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SHORT COMMUNICATION

An Acclimatization Period in a Cage Promotes Site Fidelity of Hatchery-Reared Dusky Groupers to the Release Areas

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Keywords: acoustic biotelemetry | *Epinephelus marginatus* | *in-situ* conditioning | restocking trials | settlement

Abstract

The influence of an *in-situ* acclimatization period of five weeks, seeking the settlement and fidelity of hatchery-reared dusky groupers (*Epinephelus marginatus*) to the release sites in restocking actions, was tested using acoustic biotelemetry. Eight tagged adult groupers were submitted to the wild conditions in a fish farm cage, while seven remained in the hatchery and tagging location until they were all released. Ten months later, half of the acclimatized groupers were still present in the release site, while all the non-acclimatized rapidly dispersed. These results show that an *in-situ* acclimatization period of five weeks promotes the establishment and site fidelity of hatchery-reared dusky groupers, contributing to successful restocking.

1 | Introduction

The dusky grouper, *Epinephelus marginatus*, is one of the most threatened serranid species (Mitcheson et al. 2020). As a big-size and slow-growth species with late maturation and complex life traits, such as protogynous hermaphroditism and spawning aggregations (Zabala et al. 1997; Glamuzina et al. 1998; Marino et al. 2001), the dusky grouper is highly vulnerable to overfishing (Mitcheson et al. 2020). As a consequence of the historically high fishing pressure, it is classified as an 'Endangered'

species in Europe (Harmelin-Vivien and Craig 2015) and the Mediterranean basin (Cornish and Harmelin-Vivien 2011). Despite the efforts to reverse the situation, for instance, the creation of no-take areas, with local population recovery in some of those areas in the Mediterranean, the overall decrease persists (Pollard et al. 2018). In light of this, restocking programs with hatchery-reared dusky groupers have been considered and studied as a measure to restore populations throughout its distribution range, nevertheless, there is still no evidence of the effectiveness of this measure (La Mesa et al. 2008; Gallego et al. 2013; Riede et al. 2017; Silva et al. 2022).

The settlement of a fish (juvenile or adult) depends on the existence and the finding of a suitable habitat to establish a home range (Crook 2004), i.e., an area to which the fish regularly restricts its presence and movements, as it provides the necessary resources for basic life functions (Burt 1943; Gerking 1953). The fish hatcheries are typically psychosensorial deprived environments, so the hatchery-reared fish are commonly unable to perceive environmental stimuli useful for their settlement or to exploit available feeding resources (Olla et al. 1998). Such a condition may hamper the search for a suitable home range area, extending the ranging movements of the fish for a longer period and outside the release area (Taylor et al. 2017). This is even more complex when species have social hierarchical systems (Olla et al. 1998; Crook 2004), which is the case of the dusky grouper (Chauvet and Francour 1990; Zabala et al. 1997). Moreover, the stress related to handling and transportation may also alter the hatchery-reared fish behaviour when released in the wild (Olla et al. 1998) and consequently may influence the likelihood of their establishment in the release sites. Providing an *in-situ* acclimatization period to the natural environment (e.g., ambient noise, hydrodynamics, olfaction clues, and part of the fish community) for an extended period could, therefore, reduce the early escapement and promote the establishment of the hatchery-reared dusky groupers in the release areas (Silva et al. 2022).

The southwestern and the central mainland coast of Portugal, along with the Azores archipelago, are the northernmost areas with known resident populations of dusky grouper in the Atlantic (Pierre et al. 2007; Mahé et al. 2012; Horta e Costa et al. 2018). Along the southwestern coast, the species is strictly protected from fishing since 2011 inside the 'Sudoeste Alentejano' and 'Costa Vicentina' Marine Park (SACVMP), from south of Sines (Alentejo) to Burgau (Algarve), up to 2 km offshore (Figure 1A). Four small (*ca*. 6 km^2 each) no-take areas (where almost all extractive activities are forbidden, with the only exception of the commercial harvesting of stalked barnacles in the mainland coast) provide additional protection to the species in SACVMP. Two prior releasing trials of hatchery-reared adult dusky groupers were conducted in two no-take areas of SACVMP: the Pessegueiro island no-take area and the Martinhal islets no-take area. The groupers, that were released directly into the wild, did not settle in the area, and within the first two days they dispersed to unprotected areas (Silva et al. 2022).

Following our first attempts previously mentioned, the present work consisted of the first releasing trial to test the influence of an *in-situ* acclimatization period on the establishment (site attachment and fidelity) of hatchery-reared adult dusky groupers in the release area, the Port of Sines. The hypothesis tested is that to pre-expose the groupers to the wild environmental conditions, stocking temporarily in a fish farm cage, would reduce the post-release dispersion behaviour, fostering the search for a territory in the release area, with higher number of fish and time spent in the area, and consequently the increase of likelihood of more fish to settle in.

2 | Methods

The releasing trial was carried out inside the Port of Sines (Figure 1B) because: 1) it is a sheltered area, protected from the dominant swell and wind (N and NW); 2) fully protected from commercial fishing, and mostly protected from recreational fishing, with the only exception of the shore angling in three restricted areas, including a sandy beach in the Sines bay; 3) adjacent to the SACVMP (Figure 1A); 4) with suitable habitat where dusky groupers are frequently observed; and 5) where a fish farm with net-cages operates, securing logistical support. In May 2021, 15 adult dusky groupers (7–8 years, Table 1) produced and reared in the Aquaculture Research Station of Olhão (EPPO), managed by the Portuguese Institute for Sea and Atmosphere (IPMA), were surgically implanted with acoustic transmitters (Table 1) and tagged with external dart-tags (see Supporting Information video S1, track 1). These fish were all from the same offspring, with the same age and similar size (mean $TL_{Treatment}$ = 54.2 cm, mean $TL_{Control}$ = 54.5 cm [U (Mann–Whitney) = 25.5, p-value = 0.779]; mean $W_{Treatment}$ = 2911.9 g, mean $W_{Control}$ = 3019.6 g [U = 26.0, p-value = 0.867]) and were submitted to the same cultivation, *ex-situ* conditioning, and tagging protocols of the groupers released in the above-mentioned previous trials (Silva et al. 2022). After the tagging procedures, all groupers remained for three weeks in the holding tanks to recover. During this stage, and the previous weeks, their diet was entirely based on hatchery-reared live fish prey of suitable size (e.g., mackerels, seabass and seabreams), promoting the transition from inert diets used in aquaculture conditions to the new feeding regime in the wild. After this period, eight groupers were translocated to a fish farm net-cage in the Port of Sines (treatment group), following the transportation protocols described in Silva et al. (2022). The remaining seven groupers (control group) prolonged their stay in the holding tanks (see Supporting Information Video S1, track 2). Live fish prey and artificial shelter (polyethylene tubes, *ca*. Ø 30 cm) were provided for both sets of groupers. Five weeks later, in July 2021, all groupers were released in the port, on the same day: the cage was opened near the surface, and the acclimatized groupers were encouraged to leave the cage, while the other seven groupers (control group) were directly released in the same location from a vessel (see Supporting Information Video S1, track 3). The presence of the groupers was initially tracked through five fixed biotelemetry passive acoustic receivers (VEMCO, models VR2W, VR2Tx, 69 kHz listening frequency) (R1–R3, R5, R6, Figure 1B), deployed between 10 to 25 m of depth inside the Port of Sines and its vicinities (see Supporting Information Video S1, track 4). Four months later (November 2021), due to the report of sightings of one tagged grouper around the fish farm cages, the array was reinforced with an additional acoustic receiver deployed in a buoy of the fish farm (R4, Figure 1B). In July 2022, in the scope of ATLAZUL Project (https://atlazul.eu/) with the maintenance

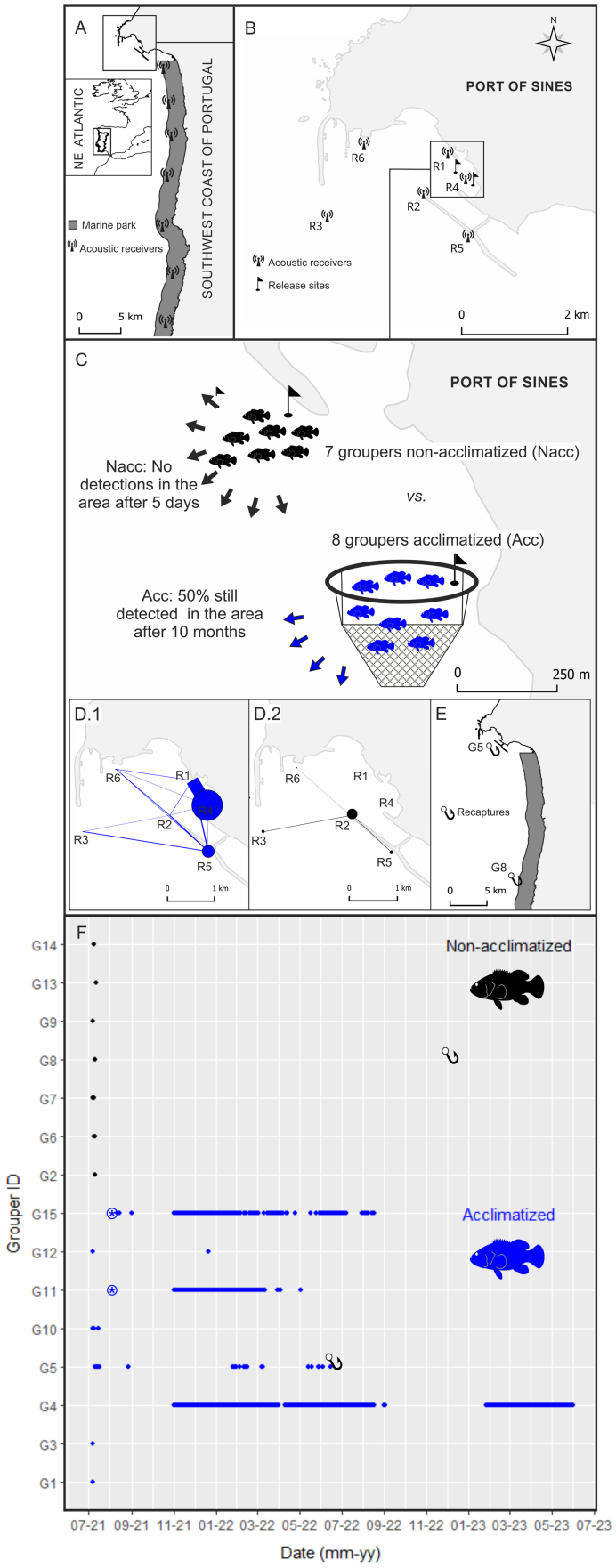

FIGURE 1 | Legend on next page.

FIGURE 1 | (A, B) Location of the study area, including the release sites and the acoustic biotelemetry receivers' arrays (in the Port of Sines array (B), each acoustic receiver was identified with the letter R and a number). (C) Experimental design and major results. (D) General pattern of space use of the release area (where nodes represent the receivers, and node size is proportional to the number of detections in each receiver, and links represent movements between receivers, with link width proportional to the frequency of consecutive detections in each two receivers) (d1) by the acclimatized groupers, (d2) by the control group. (E) Location of the dusky groupers' recaptures (G5, G8). (F) Plot of the daily presence of the released dusky groupers (dots represent presences detected by the passive acoustic receivers, while the asterisk symbol represents the presence detected with active manual tracking, hooks indicate the recapture of the groupers G5 (in June 2022, 2 km from the release site) and G8 (in December 2022, 16 km from the release site)).

TABLE 1 | Summary data of the tagged and tracked dusky groupers.

Fish ID	Total length (mm)	Weight (g)	Transmitter ID/model[a]	Acc. (T/C)	Nr. days with detections	Nr. detections	Last detection Date Time_Receiver[b]
G1	542	2620	22971/V13-1 L	T	1	4	2021-07-08 00:12_R2
G3	515	3920	22973/V13-1 L	T	1	21	2021-07-08 01:55_R3
G4	508	2333	22974/V13-1 L	T	430	134,910	2023-07-31 14:59_R5
G5	500	3020	22975/V13-1 L	T	27	1801	2022-06-16 12:30_R5
G10	488	3180	2090/HP16	T	4	52	2021-07-16 00:35_R3
G11	515	2962	2091/HP16	T	140	69,020	2022-05-03 15:00_R1
G12	489	2440	2092/HP16	T	5	10	2021-12-22 19:28_R4
G15	530	2820	2095/HP16	T	174	10,369	2022-08-17 07:15_R5
G2	538	3444	22972/V13-1L	C	1	14	2021-07-11 22:34_R3
G6	518	2911	8035/V16-6H	C	2	43	2021-07-10 17:19_R3
G7	495	2755	8036/V16-6H	C	3	114	2021-07-09 22:01_R3
G8	517	2980	8039/V16-6H	C	1	24	2021-07-11 22:38_R2
G9	517	2285	8041/V16-6H	C	1	7	2021-07-07 21:42_R2
G13	502	3944	2093/HP16	C	2	49	2021-07-12 07:42_R2
G14	500	2818	2094/HP16	C	1	15	2021-07-09 22:12_R2

[a]Transmission frequency = 69 kHz, V13-1L and V16-6H exclusively detectable by VEMCO receivers, i.e., the Port of Sines' array, Mean tag-to-body-weight ratio = 0.4% (range: 0.2%–0.7%).
[b]Release on 2021-07-07 (year-month-day), last data retrieval on 2024-07-19, receivers (R#) are georeferenced in Figure 1B, Acc.: Acclimatization (T – treatment, C – control).

ensured by COASTNET Infrastructure (https://coastnet.pt/), seven additional acoustic receivers (Thelma Biotel, model TBR800 Release, 69 kHz listening frequency) were deployed and became available to register the presence of the groupers along 30 km from Sines to south, inside the SACVMP (Figure 1A). The first retrieval of detection data logged by the biotelemetry receivers took place after three months of continuous tracking in the Port of Sines. This initial monitoring period was complemented with a manual tracking session, in August 2021. Three additional data retrievals were performed in January and June 2023, and in July 2024.

3 | Results

A total of 216,453 detections of different acoustic signals were logged in the six receivers deployed in the Port of Sines throughout the study period. The total number of detections of each fish in the case of the acclimatized groupers (treatment group) varied between 4 and 134,910 (median = 926.5), while the non-acclimatized groupers (control group) were detected between 7 and 114 times (median = 24). The number of days with detections in the treatment group varied between 1 and 430 (median = 16), which was statistically different from the control group, with only 1 to 3 days with detections (median = 1) ($U = 46.0$, $p = 0.040$). Four groupers of the control group (60%) were detected in a single day only, and by the fifth day after the release, no further groupers of the control group were detected (Table 1, Figure 1C,F). Six groupers out of the eight (75%) from the treatment group were detected in the release area, i.e., the Port of Sines, until five months after the release, two of them (25%, groupers G4 and G15) near the fish farm cages where they were acclimatized and released (receivers R1 and R4, Figure 1B). Ten months after the release, 50% ($n = 4$) were still detected in the release area (Table 1, Figure 1C,F). One of the groupers (G4) of the treatment group was still detected after two years of monitoring

(until July 2023) in the receiver R5 (Figure 1B), where it seems to have set residency since this receiver continuously detected it for more than a year. Another grouper (G5) of the treatment group was recaptured by a fisher almost one year after the release also near to the receiver R5 (June 2022), while one of the groupers (G8) of the control group was caught about 16 km south, six months later (December 2022) (Figure 1E). Both groupers were caught by professional fishers with baited traps.

4 | Discussion

The rapid escape of translocated animals from the release areas is a very commonly observed behaviour (Tetzlaff et al. 2019), namely in the case of hatchery-reared fish released in the wild (Uglem et al. 2008; Fairchild et al. 2009; Lino et al. 2009; Ducos et al. 2022; Silva et al. 2022). The results of the present investigation demonstrate that pre-exposing the hatchery-reared dusky groupers to the release environment conditions in a fish farm cage during an *in*-situ acclimatization period of five weeks significantly reduces such dispersive behaviour. It promoted the settlement and the fidelity of the groupers to the release sites, as Silva et al. (2022) hypothesized and as other authors tested with different fish species, with distinct life traits (Creswell and William 1993; Jonssonn et al. 1999; Brennan et al. 2006; Fairchild et al. 2009) or even with different taxa (Tetzlaff et al. 2019). Beyond the obvious difference between both sets of groupers in the present study, the proportion of acclimatized groupers detected over time in the study area (50% after 10 months) largely contrasts with the observed in previous releases of non-acclimatized hatchery-reared dusky groupers (La Mesa et al. 2008; Riede et al. 2017; Silva et al. 2022). In two artificial reefs in the Mediterranean, sighting rates in the release sites ranged between 15 and 20% one month after the release, while some groupers were recaptured more than 10 km away (La Mesa et al. 2008). In Brazil, *ca*. 35% of non-acclimatized released groupers were detected by the seventh month of monitoring in the release sites (Riede et al. 2017). In more recent attempts, in the 'Sudoeste Alentejano' and 'Costa Vicentina' Marine Park, all non-acclimatized groupers left the release sites within the first two days and, at least, 40% dispersed more than 10 km (Silva et al. 2022). In the current study, the presence of one acclimatized dusky grouper in the study area after two years indicates the existence of a suitable habitat and, most importantly, that the inability to perceive environmental stimuli useful for the settlement and the establishment of a home range was totally overcome by this grouper. This result reinforces the importance of the acclimatization of the fish in restocking actions, particularly when it aims at restocking a specific protected area, such as in the present case. The observed detection pattern also confirms that 50% of the acclimatized hatchery-reared dusky groupers released were able to survive in the wild for at least 10 months. This reflects a certain resilience of the individuals, probably due to their good body condition at release combined with the acclimatization period that the fish underwent, suitable habitat and human use (fishing) conditions of the release site. The success of the restocking actions does not uniquely rely on the conditioning of the fish, but rather on an integrated and adaptative approach depending on the goals and the species to restock (Kuwada et al. 2004; Støttrup 2004; Bartley and Bell 2008; Trushenski et al. 2010). For instance, the social aspects of the species should also be considered (Kleiman et al. 1986; Brown and Day 2002). The complex social hierarchical system and the territorial behaviour of the groupers involve intense agonistic interactions, most particularly in adulthood (Chauvet and Francour 1990; Zabala et al. 1997; Gerhardinger et al. 2006). Therefore, the release of juveniles instead of adults may help to reduce intraspecific competition and enhance the settlement of the fish in the release sites. However, that would involve greater mortality rates due to a higher vulnerability to predation, which, in turn, implies releasing larger numbers of fish. Thus, the successful restocking of the SACVMP with hatchery-reared dusky groupers may require the release of a high number of juvenile fish, instead of a limited number of adults. The task may be challenging and will require more information on juvenile dusky groupers' behaviour and movements, but is one potential next step to be taken to effectively restore an endangered flagship species on the edge of its distribution range, i.e., the dusky grouper on the southwest coast of Portugal.

4.1 | Implications for Conservation

Despite the local success of specific conservation measures taken in recent decades, such as a ban on spearfishing in part of the species' range and the creation of Marine Protected Areas (MPAs) with no-take areas, the dusky grouper remains in decline. Extending these measures to the entire range of the species would be certainly an important step towards the reversal of this trend. Nevertheless, additional conservation actions may be necessary in very depleted areas. In such cases, restocking the MPAs with hatchery-reared specimens is a promising solution. Not only could it restore dusky grouper populations in no-take areas, but it could also promote spillover into adjacent areas. However, to be effective, it would be necessary to ensure that sufficient numbers of hatchery-reared specimens settle within the protected areas. Given that hatchery-reared fish tend to disperse rapidly once released into the wild, this study, with unparalleled results on this species, describes a method of promoting settlement of released groupers (i.e., by submitting the fish to an *in-situ* acclimatization extended period). It will therefore contribute to the future success of restocking programs throughout the dusky grouper's range, as a complementary tool to fishing restrictions and MPA designation, and thus contributing to the conservation of the species. Furthermore, the results of this study can be applied to other epinephelids and other reef species with similar life history traits and conservation status.

Author Contributions

Conceptualization, A.F.S., B.R.Q., J.L.C and P.R.d.A.; methodology, A.F.S., B.R.Q., J.L.C., P.R.d.A.; software, A.F.S. and B.P.; formal analysis, A.F.S.; investigation, A.F.S., J.P.M., E.P., B.R.Q., T.S., A.C.-M. and P.G.L.; resources, P.P.-F., and B.R.Q.; data curation, A.F.S.; writing – original draft preparation, A.F.S. and B.P.; writing – review and editing, A.F.S., J.L.C., E.P., J.P.M., B.P., J.J.C., T.S., P.G.L., A.C.-M., P.P.-F., P.R.d.A. and B.R.Q.; visualization, A.F.S. and B.P.; supervision, B.R.Q., P.R.d.A., J.L.C.; funding acquisition, B.R.Q., J.L.C. and J.J.C. All authors have read and agreed to the published version of the manuscript.

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Ethics Statement

Handling, tagging procedures and the release of fish in the wild were performed under the approval of the Institute for Nature Conservation and Forests (Portuguese: Instituto da Conservação da Natureza e das Florestas, I.P.) in the scope of MARSW project (Licence Nr. 338/2021/CAPT).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Datasets analysed in this study are available through the European Tracking Network data management portal - https://www.lifewatch.be/etn/.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.