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Let them go free: Spatial behaviour following the experimental release of hatchery-reared juveniles *Sciaena umbra* and *Dentex dentex*

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ABSTRACT

As marine stocks and biodiversity are rapidly declining, the release of hatchery-reared individuals into the wild is a species conservation measure aiming at replenishing stocks of freshwater, anadromous and marine fish species. The aim of this study is to assess for the first time the post-release exploration behaviours, survival and dispersal in the wild of released hatchery-reared juveniles of *D. dentex* and *S. umbra*. For this purpose, twenty fish from each species were tagged with acoustic transmitters and released in a marine no-take zone located in Corsica (France). Most individuals were considered alive until their last detection (95% of *S. umbra* and 74% of *D. dentex* detected individuals). Fish were detected within the receiver array on average up to 11.3 (± 20.7) and 10.3 (± 10.3) days after being released for juveniles of *D. dentex* and *S. umbra*, respectively. *Dentex dentex* juveniles dispersed mostly southward along the shore whereas *S. umbra* juveniles tended to disperse along the shore both northward and southward from the acoustic receiver array. Before their rapid dispersion (*i.e.* within a few days) outside the studied zone, juveniles of both species displayed increasing cumulative MCP areas and increased their vertical niche revealing their gradual coverage and exploration of deeper habitats in the area. The results from our study highlighted that hatchery-reared juveniles of both species did not settle inside the studied area but seemed to start to acclimate to the natural environment. This work is a first step towards the assessment of the viability of restocking for both species.

1. Introduction

The Mediterranean Sea is a rich marine ecosystem, where a wide range of endemic and emblematic species dwell, making this area a biodiversity hotspot (Coll et al., 2010). The biodiversity richness of this geographical region suffers, however, from severe anthropogenic impacts (e.g. pollution, fisheries pressure). The latter notably has caused habitat loss and led several marine species becoming threatened (Coll et al., 2010, 2012). For example, ten species of fish belonging to Osteichthyes are nowadays considered threatened in the Mediterranean region, as reflected in the IUCN red list (IUCN, 2020).

The release of hatchery-reared juvenile fish for restocking purposes is a species conservation approach that is increasingly used for threatened species (e.g. Brown and Laland, 2001; Esquivel-Muelbert et al., 2018; Taylor et al., 2017). This ex situ conservation strategy has already been applied to numerous freshwater or anadromous fish species (e.g. salmonids, sturgeon) but is not a common practice in the marine environment (Brown and Day, 2002). However, as stocks of most marine fish species are declining (Dulvy et al., 2003) and aquaculture of many species is developing (Lorenzen et al., 2013), this conservation tool is becoming an interesting option to consider for marine fish species. This tool should be used with the scientific certainty that the wild populations will not be negatively impacted. Therefore, several parameters such as genetic diversity, parasites or diseases must receive particular attention in hatchery-reared stocks before their release into the wild.

Despite the real relevance and interest of this conservation measure, it is currently controversial, mainly in regards to the high mortality rate of hatchery-reared individuals after their release in the wild (e.g. Leber, 2011; Olla et al., 1998).

Recently, several pilot studies in the marine environment aimed to assess the viability of releasing hatchery-reared species for restocking purposes (e.g. Lee et al., 2015; Lino et al., 2009).

In the wild, the effectiveness of a release experiment, and more broadly the success of a conservation program, depends on a few main factors that include acclimation, habitat colonization and survival of the released organisms, and in the long-term on their ability to reproduce in

the wild (Brown and Day, 2002; Esquivel-Muelbert et al., 2018). These factors are known to be closely linked to the natural environment in which they are released (i.e. through food availability, predation rate, presence of preferred habitat) and to the released organisms themselves, especially regarding their health, their size-at-release (e.g. Lee et al., 2015) and their behaviours and performances (Esquivel-Muelbert et al., 2018). Hatchery-reared fish health, behaviours and

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performances are individual components known to be strongly influenced by rearing conditions (Braithwaite and Ahlbeck Bergendahl, 2020). However, mastering the larval and juvenile production alone is not sufficient for the success of a species restocking program. All other components influencing the success of a restocking program are rather linked to the release itself, *i.e.* the transitional stage between the rearing environment and the wild with the discovery of this new environment

Acoustic telemetry is an appropriate tool to monitor the movements and the presence in a studied area (whenever the fish is within the range of the receiver) of hatchery-produced fish after their release in the wild (Matley et al., 2022). This tool consists in tracking individuals that were tagged with an electronic transmitter allowing the estimation of their survival (Villegas-Ríos et al., 2020), the assessment of behaviours (e.g. Gualtieri et al., 2013) and dispersal (e.g. Eder et al., 2015). This method has already constituted the experimental strategy of several studies aiming to study hatchery-reared fish behaviour in the wild for

restocking purposes (e.g. Eder et al., 2015; Jordan et al., 2006; Klinard et al., 2020; Lee et al., 2015; Lino et al., 2009; Taylor et al., 2017; Wada et al., 2017). Some studies demonstrated acclimation to the natural environment and even settlement (e.g. Taylor et al., 2017), quick dispersion (e.g. Klinard et al., 2020), difference of dispersion patterns between sizes-at-release (e.g. Lee et al., 2015) and some behavioural differences between wild and hatchery-reared juveniles (e.g. Lino et al., 2009).

The brown meagre, *Sciaena umbra* (Linnaeus, 1758), and the common dentex, *Dentex dentex* (Linnaeus, 1758), are two coastal and demersal marine fish species, respectively from the Sciaenidae and Sparidae families, that are found in the Mediterranean Sea. Both species are currently assessed as "Vulnerable" in the Mediterranean region on the IUCN Red List of threatened species (Bizsel et al., 2011a; Bizsel et al., 2011b). Along Corsican coasts (France), *D. dentex* is among the five most targeted species by fishing nets and bottom longlines of small-scale fisheries (Bousquet et al., 2022). It is also highly targeted by recreational fishery especially trolling by boatfishing (Marengo et al., 2015). However, management policies such as the implementation of Marine Protected Areas (MPAs) can positively affect *D. dentex* stocks (Marengo et al., 2014). MPAs have also been described as effective for *S. umbra* (Di lorio et al., 2020) whose stock abundance is declining in the Mediterranean region (Abdul Malak et al., 2011). It is for this reason that this latter species is currently subject to a moratorium on recreational fishery in French Mediterranean waters (2013–2023).

Some field studies have already investigated *D. dentex* and *S. umbra* behavioural ecology of wild adult individuals through the use of passive acoustic telemetry (e.g. Alos and Cabanellas-Reboredo, 2012'; Aspillaga et al., 2017, 2019; Brazo et al., 2021; Picciulin et al., 2005). They emphasized that the common dentex presents preferential depths linked to the location in depth of the thermocline (Aspillaga et al., 2017) and displays a gregarious behaviour pattern during the breeding period (Aspillaga et al., 2019). Regarding *S. umbra*, wild adult individuals are rather sedentary with a limited space use (Alos ' and

Cabanellas-Reboredo, 2012) and display vertical movements in relation with the seasons (*i.e.* deeper depths visited in the cold season and shallower depths visited in the warm season; Brazo et al., 2021). Two studies assessed the dispersal capacity of *S. umbra* individuals, and presented conflicting results potentially due to the release method used. A very low dispersal tendency was recorded when four wild individuals were released at the same site where they were fished (Alos and ´Cabanellas-Reboredo, 2012). However, when two translocated wild individuals of this species were studied, they presented quick dispersal, within a few days, from the release site and, more broadly, from the studied zone (Picciulin et al., 2005). Therefore, it has been estimated that translocation in a new habitat could explain the difference of dispersal in both studies.

Thus, monitoring hatchery-reared and produced juveniles that never experienced living in the wild could be another step towards understanding the ecological behaviour of *S. umbra* and *D. dentex*, in combination with acclimation in a novel environment, which could in turn contribute to assess the viability of restocking programs.

Both species are the subject of breeding programs for potential conservation and restocking purposes (e.g. Chatzifotis et al., 2006; Efthimiou et al., 1994; Glamuzina et al., 1989; Hamzaçebi and Can, 2021; Koumoundouros et al., 2004). However, no experimental study, as far as we know, has focused on hatchery-reared *S. umbra* and *D. dentex* juvenile behaviours for restocking purposes. This study's main aim was to study the short-term spatial and temporal behaviours, movements and habitat use of *S. umbra* and *D. dentex* hatchery-reared juveniles following their release into the wild. This aim was achieved through the use of two different acoustic telemetry methods (a real-time tracking array at a fine spatial scale and a passive acoustic method at a larger spatial scale) in order to monitor the release dindividuals around the release site at different resolutions.

Moreover, as both species are known to display behavioural differences in the wild, this study also aimed to compare the post-release behaviour of both species as they can respond differently to this management action and one species could be more suited to restocking programs. Any difference could lead to adapt the release procedure to each species to reach successful management outcomes.

This experimental survey was a preliminary study testing the viability of releasing *S. umbra* and *D. dentex* hatchery-reared juveniles for restocking and conservation purposes.

2. Materials & methods

The rearing as well as the surgical and experimental methods used on the fish in this study were approved by the French National Ethics Committee for Animal Experimentation (CNREEA).

2.1. Fish rearing and tagging

For each fish species, the individuals used in this study came from the same batch of eggs. *Dentex dentex* and *S. umbra* stocks were hatched in March and in May 2019, respectively. From the juvenile stage, fish individuals were maintained in rearing tanks (open circulation system; volume 4.5 m³) lit by a natural photoperiod and with water at a natural, seasonally fluctuating temperature.

Fish tagging was performed between September and October 2020 for both species, thus on 1-group individuals. Prior to tagging, individuals were individually anaesthetized (Benzocaine, 200 ppm) and a transmitter was then implanted, by a qualified staff member, in each fish's intra-peritoneal cavity *via* a surgical incision. An antibiotic injection was individually carried out after each surgical procedure (Nuflor, 0.1 mL/kg). In order to allow proper healing and to maximise the welfare and survival of tagged individuals, each tagging followed the "2%" rule requiring that a tag must weigh less than 2% of the corresponding fish body weight (Winter, 1983).

Thelma Biotel D-LP7 coded transmitters (W = 1.2 g; L = 21.5 mm; Ø = 7.3 mm) were used for S. umbra individuals and Thelma Biotel AD-

2LP7 transmitters (W = 1.8 g; L = 27.9 mm; \emptyset = 7.3 mm) were used for *D. dentex* individuals. Both types of transmitters emit at a frequency of 69 kHz and have an estimated battery life of 6–7 months. Tags recorded depth and acceleration for *D. dentex* and only depth for *S. umbra*. This choice was guided by the fact that *D. dentex* can be considered as a semi-pelagic species in behaviour, predates pelagic fish species (Morales-Nin and Moranta, 1997) and might display high acceleration values, in contrast to *S. umbra* that is a rather slow swimming fish, often found hiding stationary in rocky caves and that might display small acceleration values.

After tagging procedures, individuals were measured (total length, TL; in cm) and weighed (W; in g).

Following this procedure, fish were maintained at least 15 days within the fish rearing facility to monitor optimal healing and surgery recovery. The water temperature as well as the photoperiod were identical to the natural conditions found at the release site in order to minimize stress. For the same purpose, all fish were not fed for 24 h prior to the transport and release.

Finally, 20 individuals of *S. umbra* and 20 individuals of *D. dentex* were released in the wild after complete healing.

All forty individuals were released on the same site (white star in Fig. 1) but to avoid signal collisions, fish were released in four groups corresponding to distinct days. *Dentex dentex* individuals were released on the 21st of October (n = 10; 598 Days Post-Hatch, DPH) and on the October 22, 2020 (n = 10; 599 DPH). *Sciaena umbra* individuals were released on the 02nd of November (n = 10; 530 DPH) and on the 04th of November 2020 (n = 10; 532 DPH).

The total length of *S. umbra* individuals released in this study ranged between 17.5 cm and 24 cm (mean TL = 21.45 ± 1.55 cm; mean body weight = 155.70 ± 34.94 g). The total length of *D. dentex* individuals ranged between 24 cm and 28 cm (mean TL = 25.80 ± 1.33 cm; mean body weight = 315.00 ± 73.14 g). All released individuals were estimated sexually immature and hence considered as juveniles.

2.2. Study site and acoustic receiver arrays

The study area of this coastal field experiment is located in the northwest of Corsica, France (Fig. 1). This zone is part of a French Marine Natural Park and, at the same time, a no-take zone meaning, among others restrictions, that scuba-diving as well as any type of fishing are prohibited inside the borders of the studied area.

The monitored area in our study is mainly composed of rocky substrate and *Posidonia oceanica* meadows that represent the preferential habitats of both species (*D. dentex*: Marengo et al., 2014; *S. umbra*: Harmelin-Vivien et al., 2015). A total of thirty acoustic receivers (Thelma Biotel; TBR 700 model) were deployed in this area (Fig. 1). The network of receivers allowed a coastal acoustic coverage of approximately 1.5 km², with a distribution of approximately 3 km along the linear coast, ranging between approximately 5 and 50 m depth. The specific localisation where all fish were released (white star on Fig. 1), corresponds to the position of a Thelma Biotel receiver and was approximately 5.5 m depth on a specific rock that could serve as a shelter. In the surrounding area, the network was dense with receivers 50 m apart from each other in order to survey the fine-scale movements around the release site.

In addition, seven floating real-time tracking devices developed by the lab were also deployed around the release site (black diamonds in Fig. 1). This prototype technology enables remote tracking of the fine-scale movements of tagged fish by returning in real-time the fine-scale

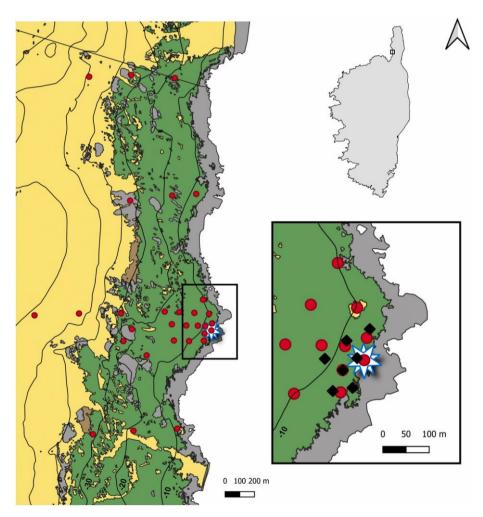


Fig. 1. Acoustic receiver array for this study composed of 30 Thelma Biotel receivers (TBR 700 model; red circles) and 7 real-time fine-scale tracking devices (black diamonds). Background colours represent the substrate type (Andromede Oceanologie, 2014) with sandy substrate (yellow), rocky substrate (grey), dead matte (brown) and Posidonia oceanica meadow (green).

position of fish using Time Difference Of Arrival (TDOA) of the signal and a trilateration algorithm (Manicacci et al. *submitted*). These devices are autonomous in terms of energy supply. This technology was used to document the short-term fine-scale behaviour of tagged fish just after their release.

2.3. Range testing of Thelma Biotel TBR 700 receivers

In order to assess the detection range of Thelma Biotel TBR 700 receivers when using Thelma Biotel D-LP7 and AD-2LP7 transmitters, range tests were carried out *in situ* within the studied coastal area using two receivers and a Thelma Biotel R-LP7 transmitter. This transmitter has the same range of emissions as the D-LP7 and AD-2LP7 transmitters but allows, through a smaller transmitting interval and a counter of the emissions, easier and more reliable assessment of the range of detections. This range testing procedure consisted in linearly immersing the transmitter during 5 min every 10 m moving away from the receiver (detections at 0, 10, 20, 30, 40 and 50 m away from the receiver position), and then every 50 m from 100 to 250 m away (detections at 100, 150, 200 and 250 m away from the receiver position). This range testing procedure has been conducted both along the coastline, with homogeneous depth, and perpendicularly, with increasing depths. Moreover, in both experiments the transmitter was tested at two depths: immersed 5 m from the surface and immersed 1.5 m above the substrate. The area in which this range testing was performed was mainly composed of a rocky substrate with *P. oceanica* patches. Acoustic signals from the TBR 700 transmitter were detected up to 50 m away from the tested receiver. A detection frequency of 50% was located up to 25 m away from the tested receiver.

Similar range tests were conducted for the real-time acoustic tracking system and provided slightly larger detection ranges with 50% of detections recorded at about 50 m from the device. Therefore, the real-time acoustic devices had overlapping detection ranges allowing positioning using TDOA inference.

2.4. Data analysis

Using the acceleration and depth data, we were able to estimate the survival of each individual during the time they were present in the array (Villegas-Ríos et al., 2020). Indeed, individuals that displayed no vertical movements and, for *D. dentex*, no variations in acceleration values (*i.e.* with a continuous emission of 0 cm s⁻¹ values or continuously emitting the same value) were considered dead. Careful consideration has been also given to aberrant behaviours (*e.g.* abnormally high swimming speeds, net shift in the pattern of displacements), that would reflect a case of predation and therefore natural mortality. All the analysis described below (*i.e.* on dispersal, exploration behaviour or spatial behaviour) were completed only over the time each individual was estimated alive.

In order to focus on the exploration behaviour of the two species, cumulative minimum convex polygon areas (MPC areas; in km²), Kernel utilization distributions (KUDs), acceleration (in m.s⁻²) and depth (in m) were investigated. Given that the number of detections displayed a strong decrease through time likely due to rapid dispersal out of receiver range, it was decided to sub-sample those variables in order to build reliable models. Thelma Biotel receiver detections were thus sub-sampled until 325 h after release (i.e. approximately 14 days post-release) for those variables, after which time threshold the number of detections dropped dramatically making robust modelling difficult. Therefore, the following models and statistical analyses based on the TBR 700 detections were performed during this period. For each species, MCP areas were cumulated per day for the Thelma Biotel network (or per hour for the real-time tracking system) in order to obtain a measurement of the total area gradually explored by an individual. MCP areas and KUDs are complementary methods for studying habitat use where the MCP area represents the area in which all detections of the animal were recorded and the KUDs represent the probability of finding an animal within a given surface area (Marshell et al., 2011).

For Thelma Biotel receiver detections, cumulative MCP areas and KUDs were calculated from the localisation of centres of activity (COA) computed for each 15 min interval (Simpfendorfer et al., 2002) using the 'ATT' functions (Udyawer et al., 2018) in the VTrack package in R (Campbell et al., 2012). MCP areas ('mcp.area' function) and KUDs ('kernelUD' function) were computed using the adehabitatHR R library and were calculated per day post-release for detections. The KUDs were estimated for each species (all individuals combined) and were depicted on a map for a geographical overview.

The real-time tracking devices also enabled individual measurement of the cumulative MCP areas directly on the positions returned from the system but, as the spatial grid is much smaller than the Thelma Biotel receiver array resulting in rapid dispersal outside the array, the calculation was made per hour post-release and was examined until a maximum of 10 h post release.

We used two Linear Mixed-Effects Models (LMMs) using the 'Imer' function from the *Ime4* R library in order to investigate whether the two species displayed significantly different cumulative areas explored (100% MCP) using both the Thelma Biotel receiver array and the real- time tracking devices array (with individual and time as random effects). Both models were performed on square-root transformed values.

All individual acceleration (for *D. dentex* individuals) and depth data (for both species) were averaged per hour leading to individual-specific hourly values. With these data, Generalized Additive Mixed Models (GAMMs) were used to investigate, in these two species, the effect of time post-release (in hour) on acceleration and depth variables. Those models were constructed with fish ID as a random effect and using a Gaussian family argument. For each model used, normality and homogeneity of variance assumptions were visually checked.

In order to study dispersal, the localisation of the last detection of each individual was used. This geographical coordinate was thus considered as the site where the fish dispersed outside the Thelma Biotel receivers array. All last individual locations allowed calculation of an angle of dispersal between the release site and the exit site. A non- parametric Watson's U² test was used (*circular* R library) on all these angular data in order to determine whether the two species presented a significant difference in dispersal distributions. This statistical test is the most widely performed when aiming to assess differences in angular distributions between two samples (Landler et al., 2021).

All data analyses used in this study were conducted *via* the R software (version 4.1.1) and all statistical analysis were considered with the significance threshold of 5%.

3. Results

All the 40 released juveniles, except one *D. dentex* individual, were detected by the Thelma Biotel receivers. Out of 20 *S. umbra* juveniles, nineteen individuals were considered alive until their last detection (95% of the twenty tagged fish). Out of 19 *D. dentex* juveniles, fourteen individuals were estimated alive until their last detection (74% of the nineteen detected individuals). For each fish, all the analyses were conducted for the period they were considered alive.

Dentex dentex juveniles were detected until a mean of 11.3 (± 20.7 SD; range: 0 to 90) days post-release. Sciaena umbra juveniles last detections corresponded to a mean of 10.3 (± 10.3 SD; range: 0 to 35) days post-release.

Some of these variables are individually presented in Appendix A.

3.1. Exploration inside the studied area

Even though inter-individual variation was present in both species, species-specific exploratory patterns were observed (Fig. 2). During the first hours after release, cumulative MCP areas estimated by the real-

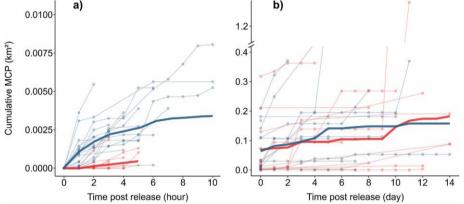
Fig. 2. Cumulative MCP areas of *Dentex dentex* (<u>blue</u>) and *Sciaena umbra* (<u>red</u>) through time, calculated using the detections of (a) the real-time tracking devices and (b) the Thelma Biotel receivers. Thin lines represent individual cumulated MCP areas whereas the opaque lines represent the mean cumulative MCP areas per day for each species.

time tracking devices significantly differed between species (LMM, F = 32.1, P < 0.001) with D. dentex displaying significantly higher values than S. umbra juveniles (Fig. 2a).

The real-time tracking devices allowed measurement of the estimation of the high-resolution fish horizontal movements (through trilateration), revealing that *D. dentex* individuals seemed to be more active and explore wider areas around the release site than *S. umbra* during the first hours post-release (Appendix B).

However, when looking at the first 14 days after release, both species displayed no significant differences in cumulative MCP area patterns (LMM, F = 0.98, P > 0.05) for detections from Thelma Biotel receivers (Fig. 2b).

In conjunction with the increase of the cumulative MCP areas of the released juveniles through time, KUDs showed changes in space use between days (Fig. 3) for both species. The first 24 h (referred to as "Day 0" in Fig. 3) showed intensive use of the area around the release site (in the centre of the receiver array).



As the days passed, *D. dentex* juveniles tended to preferentially use the southern part of the array and, conversely, the *S. umbra* juveniles tended to use the northern side of the array.

In addition, depth and acceleration values were used to study the overall exploratory behaviour during the first 325 h post-release.

Both species showed a significant relationship between depth and time post-release (GAMM, *P* < 0.001; Fig. 4). The depth used by fish tended to increase through time with the use of deeper habitats from about three days post-release, until reaching approximately 20 m depth at the end of the monitoring period. Moreover, *D. dentex* show a declining trend in the swimming

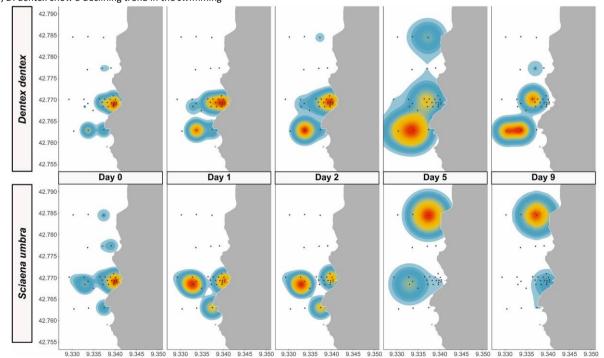


Fig. 3. Kernel utilization distributions (KUD) per day after release for each species using Thelma Biotel receiver detections. The darkest red colour stands for 5% KUD, the yellow colour stands for 50% KUD and the brightest blue colour stands for 95% KUD. The black dots represent the localisation of all Thelma Biotel receivers used in this study.

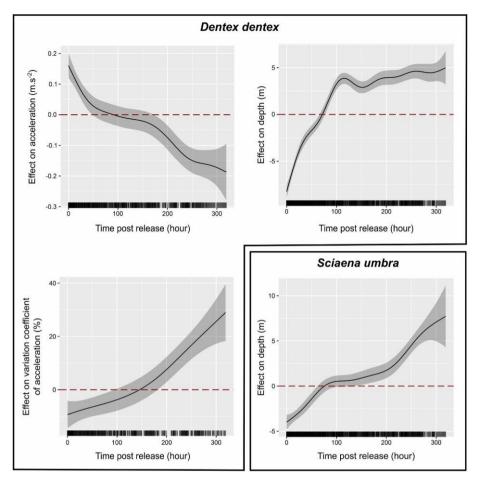


Fig. 4. Effect of time post-release (hour) on *Dentex dentex* and *Sciaena umbra* behavioural variables (acceleration and coefficient of variation (CV) of acceleration for *D. dentex* and depth for both) from Thelma Biotel receiver acoustic detections. Results of GAMMs on both species. All y-axes represent the standardized residuals from the corresponding GAMM. Black lines represent the smooth terms. The grey bands represent the 95% confidence intervals around the black line models.

acceleration values (GAMM, *P* < 0.001; Fig. 4) after release but an increase of the coefficient of variation of acceleration values (GAMM, *P* < 0.001; Fig. 4). This

indicates that if average acceleration decreased, the fish may have gradually shifted from high activity behaviour to behaviour characterised by a large number of resting phases and few periods of bursts of activity. 3.2. Dispersion outside the studied area

As the days passed and the juveniles explored the studied area (Figs. 2 and 3), number of detections decreased. For the most part, the last detections of individuals were localized on the periphery of the Thelma Biotel receiver array suggesting that these fish started to explore outside the monitored area and never came back.

Dentex dentex juveniles showed the majority of the last detection angles directed southward (between 180 and 270° from the release site; Fig. 5a) leading to a median value inferior to that of *S. umbra* individuals (median of *S. umbra* angular data = 263.33°; median of *D. dentex* angular data = 244.66°; Fig. 5b). Sciaena umbra juveniles indeed seemed to be detected for the last time either northward (with 9 individuals having a last detection localized between 315 and 45° from the release site) or in the same direction as *D. dentex* individuals. Both species showed significant different angular distributions (Watson's U², P < 0.01). Note too that locations between 0 and 180° from the release site were difficult to reach since it corresponded to the shore (Figs. 1 and 5).

4. Discussion

Acoustic telemetry has been used since the 1950s (Hockersmith and Beeman, 2012) to remotely monitor the horizontal and vertical movements of fishes in their natural environment and is an effective tool allowing the study of the relationship between an organism and its environment through its horizontal and vertical movements (Aspillaga et al., 2019).

We conducted a preliminary investigation of the behaviour of hatchery-reared juveniles of two targeted emblematic fish species in order to assess the potential of release programs for restocking local populations in Corsica. This study used two combined acoustic telemetry methods to study movements of hatchery-reared *S. umbra* and *D. dentex* juveniles at a fine spatial scale immediately after release (during the first hours) as well as at a larger spatial scale during the first days after their release. This monitoring revealed that individuals of both species seemed to increasingly explore the studied area while they tended to progressively swim into deeper habitats with decreasing acceleration values (for *D. dentex*). They also tended to quickly disperse outside the studied area with a differentiation of preferential explored areas between both species.

4.1. Survival

In general, *S. umbra* and *D. dentex* juveniles were not detected by acoustic receivers for a long time after their release, on average not more than 10 and 11 days post-release for *S. umbra* and *D. dentex*, respectively. Some individuals of both species were even lost after a few hours post-release and were not detected anymore from the day after their release. The very short monitoring period made the estimation of the survival of these individuals difficult as it required distinguishing between dispersal and mortality to explain the loss of detection. However, post-release mortality of tagged fishes often occurs over the first hours

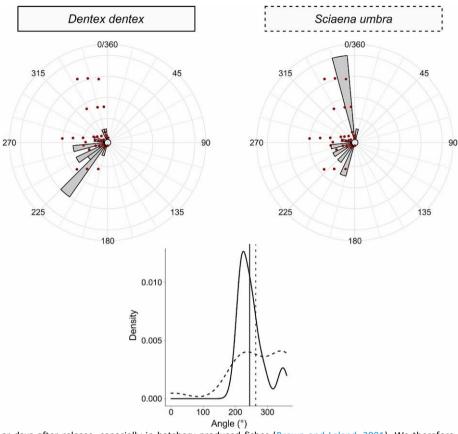


Fig. 5. (a) Angular distributions (in degrees) between individual last detection for live *Dentex dentex* and *Sciaena umbra* juveniles and the release site. The y axis (visualized through the length of the bars) represents the number of individuals with the same direction within a 10° range. The red circles represent the localisation of all Thelma Biotel receivers used in this study. **(b)** Density distribution of individual direction of the last detection for *Dentex dentex* (solid line) and *Sciaena umbra* (dashed line) juveniles. Vertical grey lines represent the median value for each species (*Dentex dentex*: solid line; *Sciaena umbra*: dashed line).

or days after release, especially in hatchery-produced fishes (Brown and Laland, 2001). We therefore relied on several behavioural parameters to estimate mortality (Villegas-Ríos et al., 2020) to infer if and when mortality occurred, and then filter our data accordingly to monitor fish behaviour. Our findings emphasized a good survival rate, as the majority of the released individuals detected (i.e. 74% of D. dentex and 95% of S. umbra) were estimated alive until their last acoustic detection. The high loss of detection, in the Thelma Biotel receiver array, occurring several days after release is thus most likely due to dispersal outside the monitored zone, that does not allow estimation of survival over a longer term.

Hatchery-reared juveniles are usually more susceptible than wild individuals to die after being released in the wild (Larocque et al., 2020). Hatchery-reared individuals are naïve concerning predation pressure, as they have never encountered or been chased by a predator, and concerning natural feeding, as they are usually fed with dry pellets or other inert food during their captivity. Yet predation avoidance and feeding efficiency are two essential requirements for the survival of individuals in the wild. Therefore, our estimated survival rate is quite promising as mortality after release in the wild is generally estimated to be high for hatchery-reared fish (e.g. more than 50% of mortality for Salmonidae fish after a few days post-stocking; Klinard et al., 2020).

In restocking programs, individuals used are usually at the juvenile stage. Juvenile individuals display a higher survival rate than larvae in the natural environment and are kept less long in captivity than adult individuals, which is valuable to increase their capacity to adapt to the wild (Philippart, 1995). Wild D. dentex individuals from the western Mediterranean (Mallorca) showed the occurrence of 50% maturity at approximately 34 cm total length (Grau et al., 2016). In the same geographical region, S. umbra individuals were estimated as having their first sexual maturity at around 20–21 cm standard length (Chakroun-Marzouk and Ktari, 2003). Previous unpublished work on S. umbra juveniles enables an estimation of the standard length (SL) of our individuals on the basis of the measured

TL (SL in mm = $0.7878 \times TL - 1$, 1365; $R^2 = 0.9974$). A total length of 24 cm might correspond to a standard length of 18.8 cm, which is the length of the largest *S. umbra* individuals in this study. Therefore, all released individuals in this study were assumed to be sexually immature and hence considered as juveniles. However, in restocking programs, sizes-at-release for finfish juveniles are preferentially small, mostly between 3 and 10 mm TL (*e.g. Li*, 1999; Masuda and Tsukamoto, 1998), and are obviously smaller than the body sizes used in this work. It must be kept in mind that individual sizes- and ages-at-release are known to potentially affect post-release survival (Willis et al., 1995) and colonization (Lee et al., 2015) of hatchery-reared fish. Therefore, the fish used in this study would not be subjected to the same predation pressure as fish of the body size used for usual restocking.

4.2. Exploration of the studied zone and acclimation to a new and natural environment

A few hours after being released in the wild, juveniles of both species moved away from the area surrounding the release site (confirmed by the fine-scale real-time tracking array). Only a few individuals were still detected by the real-time tracking devices located directly around the release point after 5 h post-release whereas the other ones progressively explored the whole studied area.

Both species have been shown to differently explore their novel environment during the first hours: *D. dentex* individuals displayed a higher cumulative rate of MCP areas and were detected for a longer time by real-time tracking devices than *S. umbra*. Moreover, after release,

D. dentex seemed to be more active than S. umbra around the release site.

This could be a display of *D. dentex* stress-induced behaviour just after release, driving these individuals to frenetically swim around the release site. This stress-induced behaviour is less noticeable for *S. umbra* individuals.

Then, as *D. dentex* individuals moved away from the release site and were studied through the Thelma Biotel receiver array during the first days after release, the difference in cumulative MPC area patterns between the two species disappeared. This could be a sign that *D. dentex* started to display a more natural exploratory behaviour after several hours, through the decrease of stress-induced behaviours. This hypothesis is supported by *D. dentex* acceleration data demonstrating that average acceleration decreased through time but variation in acceleration increased through time. Thus, these data suggest that fish transited from a stressed state, characterized by continuous high activity, to a more natural behaviour with alternating resting and active phases (which might include predator avoidance and foraging), as individuals progressively acclimated to their novel environment. The alternation between those resting and active phases does not support the hypothesis of the exhaustion of the individuals who finally might have slowed down their active food search.

As fish progressively moved away from the release site (5.5 m depth), both species seemed to explore deeper habitats. Wild juveniles of the two species are usually found in shallow depths (*S. umbra*: Grau et al., 2009; *D. dentex*: Marengo et al., 2014). *Sciaena umbra* is generally found within the first 20 m depth. For instance, Harmelin and Ruitton (2007) suggested that *S. umbra* with body sizes between 15 and 35 cm (size range of our fish) were most frequently observed in 2–23 m depth. Moreover, a recent study using a passive acoustic method on adult *S. umbra* (39.7 ± 7 cm TL) highlighted the fact that, during the cold months of the year (during which our study was carried out), wild individuals were found at a mean depth of 22.5 m (Brazo et al., 2021). For *D. dentex*, wild individuals show preferential depths according to body size with the smaller juveniles inhabiting shallow depths (fish below 15 cm length mostly found down to 10 m depth; Chemmam-Abdelkader, 2004) and intermediate size fish mostly found between 15 and 50 m depth (Chemmam-Abdelkader, 2004; Marengo et al., 2014). Therefore, increasing depths of the hatchery-reared juveniles in this study, with individuals detected between 15 and 25 m depth after a 300 h period following release, indicates that the individuals who have always lived in shallow tanks and under controlled rearing conditions may have slowly adopted a natural pattern of behaviour and habitat use

Previous acoustic telemetry studies also investigated acclimation and settlement processes of hatchery-produced fish after their release in the wild (e.g. Kawabata et al., 2007; Taylor et al., 2017a,b; Yokota et al., 2006). For instance, hatchery-reared Coregonus hoyi individuals were monitored at depths similar to findings on wild individuals of this species, indicating that these individuals displayed a natural habitat use some hours after release (Klinard et al., 2020). In addition, Yokota et al. (2006) showed that hatchery-reared fishes from the Malacanthidae family seemed to settle after 10 days in the wild. Using acoustic telemetry, settlement is often estimated when horizontal movements are reduced and when habitat use and spatial niches become narrower. In our study, the juveniles did not seem to settle as their cumulative MCP areas increased until the end of the detection period without reaching an obvious plateau. Thus, this indicates that fish did not settle in the studied area but continued to explore and likely quickly dispersed after their release. However, as previously mentioned, the gradual exploration of deeper habitats, that is consistent with the preferences of these species, and the decrease of *D. dentex* acceleration values suggesting a decrease in stress, could be considered as the first stage of an acclimation period. It is thus likely that juveniles started to acclimate to their natural environment during this period but that the exploration phases before settlement in a stabilised home range would require a longer period and a larger monitored area (i.e. larger network of receivers).

In the wild, the two species tend to display differences in ecological behaviours. For instance, *D. dentex* is known to be, at least for body sizes over 20 cm TL, a piscivorous predator (Morales-Nin and Moranta, 1997) mostly observed swimming in open water, whereas *S. umbra* feeds essentially on benthic crustaceans (Fabi et al., 1998) and is generally observed swimming close to the substrate or hidden in rocky crevices. Despite the differences in behavioural traits, both species quickly dispersed outside the studied area in our study. This rapid dispersal and the extensive horizontal movements of individuals could potentially be linked to the carrying capacity of our studied area. It should be recalled that the studied area is part of a MPA (where any type of fishing is notably forbidden) which could have potentially led to an increase of biomass and abundance of organisms as has already been observed in other protected zones (*e.g.* Edgar et al., 2014; Rodwell et al., 2003). This could thus be another potential factor causing hatchery-reared fish to disperse.

4.3. Dispersion outside the monitored area

Even if they were released at exactly the same spot, the two species clearly showed different strategies in the way they explored their new environment. Juvenile *S. umbra* explored the area northward while *D. dentex* mainly directed their exploration southward.

As *D. dentex* and *S. umbra* juveniles were not released on the same day (*D. dentex* at the end of October and *S. umbra* at the beginning of November), the directions of emigration outside the studied area could be linked to different meteorological and environmental conditions. For example, Klinard et al. (2020) explained their hatchery-reared fish post-release movements to probably be related to the currents prevalent in the area. In the present study, the choice of dividing the number of juveniles to be released between four release days was designed to minimize potential signal collisions leading to data loss (Melnychuk, 2012). Moreover, it has been shown through acoustic telemetry that release density can have an impact on fish dispersal with *Argyrosomus japonicus*, a Sciaenidae fish species, showing a higher tendency to disperse faster and further when they are released at a higher density (Taylor et al., 2013).

Alternatively, the differences in dispersal patterns found in our study could reflect differences in habitat preferences in the two species. For example, numerous detections of *S. umbra* juveniles were obtained on two receivers located approximately at a 270° angle from the release site, corresponding to the location of a big rock. During days 1 and 2, *S. umbra* tended to substantially explore this specific area as this rock might probably provide places to hide for a species that is known to take refuge inside holes in rock formations (Alos and Cabanellas-Reboredo, ´2012). As the studied zone is entirely composed of a mixture of *Posidonia* meadows as well as sandy and rocky substrates and the dispersion distributions only give us movement tendencies, it is difficult to reach a conclusion for both species regarding dispersal patterns linked to the colonization of a specific habitat. *4.4. Considerations for population restocking*

Our findings allow better understanding of the short-term movements, spatial behaviour and habitat use of *D. dentex* and *S. umbra* hatchery-reared juveniles released in the wild. They revealed that these juveniles displayed good short-term survival rates, which is a positive outcome for restocking purposes. Moreover, these hatchery-reared juveniles seem to start acclimating to the natural environment and thus showed promising preliminary results for restocking.

However, in the present study, the low number of daily detections did not allow examination of foraging or escape behaviours of *D. dentex* in detail through their acceleration values. Foraging is a critical parameter for survival, especially for fish born in captivity that will have to find food in the wild. In the future, it

could be useful to study the foraging performances of *D. dentex* and *S. umbra* hatchery-produced juveniles which have only been fed with dry pellets in captivity, except from the larval stage that required live prey.

Future *in situ* experiments could also benefit from a finer detection coverage in order to obtain a greater number of detections. The small size and associated low power signal output of the Thelma Biotel LP7 transmitters used in this rocky area resulted in short transmission distances and therefore low detection efficiency. In order to cover a sufficiently wide area around the release site, the receivers were deployed in a relatively dense network directly around the release site but as we move away from this location the density of receivers decreased (Fig. 1). Thus, even if this study allowed the detection of the studied juveniles over a wide area, a denser receiver array could have increased detection rate and monitor fish movement and behaviour at high resolution. Moreover, it could be interesting to assess long-term dispersion and survival data of the released individuals in the wild, our study monitoring period being limited by the short battery life of our small tags. It could thus be very informative to conduct a parallel study aiming to use a capture-recapture method in order to include long-term information in the estimation of dispersion and survival of hatchery-reared *D. dentex* and *S. umbra* juveniles.

Another work, performed by the same lab team, consisted in the release of small juveniles of the two species tagged with fluorescent elastomer tags (Northwest Marine Technology), and highlighted the fact that those juveniles tended to quickly disperse after release and were thus rarely observed again in the area (Ducos, unpublished data), confirming the results from the present study. Ultimately, to ensure successful conservation replenishment programs, future studies would need to investigate whether released fish are able to feed and reproduce in the wild.

Our findings complement the currently limited knowledge with the aim of better understanding behaviours and performances of hatchery- reared juveniles in the wild for conservation purposes. Through the use of passive acoustic telemetry, post-release movements of released individuals were studied to investigate the potential effectiveness of such restocking programs. Highlighting post-release survival, movements and behaviours of hatchery-produced individuals of the two species is a first step when trying to assess the viability of using those juveniles for restocking purposes and can thus be considered as a first pilot study with the aim of achieving future larger-scale restocking programmes of juvenile hatchery-reared *D. dentex* and *S. umbra*.

Author statement

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Declaration of competing interest

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

Data availability

Data will be made available on request.

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Individual biometry, survival and detections (through Thelma Biotel receivers) information on each of forty released juveniles. The Survival column indicates the individuals that were estimated to be alive until the last detection ("Yes"), the individuals that were estimated dead before the last detection ("No") and the individuals that were complexly identifiable as dead or alive ("?"). One juvenile (i.e. 20352705 ID Tag) was not detected by the Thelma Biotel receivers and was thus noted "-" in the Survival column. The assessments of the Total period of post-release detection (i.e. number of 24 h between the release and the last detection; in days) and the Total of monitoring post-release days were carried out only over the time each individual was estimated alive. The Residence Index (RI column) was calculated as the total of monitoring post-release days divided by the total period of post-release detection (in days).

Species	ID Tag		Total length (cm)	Weight (g)	Release yyyy)	day (dd/mm/	Survival	Total period of post-release detection (days)	Total of monitoring post- release days	RI
Sciaena 2035	2681	20.5	142	02/11/2021	'es	13	4	0.3077 umbra		

Sciaena 20352682	20.5	126	02/11/2021 Ye	es :	15	4	0.2667 umbra
Sciaena 20352683	23	190	04/11/2021 Ye	es :	1	1	– umbra
Sciaena 20352684	22.5	180	04/11/2021 Ye	es :	1	1	– umbra
Sciaena 20352685	22	168	02/11/2021 Ye	es :	24	3	0.125 <i>umbra</i>
Sciaena 20352686	21	144	02/11/2021 Ye	es :	1	1	– umbra

(continued on next page)

Species	ID Tag	Total length (cm)	Weight (g)	Release day (dd/m yyyy)	m/ Survival	Total period of po (days)	st-release detection Total of monitoring po days	st- release RI
Sciaena	20352687	21	136	04/11/2021	Yes	13	2	0.15
umbra Sciaena	20352688	21	128	04/11/2021	Yes	1	1	-
umbra Sciaena	20352689	21	148	02/11/2021	Yes	1	1	-
umbra Sciaena umbra	20352690	19	94	04/11/2021	Yes	5	5	1
Sciaena umbra	20352691	23	174	04/11/2021	Yes	7	7	1
Sciaena umbra	20352692	20.5	124	04/11/2021	Yes	1	1	-
Sciaena umbra	20352693	24	222	02/11/2021	Yes	15	6	0.4
Sciaena umbra	20352694	21.5	176	04/11/2021	Yes	9	2	0.22
Sciaena umbra	20352695	21.5	166	02/11/2021	Yes	30	18	0.0
Sciaena umbra	20352696	21.5	156	02/11/2021	Yes	1	1	-
Sciaena umbra	20352697	22.5	198	02/11/2021	Yes	9	2	0.22
Sciaena umbra	20352698	24	202	04/11/2021	Yes	35	12	0.34
Sciaena umbra	20352699	21.5	154	02/11/2021	?	NA	NA	N.
Sciaena umbra	20352700	17.5	86	04/11/2021	Yes	13	10	0.76
Dentex dentex	20352701	24	230	22/10/2021	No	3	3	1
Dentex dentex	20352703	24	230	22/10/2021	Yes	1	1	-
Dentex dentex	20352705	25.5	264	22/10/2021	-	NA	NA	N
Dentex dentex	20352707	24.5	252	21/10/2021	Yes	1	1	-
Dentex dentex	20352709	24.5	294	22/10/2021	?	1	1	-
Dentex dentex	20352711	27.5	338	21/10/2021	Yes	12	11	0.93
Dentex dentex	20352713	28	428	21/10/2021	No	15	14	0.93
Dentex dentex	20352715	27	320	22/10/2021	Yes	1	1	-
Dentex dentex	20352717	25	214	22/10/2021	No	7	7	1
Dentex dentex	20352719	25	338	22/10/2021	Yes	90	11	0.12
Dentex dentex	20352721	25,5	228	22/10/2021	Yes	1	1	-
Dentex dentex	20352723	25	254	21/10/2021	Yes	8	7	0.8
Dentex dentex	20352725	28	456	21/10/2021	?	10	10	1
Dentex dentex	20352727	27	328	22/10/2021	Yes	7	4	0.57
Dentex dentex	20352729	25	324	22/10/2021	Yes	11	8	0.72
Dentex dentex	20352731	26	368	21/10/2021	Yes	1	1	-
Dentex dentex	20352733	25	294	21/10/2021	Yes	1	1	-
Dentex dentex	20352735	25	304	21/10/2021	Yes	1	1	-
Dentex dentex	20352737	27,5	410	22/10/2021	Yes	34	9	0.26
Dentex dentex	20352739	27	426	21/10/2021	Yes	10	6	0.

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