by moving south or southwest.

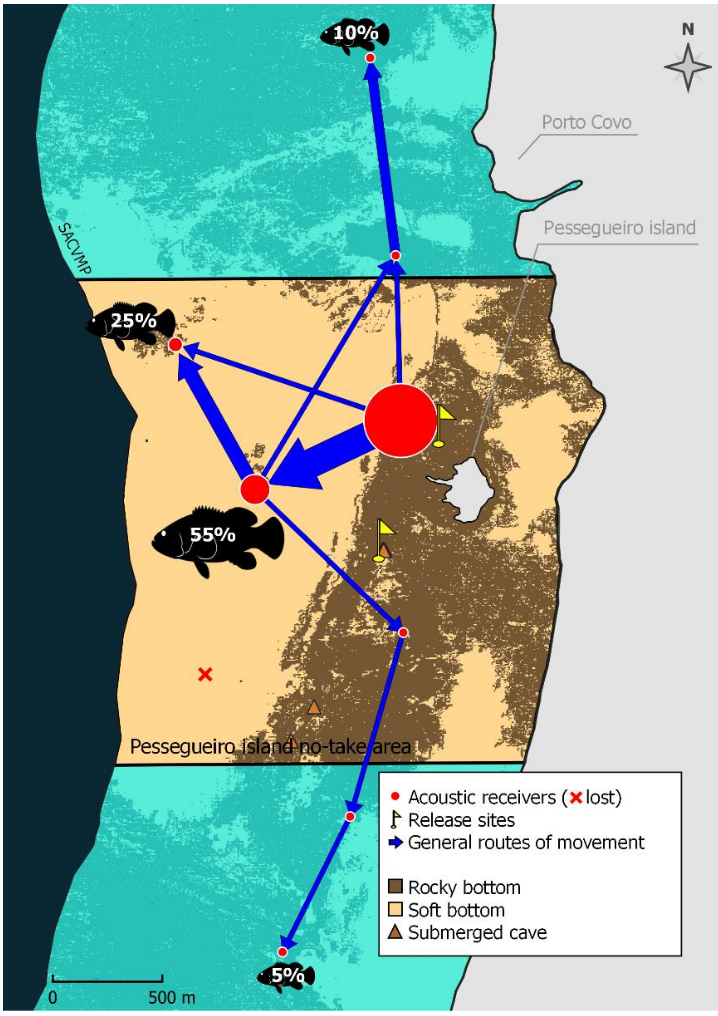


Figure 5. General routes of short-distance ranging movements of the 20 dusky groupers released in the Pessegueiro island no-take area. Nodes represent acoustic receivers, and the size of the circles is proportional to the number of detections logged in each receiver. The edge's thickness is proportional to the number of movements between two nodes and arrows represent the direction of the predominant movement directionality. Percentages associated with the size of the dusky groupers' silhouettes represent the proportion of individuals detected for the last time in the respective location. The light blue indicates 'buffer areas' of the marine park (SACVMP).

Five dusky groupers (55%) were later detected inside the Martinhal no-take area for a short time (four hours in maximum): four of them within the following four days (G23, G24, G27, and G28) and another one 40 days later (G26), possibly just passing through.

3.3. Long-Distance Ranging Movements (Outside the Study Areas)

After leaving the no-take areas, 43% of the dusky groupers (N = 13) were detected in other biotelemetry acoustic receivers' arrays (N = 7, 23%), caught by fishers (not released) (N = 6, 20%), or both (N = 1, 3%) (Table 6 and Figure 7). Additionally, one of the fish detected in other acoustic receivers' arrays was later sighted by a free diving researcher (Table 6 and Figure 7). The majority of these groupers (N = 7, 54%) were detected or recaptured within the first month after the release, while only two individuals (15%, G10 and G23) were detected more than three months later (Table 6). The groupers traveled, at least, between 3.5–168 km, and more than half of them (N = 7, 54%) traveled more than 30 km. The estimated travel speed was 540 m per hour, on average (Table 6), predominantly northwards (70%).

Table 5. Estimated short-distance ranging movements' speeds based on minimum distances and time traveled by each dusky grouper in the Pessegueiro island and Martinhal Islets study areas.

Study Area	Grouper [1]	Distance Traveled (m) [2]	Time Traveled (hh:mm) [3]	Ranging Speed ($m.h^{-1}$)
Pessegueiro island	G1	4379.77	3:56	1113.48
	G2	911.87	1:12	755.52
	G3	5340.70	20:55	255.33
	G4	-	-	-
	G5	8233.89	6:09	1338.84
	G6	734.91	3:24	216.15
	G7	-	-	-
	G8	734.91	30:31	24.08
	G9	-	-	-
	G10	3744.90	8:05	463.29
	G11	741.61	5:59	123.95
	G12	1158.21	16:52	69.13
	G13	734.91	3:29	210.98
	G14	747.60	0:35	1281.60
	G15	747.60	25:49	694.99
	G16	734.91	0:51	864.60
	G17	1823.74	0:38	2879.59
	G18	911.87	0:39	1402.88
	G19	2631.52	2:45	956.92
	G20	734.91	26:10	28.09
Martinhal islets	G21	3421.87	29:37	115.54
	G22	3344.15	32:55	101.59
	G23	7594.19	16:06	471.69
	G24	4883.05	13:46	354.33
	G25	2011.72	11:11	179.69
	G26	1473.46	2:52	513.90
	G27	1183.33	7:27	158.74
	G28	-	-	-
	G30	6035.60	33:53	178.07
Mean		2547.60	12:14	590.12

[1] G4, G7, G9, and G28 were detected in one single receiver, thus this analysis was not performed for these individuals; [2] computed by Refined Shortest Paths (RSP) R-tool; [3] time interval between the first and the last detection.

Detections in other arrays occurred in different receivers in tandem, during a few minutes to a few hours in each receiver (maximum of 13 h, in Arrábida MPA), which is consistent with unidirectional movements along the coast. When outside the study areas, some groupers released in the Pessegueiro island no-take area were detected by receivers in other no-take areas of the 'Sudoeste Alentejano' and 'Costa Vicentina' Marine Park, such as the Rogil no-take area, ca. 50 km to the south (location 9, Figure 7; G8, G10, Table 6). Three individuals were detected in other marine parks, such as the Arrábida MPA, 70 km to the north (locations 5 and 6, Figure 7; G5, G17, G18, Table 6). One dusky grouper released in the Martinhal islets no-take area was later detected in a diving site of an underwater park, approximately 30 km northeast of the Martinhal islets (location 16, Figure 7; G26, Table 6). Another grouper was detected almost five months later in the Port of Sines, 113 km to the north (location 15, Figure 7; G23, Table 6).

After leaving the Pessegueiro island no-take area, G5, G17, and G18 headed north. G5 and G17 arrived at the easternmost receiver of the Arrábida acoustic array (location 5, Figure 7) almost simultaneously (5 min interval), taking 96 and 101 h, respectively, to travel 70 km (ground speed G5 = 730 $m.h^{-1}$, G17 = 690 $m.h^{-1}$). G18 took more time to reach that location (nine days), despite leaving the no-take area nearly at the same time as G5 and G17. The presence of these three groupers was registered in 26 receivers deployed linearly along ca. 20 km of coast, from the eastern limit (location 5, Figure 7) to the western limit of

Arrábida MPA (Cape Espichel, location 6, Figure 7), quite near the shore (up to 1 km off the coast). Those detections were logged in chronological sequence from east to the west. After arriving at Arrábida MPA, G5 and G17 kept moving relatively synchronized in the first 3 to 4 h on course along the coast. On the Sesimbra shore (Figure 7), where there is a pontoon and a wreck on the sea bottom, G17 remained in the area for 10 h, while G5 kept going. All the three groupers were detected for the last time in the westernmost receiver within two days. G17 was later detected at the mouth of the Tagus estuary (location 10, Figure 7 and Table 6), but kept going towards the north where was recaptured by professional fishers 2 km off the coast of Ericeira, 168 km away from the releasing site, two months later (location 11, Figure 7 and Table 6).

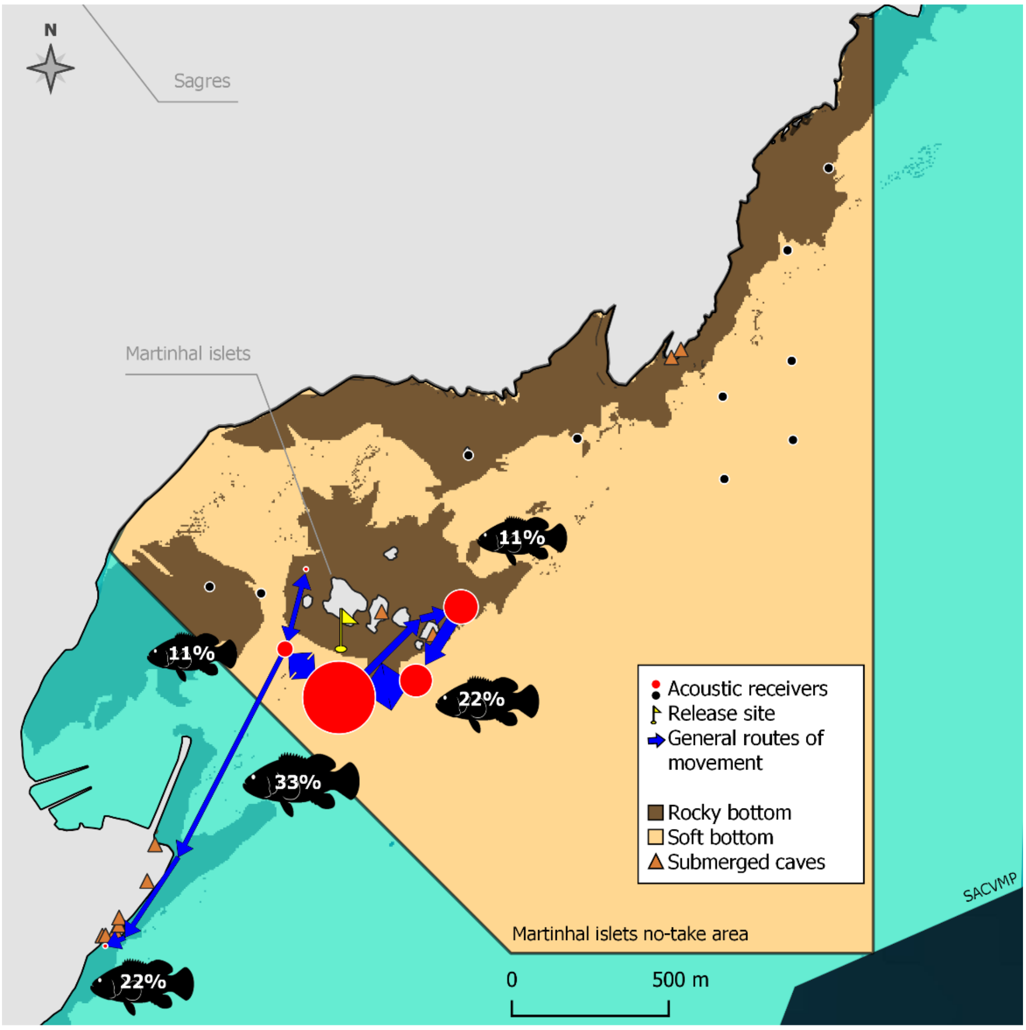


Figure 6. General routes of short-distance ranging movements of nine of the ten dusky groupers released in Martinhal islets no-take area. Nodes represent acoustic receivers (with detections—red; without detections—black) and the size of the circles are proportional to the number of detections logged in each receiver. The edge's thickness is proportional to the number of movements between two nodes and arrows represent the direction of the predominant movement directionality. Percentages associated with the dusky groupers' silhouettes represent the proportion of individuals detected for the last time in the respective location. The light blue indicates buffer areas of the marine park (SACVMP).

Table 6. Descriptive data of dusky groupers' long-distance ranging movements along the coast, reported through a combination of detections with acoustic biotelemetry (passive or active tracking) and recaptures (by professional or recreational fishers) after leaving the no-take areas.

Grouper.	Movement [1] (A → B)	Type of Reported Event [2,3] (A—B)	Date Time of Reported Event [4] (A–B)	Minimum Distance Traveled (km)	Maximum Time Traveled (Hours) [5]	Ground Speed (m.h^{-1})
G1	1 → 2	detection—recapture	23 May 2019 21:19–30 May 2019	23.3	-	-
G3	3 → 4	detection—recapture	23 May 2019 20:38–4 June 2019	3.5	-	-
G5	3 → 5	detection—detection	22 May 2019 21:08–26 May 2019 21:13	70.0	96.07	730
	5 → 6	detection—detection	26 May 2019 21:13–28 May 2019 21:36	17.4	48.38	360
		∑		87.4	144.45	-
G6	3 → 7	detection—recapture	22 May 2019 20:43–17 June 2019	25.0	-	-
G7	3 → 8	detection—recapture [6]	28 May 2019 21:48–24 June 2019	16.0	-	-
G8	3 → 9	detection—detection	23 May 19 20:26–27 May 2019 01:32	51.5	77.08	670
G10	3 → 9	detection—detection	22 May 2019 22:06–26 May 2019 20:22	49.0	94.25	520
	9 → 3	detection—detection	27 May 2019 01:32–29 May 2019 21:33	52.0	68.00	760
	3 → 8	detection—detection [7]	29 May 2019 22:07–7 September 2019; 21 September 2019; 6 January 2020	16.0	-	-
		∑		117.0	-	-
G17	3 → 5	detection—detection	22 May 2019 15:52–26 May 2019 21:18	70.0	101.42	690
	5 → 6	detection—detection	26 May 2019 21:18–28 May 2019 01:36	17.4	28.30	620
	6 → 10	detection—detection	28 May 2019 01:36–31 May 2019 22:56	28.6	93.32	310
	10 → 11	detection—recapture	2 June 2019 02:01–29 July 2019	52.0	-	-
		∑		168.0	-	-
G18	3 → 5	detection—detection	22 May 2019 21:31–1 June 2019 02:44	70.0	221.20	320
	5 → 6	detection—detection	1 June 2019 02:44–2 June 2019 21:28	17.4	42.70	410
		∑		87.4	263.90	-
G22	12 → 13	detection—recapture	20 April 2021 21:15–June 2021	21.0	-	-
G23	14 → 15	detection—detection	22 April 2021 22:20–15 September 2021 19:55	113.0	3501.58	30
G26	12 → 16	detection—detection	19 April 2021 14:51–28 May 2021 01:27	30.8	922.60	30
	16 → 12	detection—detection	28 May 2021 01:48–29 May 2021 01:09	29.0	23.33	1240
		∑		59.8	-	-
G30	12 → 13	detection—recapture	20 April 2021 21:56–June 2021	21.0	-	-
			Mean	61.1	-	540

[1] location of each reported event, georeferenced in Figure 7; [2] detections from passive tracking and recaptures by commercial fishers, unless a different kind is indicated; [3] reported recaptures with no release of the fish; [4] date–time of the last detection in location A and the first detection in location B; [5] only estimated for passive acoustic detections; [6] recapture by recreational fisher; [7] detections through manual tracking surveys after a sighting by a researcher while free diving on 7 September 2019.

Similar to G5 and G17, G22 and G30, released at the Martinhal islets, moved relatively synchronously at least from the last six hours inside the no-take area. They were detected for the last time at the same receiver less than one hour apart and finally were recaptured by fishers in the same location (Castelejo beach, location 13, Figure 7) two weeks apart.

Most of the reported dusky groupers recaptured by professional or recreational fishers were caught in rocky bottoms (G3, G6, G7, G22, and G30, Table 6), some of them in highly complex bedrock areas (locations 4, 7, and 8, Figure 7). In the same way, G10 was spotted by a free diving researcher four months after the release on a highly complex rocky shore (Cape of Sines, location 8, Figure 7). Its presence was posteriorly confirmed through manual tracking surveys, which confirmed its ID, recurrently using the area until ca. four months later (eight months after the release) in the same location (Table 6).

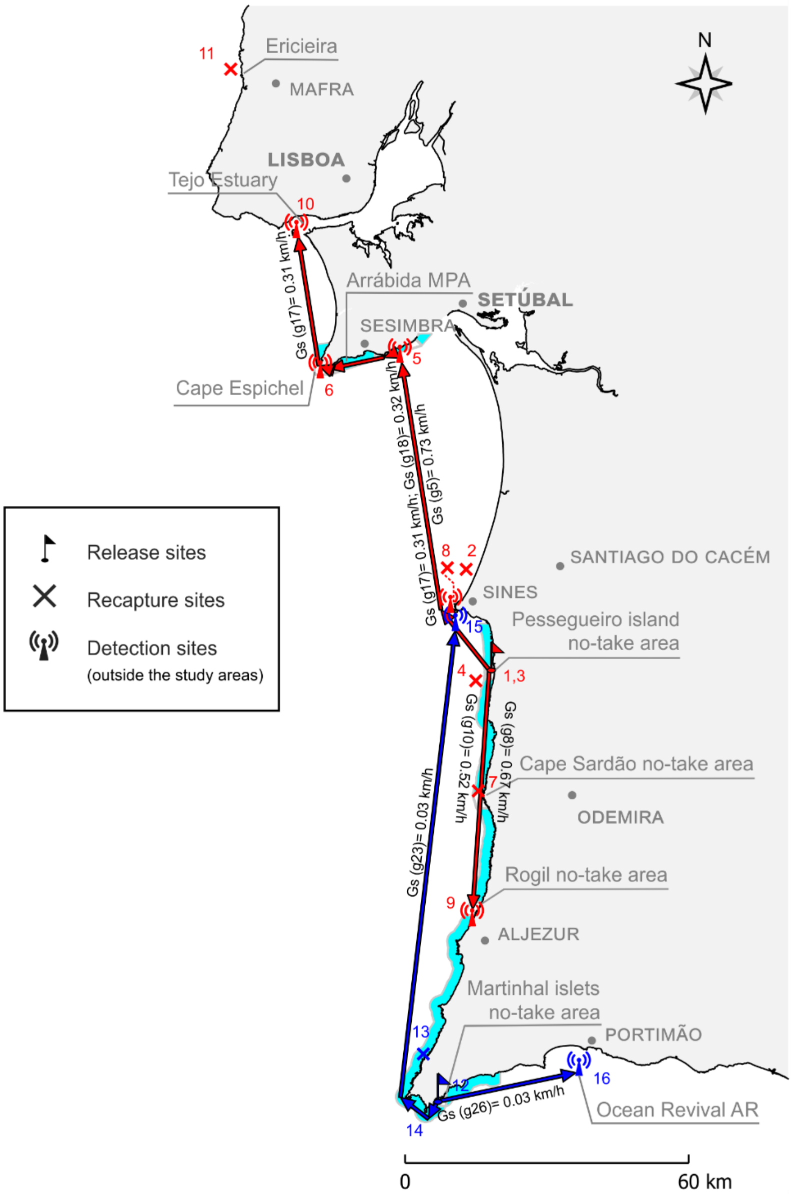


Figure 7. Locations of dusky groupers detections in other biotelemetry acoustic receivers' arrays, by manual tracking surveys and recaptures by professional or recreational fishers, after leaving the no-take areas. Numbers are not necessarily by timeline order of records but by the order in which they are indicated in Table 6. Some of the movements are represented by arrows with the respective ground speeds (Gs) and groupers (g#). Red and blue arrows correspond to long-distance ranging movements performed by dusky groupers released in the Pessegueiro island no-take area and Martinhal islets no-take area, respectively.

3.4. Grouper 29

Contrarily to the remaining groupers, G29 stayed in the Martinhal islets study area for a longer period of 25 days. After the release, it was continuously detected in the same location until dusk. It remained undetectable for two days, and then it was sporadically detected in the next day and a half in the same area. Four days later, G29 moved onto the northeastern side of the no-take area where it remained for 15 days, mainly around M26 and M29 (Figure 2b), performing regular movements between these two locations (Figure 8). After this period, G29 moved towards the releasing site and within four hours left the no-take area through the southwestern limit, leaving the area covered by M34 five days later (Figure 8). According to the hourly circular graphs, these short-distance ranging movements occurred mainly at dusk and night periods (Figure 9).

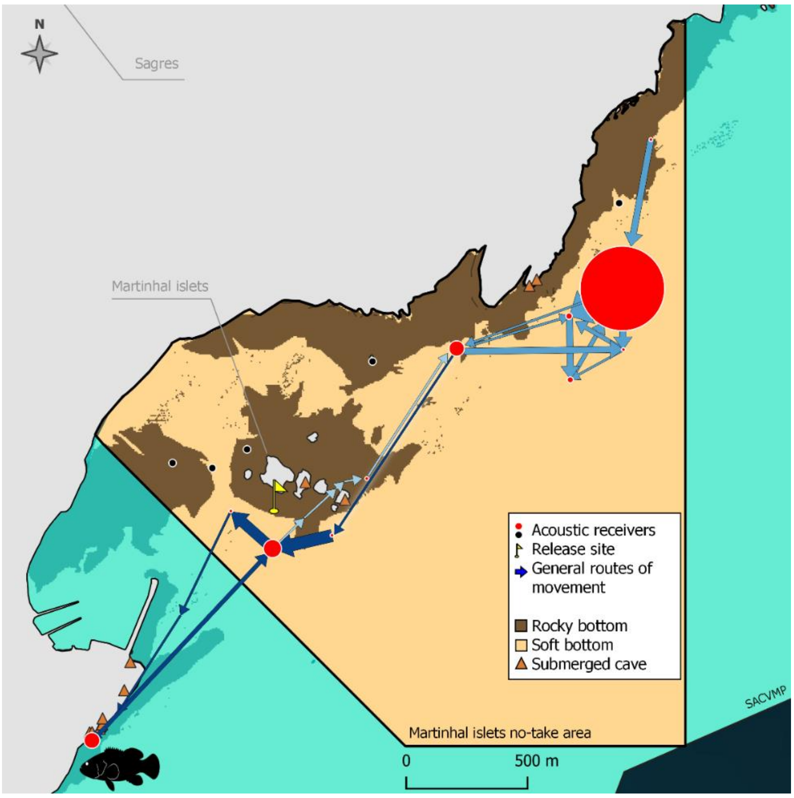
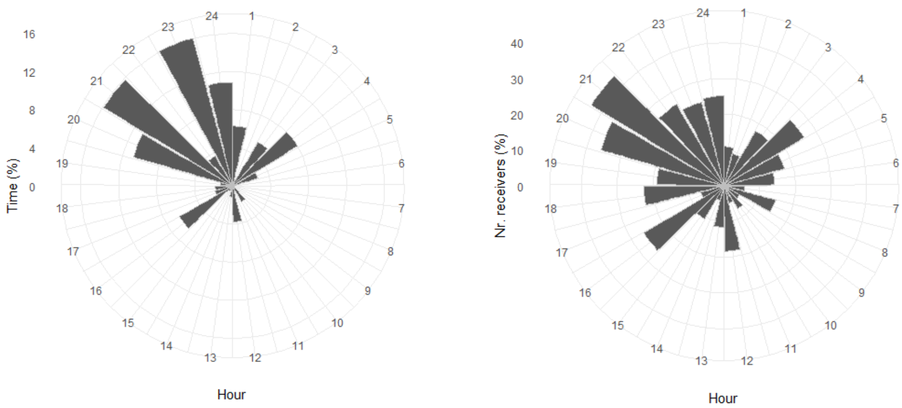


Figure 8. General routes of short-distance ranging movements of G29 in the Martinhal islets study area. Nodes represent acoustic receivers (with detections—red; without detections—black) and the size of the circles are proportional to the number of detections logged in each receiver. The edge's thickness is proportional to the number of movements between two nodes and arrows represent the direction of the predominant movement directionality. The three shades of blue of the arrows reflect the time sequence of tracked ranging movements: the lighter blue arrows represent the movements within the first four days after release, the darker ones represent the ranging movements in the last five days of presence, and the arrows with intermediate color represent the movements performed between the two periods. The dusky grouper silhouette represents the location where G29 was detected for the last time. The light blue indicates buffer areas of the marine park (SACVMP).



(**a**) (**b**)

Figure 9. Circadian activity and movement patterns of dusky grouper G29 during the 25-day period of residency inside the Martinhal Islets no-take area: (**a**) Mean percentage of the total time in which G29 was continuously active per hour of the day; (**b**) Mean percentage of the total number of receivers with detections of G29 per hour of the day. At that time of the year, dusk periods occurred between 7:30–9:30 p.m. (UTC), while dawn periods occurred between 3:30–5:30 a.m. (UTC) [51].

4. Discussion

Data collected in the present study revealed that the release of hatchery-reared young adult dusky groupers was not successful with respect to site attachment concerns, since no individual set residency inside the Pessegueiro island or the Martinhal islets no-take areas. Instead, some individuals moved for more than hundreds of kilometers along the coast, quite near the shore, which may evidence the importance of better understanding and protecting coastal corridors as connectivity promoters.

To the best of our knowledge, long-distance movements were described only once, also with hatchery-reared young adult dusky groupers released in a marine protected area (MPA) on the southern coast of Spain. Two groupers were recaptured more than 250 km away from the release location, near the original location of the breeders [36,57]. Other studies on recruitment, reproduction, and genetic patterns have already discussed the possibility of the occurrence of isolated large-scale migrations of dusky groupers in the Mediterranean and the eastern Atlantic, as a consequence of massive recruitment events and 'habitat bottlenecks' [58,59]. Yet, such events mismatch with the typical sedentary behavior and site fidelity of dusky groupers which reinforces the need for additional data on juvenile and adult movements [59]. So far, the great majority of the studies reported displacements of a few kilometers [29,32,34,35,60], including movements towards the original territories when individuals were translocated [29,34]. In a pilot study on the release of hatchery-reared juvenile dusky groupers in two artificial reefs in the Mediterranean, sighting rates in those artificial reefs ranged between 15 and 20% and the furthest recapture occurred 13 km away from the release location [35]. In the present study, 40% of the 30 released groupers were located (detected or recaptured) more than 10 km apart from the release locations, 23% traveled at least 50 km, and 3 fish (10%) moved more than 100 km.

Dusky groupers are known as highly sedentary species, showing strong site fidelity when adequate conditions are met, in particular, adequate habitat and prey availability [29–34]. According to previous studies on the habitat preferences of the dusky grouper [17,60–66], both no-take areas on which the adult dusky groupers were released in the present study had favorable conditions for settlement, concerning habitat structure and complexity. Those conditions include intricate rocky reefs with large underwater caves, rocky outcrops surrounded by sandy bottoms, and the presence of islets [41]. Considering the estimated carrying capacity of other insular Mediterranean MPA for dusky grouper (K = 3.73 kg per 100 m^2) [25] and also the possible absence, or, at least, very low abundance of wild resident adult dusky groupers inside the no-take areas of the present study, the number of individuals released was possibly very far from the respective carrying capacity. Given all these assumptions, one would say that habitat suitability in terms of structure and complexity, as a proxy for shelter availability, would not have been the cause for the total absence of site attachment to the no-take areas.

Conversely, depth and hydrodynamics could have triggered such behavior. Through a multi-scale seascape approach, Alvarez-Berastegui et al. [66] found that adult dusky groupers were most frequently found deeper than 25 m deep. This distribution pattern had been commonly explained by the spearfishing pressure which is limited to the free divers' capacity to reach deep habitats [21,24,61,63,65]. However, the target of Alvarez-Berastegui et al. [66] study was a recovered population protected from spearfishing for 20 years in a Mediterranean MPA, and yet such distribution pattern persisted, possibly as the natural behavior of the undisturbed populations. In the present study, suitable habitat for dusky groupers in the no-take areas is restricted to less than 25 m deep, with high hydrodynamic conditions, particularly on the west coast [41]. In addition to similar results between both no-take areas, it is worth mentioning that in the Pessegueiro island no-take area, the dusky groupers showed a clear preferential route to exit the area towards higher depths, unlike in the Martinhal islets no-take area where no such tendency was observed. This could have been a consequence of the shallower bottom in the Pessegueiro island no-take area. While in this no-take area, 86% of the sea bottom is shallower than 20 m, 60% of the Martinhal islets no-take area is deeper than 20 m. In the Martinhal islets, the shoreline is steeper and rapidly sinks, in some places reaching 20 m of depth less than 100 m from the shore. Furthermore, ocean conditions on the west coast of Portugal are harsher than in the Mediterranean, thus the range of depths required for shelter may be even larger than what was shown in previous studies in the Mediterranean [66]. Indeed, sea condition aggravation has been shown to cause dusky groupers' displacement to deeper areas, partly due to the high energy costs of moving and keeping position in very hydrodynamic conditions [64]. In this sense, the depth of the releasing area could have played an important role in the fish's behavior. It could have explained the longer presence, of almost one month, of one fish in the Martinhal islets (G29) and the higher number of fish re-crossing the area a few days after leaving (50%), compared to 15% in the Pessegueiro island no-take area. Sea conditions could have also influenced the behavior of the dusky groupers, as they were not ideal at release, but got considerably worse in the following days. For example, G29 became undetectable during the worst sea conditions (i.e., a period wave of more than 13 and more than 2 m of height), returning to be detected in the northeast region of the no-take area when conditions got better. It remained there while the conditions were stable and finally left the area when conditions got worse again. Thus, this is a factor to consider when planning future releases. The differences in the exposure to the dominant winds and swell of both no-take areas could have also explained the slight variations between the results obtained in both trials.

Other factors, such as prey availability, were unlikely to be a limiting factor, as both areas are abundant in benthic and benthopelagic fish and octopus [41–43,45], the most important food items to medium-big size wild dusky groupers [67–71]. In fact, these protected areas are more abundant in fish than the adjacent buffer areas [42,43,45]. Such prey availability also supports the presence of other big predators, such as conger eels and morays [42,44,45]. The high biomass of these two territorial predatory species could have also prevented the settlement of the dusky groupers, due to interspecific competition, especially in the Pessegueiro island no-take area [42].

In addition to the habitat constraints, intrinsic species characteristics could have also influenced the results. Small-scale reproductive migrations (in late spring/early summer) and complex hierarchical social behavior during the reproductive season (summer) are typical of this species. The displacement of medium-size individuals as a result of territorial intraspecific competition and agonistic behavior was already observed in several studies [16–18,32,60,72,73]. These behavioral patterns might be innate, having turned out in the natural environment and possibly triggering the observed dispersal behavior.

The ranging movements of the dusky groupers were more frequent and wider during night periods, particularly during dusk in both no-take areas and while moving along the coast, including in the MPA further north (Arrábida). These results are in line with other studies which indicate that dusky groupers are more active and forage during dusk and night periods [32,65,73,74]. After leaving the no-take areas, some of the dusky groupers moved along the coast for long distances, up to 200 km, mainly to the north, traveling between 500 and 600 m per hour. Another interesting behavior observed was the apparent association of some dusky groupers in pairs while moving, such as the case of the two individuals almost simultaneously detected in the Arrábida MPA, four days after being released in the Pessegueiro Island no-take area and after performing a relatively long movement of at least 70 km. The association of juvenile dusky groupers in groups of two or three individuals was described in other studies [63–65]. Some commercial fishers operating in the region also reported the capture of pairs of wild dusky groupers on consecutive hooks [28].

The dusky groupers' recaptures in areas of known high habitat complexity and biodiversity seem to confirm the ranging behavior of the released groupers, searching for a suitable area to settle. For example, four months after leaving the Pessegueiro island no-take area, G10 was spotted (free diving) by one of the researchers involved in this study on a rocky shore outside and north of the SACVMP (Cape of Sines), a highly complex and rich rocky area, reaching considerable depths relatively nearshore. The fish was in a good body condition and was later detected in two manual tracking surveys, the last one eight months after the release. In the exact same place, another grouper (G7) was caught from shore by a recreational fisher with rod fishing using a live crab (sardine swimming crab, *Polybius henslowii* Leach, 1820) as bait. Its stomach was full of crabs and three hooks. Such observations may support the idea that hatchery-reared dusky groupers can settle down when they find areas with adequate conditions, being able to survive as opportunistic predators. In both releasing trials ca. 50% of the hatchery-reared released dusky groupers proved to be able to survive in the wild at least until the end of the first month after the release. This reflects some resilience of the individuals, probably a result of their good body condition at the release, but it may also reflect their ability to adapt to the wild conditions.

Considering the resident behavior of dusky groupers, atypical results from corroborating trials (performed in this study by releasing hatchery-reared fish in the two no-take areas) could have been related to the origin of the released fish. The inability of hatchery-reared fish to perceive environmental stimuli useful for their settlement or to exploit available feeding resources is a consequence of a psychosensorial deprived environment, typical of fish hatcheries [75]. Consequently, the process of getting a 'normal' behavior from reared fish after release includes: (1) a short period of recovery with defense strategies, such as 'freezing', hiding, and schooling; (2) a period of wary exploratory behavior and familiarization with the new environment, and (3) a period of adjustments of feeding habits and adjustments to the new environment, to the new spatial and social frame, including adaptation to hydrodynamic conditions and choice of shelter [76]. The last two phases can occur simultaneously [76]. In the present study, all dusky groupers were able to overcome the first period, during which, at least, two of the three defense behaviors (i.e., hiding and schooling) were exhibited immediately after the release, and all groupers underwent the second phase, although it was not accomplished inside the no-take areas. Step three is quite critical to territorial species, especially when socially hierarchical systems are involved, which is the case of the dusky grouper. In such cases, a new 'spatial map' is required for the establishment of home ranges, which implies more time to explore the area. At least one grouper, G10, showed some signals of undertaking the third phase (although outside of the marine protected area), since it was spotted and detected twice (four and eight months after the release) in the exact same place, in an area with apparently good conditions for a dusky grouper to settle. Other individuals could potentially be settling into specific areas, but because they were not released after being caught, no further information on their site attachment was possible to obtain.

The results found in this study point out the necessity of altering the restocking approach with hatchery-reared dusky groupers, focusing on protected areas, to increase site fidelity to the target areas and, consequently, the efficiency of these actions. To overcome this, and to prevent the early departures of the released hatchery-reared dusky groupers from the target areas, some adjustments may be considered. For instance, providing an acclimatization period to the natural environment of an extended period of several months inside a cage could considerably reduce the dispersal tendency of the fish after the release [36]. This would introduce and familiarize the fish with natural conditions, such as ambient noise, hydrodynamics, olfaction clues, and part of the fish community, whereas small prey could enter the cage where groupers would remain. During this period, a progressive transition to the natural living prey should be ensured. The fish should be acclimatized and released in small groups in the best suitable habitat (substrate complexity, shelter, food availability, and depth), and should remain in relatively large cages provided with appropriate refuge structures [75,77].

Another possible approach to test would be the release of juveniles instead of young adults. Agonistic behavior related to a complex social hierarchical system is particularly intense in adulthood. While site fidelity was also observed in juvenile dusky groupers [29,30,34,35,72], the release of juveniles to reduce intraspecific competition is a potential alternative. Even though such agonistic behaviors are likely to occur, as observed in other hatchery-reared dusky groupers restocking programs [35], this emphasizes the need to work with low densities of fish and to take into account of carrying capacity of the releasing areas [35,75]. On the other hand, because the releasing of juveniles would involve greater mortality rates, it would imply greater releasing stocks.

According to prior evaluation, in the case of the releasing areas with carrying capacity deficit, habitat enrichment by means of artificial reefs or other appropriate structures may be considered [78,79]; as it has already been demonstrated that the use of artificial habitats can attract juvenile and adult dusky groupers and host a high density of juveniles [80–82].

However, regarding the choice of target areas, two notes on spatial management can be taken from our case study. One is that there is a challenge related to ensuring that protection measures are respected, as at least 10% of released fish were caught in this MPA, despite the prohibition of catching this species in this area. The other is that the option of releasing in the most suitable habitat, regardless of the protection level, could also be a valid approach, provided that the expected fishing effort is low. In this context, actions could also be taken to raise fishers' awareness of the conservation of this endangered species.

5. Conclusions

The restocking of specific areas of interest, such as the MPAs of the northeast Atlantic, with hatchery-reared flagship species, such as the endangered dusky grouper, could provide ecological and socio-economic benefits. Despite the potential, these actions seem to have a low success rate since the attachment of the released individuals to the releasing areas has not been observed. This is mainly due to the complex behavior of individuals reared in captivity, the uncertainties regarding the best releasing approach, and the choice of suitable area, as well as due to fishing mortality and noncompliance. Thus, efforts must be conducted in future restocking trials to improve the efficiency of such actions. Critical factors must be addressed, such as the ontogenic stage of the releasing individuals, the introduction of natural live prey prior to the release, acclimatization at the release site, the density of releasing stocks, and careful selection of target areas, considering carrying capacity and spatial enrichment, if needed. On the other hand, a positive outcome of this study was the discovery of the ability of the dusky groupers raised in captivity to survive in the wild, reinforcing future restocking actions with hatchery-reared stocks. Therefore, the present study highlights the challenges and the need for methodological improvement to efficiently apply restocking actions as a measure for the recovery of dusky grouper populations.

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