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SHORT COMMUNICATION

Preliminary observations of nursehound *Scyliorhinus stellaris* (Linnaeus, 1758) spawning areas in coastal waters of Tuscany, Italy

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Abstract: In the circalittoral zone of the Mediterranean Sea, the nursehound *Scyliorhinus stellaris* lays eggs in spawning areas located at depths between 25 and 50 m in the red gorgonian meadows. Given the evident decline of the nursehound population in the Mediterranean and its high vulnerability, investigating spawning and potential nursery areas is crucial for the conservation of this species. Using non-invasive techniques such as diving activities with the help of R.O.V. (*Remote Operated Vehicle*), spawning areas have been identified along the Tuscany coast in Italy. In addition, B.R.U.V.S. (*Baited Remote Underwater Video System*) was used as an auxiliary method to diving activities in order to record the presence of nursehound individuals. Between 2018 and 2024 (except for 2022), 66 eggs were spotted around Monte Argentario peninsula on the Argentarola Island, Grotta del Corallo, Cala Grande, Capo D'Uomo, Cala della Maddalena and around Giannutri Island in Punta Scaletta. The highest number of eggs was observed around the Argentarola Island, Grotta del Corallo, and Cala Grande, both daily and during the entire study period, which suggests that these three sites represent spawning areas. Furthermore, two adult nursehound individuals have also been sighted near Argentarola Island, suggesting this site could represent a potential nursery area. Further data will be collected in these sites through more in-depth investigation in order to understand spatial distribution patterns, relative abundance, population estimates and behavior of this species along the Tuscany coast.
***Keywords*:** *Scyliorhinus stellaris*; spawning areas; shark; non-invasive techniques; Mediterranean Sea; diving activities

Sažetak: PRELIMINARNA OPAŽANJA PODRUČJA MRIJESTA MORSKE MAČKE MRKULJE *SCYLIORHINUS STELLARIS* (LINNAEUS, 1758) U OBALNIM VODAMA TOSKANE, ITALIJA. U cirkalitoralnoj zoni Sredozemnog mora, morska mačka mrkulja *Scyliorhinus stellaris* polaže jaja na mrijestilištima smještenima na dubinama između 25 i 50 m u livadama crvene gorgonije. S obzirom na smanjenje populacije morske mačke mrkulje u Sredozemnom moru i visoku ranjivost, istraživanje područja mrijesta i potencijalnih rastilišta ključno je za očuvanje ove vrste. Korištenjem neinvazivnih metoda poput ronjenja te uz pomoć R.O.V.-a (daljinski upravljano podvodno vozilo), identificirana su mrijestilišta ove vrste duž obale Toskane u Italiji. Kao dodatna metoda korišten je i B.R.U.V.S. (daljinski upravljani podvodni video sustav s mamcem) za bilježenje prisutnosti jedinki morske mačke mrkulje. Između 2018. i 2024. godine (uz izuzetak 2022.), uočeno je ukupno 66 jaja na području oko poluotoka Monte Argentario i to oko otoka Argentarola, na lokacijama Grotta del Corallo, Cala Grande, Capo D'Uomo, Cala della Maddalena te oko otoka Giannutri na lokaciji Punta Scaletta. Najveći broj jaja opažen je oko otoka Argentarola te na lokacijama Grotta del Corallo i Cala Grande, kako po danu promatranja tako i tijekom cijelog istraživanog razdoblja, što upućuje na to da te tri lokacije predstavljaju mrijestilišta ove vrste. Nadalje, dvije odrasle jedinke morske mačke mrkulje opažene su kod otoka Argentarola, što ukazuje da bi ta lokacija mogla biti i potencijalno rastilište vrste. U budućnosti će se prikupiti dodatni podaci na navedenim lokacijama kroz detaljnija istraživanja kako bi se bolje razumjeli obrasci prostorne distribucije, relativna brojnost, procjene populacije i ponašanje ove vrste duž obale Toskane.
***Ključne riječi*:** *Scyliorhinus stellaris*; mrijestilišta; morski pas; neinvazivne tehnike; Sredozemno more; ronjenje

INTRODUCTION

The nursehound *Scyliorhinus stellaris* (Linnaeus, 1758) is a benthonic catshark belonging to the Scyliorhinidae family. The species is distributed throughout the Mediterranean Sea and in the Northeast and Eastern central Atlantic over the continental shelf, from Shetlands and Southern Scandinavia to Senegal (Micarelli *et al*., 2016; Leonetti *et al*., 2020; Micarelli and Reinero, 2021). The nursehound inhabits rocky or seagrass-covered bottoms of the continental shelves, at depths ranging between 1 and 380 m, with the highest abundance being reported at depths of 20-63 m (Compagno, 1984; Leonetti *et al*., 2020), although in the Mediterranean, this

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species was recorded even at depth of 500 m (Relini *et al*., 2010).

Unfortunately, the nursehound is subjected to fishery exploitation with approximately 5 tons of landing being reported in 2022 in the Mediterranean Sea (FAO, 2022), which has led to an inevitable decline of this species, especially in the Northwestern Mediterranean basin and around the Balearic Islands (Serena, 2005). Furthermore, the low level of interconnectivity of distant populations inhabiting islands could affect their resilience (Micarelli and Reinero, 2021).

Limited information is available on the biology of the nursehound (Micarelli *et al*., 2016). The size at maturity of *S. stellaris* is around 79 cm in total length (TL) for females and 77 cm in TL for males (Serena, 2005; Micarelli *et al*., 2016). The nursehound lays egg cases in pairs throughout the year, with a single egg case being produced *per* oviduct (Serena, 2005). Females are also capable of storing sperm for at least 2-3 years and fertilizing the eggs later (Micarelli *et al*., 2016). Spawning grounds are defined as areas in which fish species gather to spawn. Due to numerous reproductive strategies employed by fish, the term can include both oviposition sites, where eggs are laid, and parturition sites, where birth of newborns takes place. Conversely, areas in which juveniles occur are termed nursery grounds (Ellis *et al*., 2012). In spawning grounds, nursehound eggs are laid in shallow water attached to macroalgae or gorgonian branches (Micarelli and Reinero, 2021).

Nursehound breeding season in the Mediterranean generally peaks in the spring and summer months (Serena, 2005). In the circalittoral zone, during the reproductive season, females of *S. stellaris* release eggs in nursery areas located at a depth approximately between 30-40 m in the red gorgonian *Paramuricea clavata* (Risso, 1827) meadows (Micarelli and Reinero, 2021).

In the Mediterranean, eggs hatch generally after 9 months (Serena, 2005) and after a slightly shorter period (7-8 months) in a controlled environment (Micarelli *et al*., 2016). The fecundity of the nursehound is lower in captivity than in the wild (Capapè, 1977; Micarelli *et al*., 2016), as well as the survival of fertilized eggs (Capapè *et al*., 2006). Conversely, the survival percentage and growth of newborns seem to be higher and faster, respectively, in a controlled environment than in natural conditions (Skaramuca and Prtenjača, 1985; Capapè *et al*., 2006; Micarelli *et al*., 2016).

In 2018, the Sharks Studies Center-Scientific Institute of Massa Marittima (GR, Italy) activated a project around Monte Argentario peninsula in Tuscany (Italy) aimed at locating the presence of nursehound nurseries between 20 m and 50 m depth through diving activities and the use of both R.O.V. (*Remote Operated Vehicle*) and B.R.U.V.S. (*Baited Remote Underwater Video System*). First surveys between 2018 and 2020 around Monte Argentario peninsula indicated just two possible spawning areas in Cala Grande and Argentarola Island (Micarelli and Reinero, 2021). In this study, observations of nursehound spawning and potential nursery areas from 2018 to 2024 (except for 2022) along the Tuscany coast are presented and discussed.

MATERIALS AND METHODS

Monte Argentario peninsula (42°26’07” N; 11°07’00” E) and Giannutri Island (42°15’14” N; 11°06’13” E) are located in the central Tyrrhenian Sea, along the Tuscany coast (Fig. 1), and are characterized by cliffs of variable height and small beaches which slope rapidly reaching a depth up to 100 m. The seabed is characterized, up to 50 m, by Mediterranean tapeweed *Posidonia oceanica* (Linnaeus) Delile, 1813 meadows, while along rocky ridges there are aggregations of gorgonians of the genera *Eunicella* spp. and *Paramuricea* sp.

Data collection was performed from 2018 to 2024 (except for 2022) between June and November in Capo d’Uomo, Cala Grande, Argentarola Island, Isolotto, Punta Avvoltore, Punta Torre Ciana, Grotta del Corallo, and Cala della Maddalena around Monte Argentario peninsula, and in Punta Scaletta in Giannutri Island (Fig. 1; see coordinates of sampling areas in Table 1). In 22 days of observations, 47 SCUBA dives of around 45-50 minutes each were performed. Diving activities were always carried out by two divers monitoring the same areas. A total of approximately 2.350 minutes (about 39 hours) of diving observations were carried out, always in the morning and early afternoon between 9.00 AM and 4.00 PM. Diving activities took place between 20 m and 50 m depth and recorded underwater temperatures ranged between 16 °C and 21 °C (dive log data from dive computers). Diving activities were not carried out in 2018 (Table 2). The same team used several underwater video cameras with housing (GoPro Hero 7, 8, 10, 12) to film eggs in *P. clavata* meadows and capture

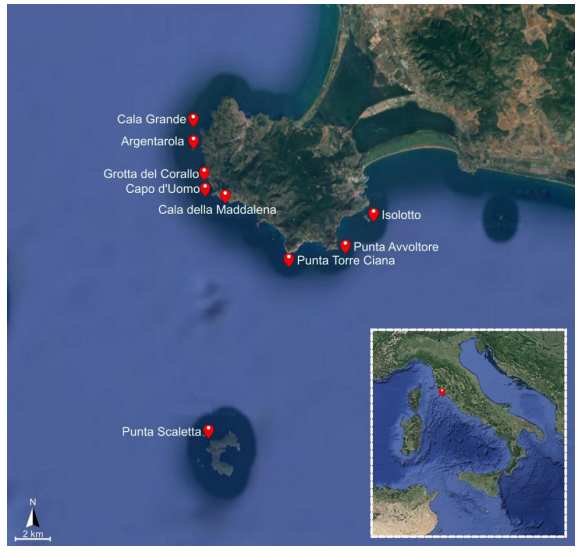

Fig. 1. Sampling areas along Monte Argentario peninsula and Giannutri Island (Tuscany, Italy).

Table 1. Summarized monitoring data showing total observation days (Days), number of eggs (Eggs) observed empty (Empty), with embryo (Embryo) or not determined (Nd) due to biofouling around and depth and water temperature (Wt) at which eggs were spotted in the sampling areas during the study period.

Year	Days	Eggs	Empty	Embryo	Nd	Area	Coordinates	Depth	Wt
2018	6	1	1	0	0	Capo D'Uomo	42°23'35" N 11°05'83" E	30-50 m	16 °C
2019	4	8	6	2	0	Cala Grande	42°26'87" N 11°06'45" E	30-35 m	17 °C
2020	2	6	5	1	0	Argentarola Island	42°25'21" N 11°04'53" E	28-38.9 m	16 °C
2021	2	5	2	1	2	Argentarola Island	42°25'21" N 11°04'53" E	33-36 m	17 °C
2023	2	7	0	2	5	Argentarola Island	42°25'21" N 11°04'53" E	37-40 m	16 °C
2023	1	2	0	2	0	Punta Scaletta	42°15'14" N 11°06'13" E	30.8 m	17 °C
2023	1	3	0	1	2	Cala della Maddalena	42°39'15" N 11°10'45" E	35 m	18 °C
2024	3	24	4	2	18	Argentarola Island	42°25'21" N 11°04'53" E	37.2-43.3 m	16-21 °C
2024	1	10	3	0	7	Grotta del Corallo	42°23'59" N 11°06'45" E	25.3-35.6 m	20-21 °C
Total	22	66	21	11	34				

the presence of nursehound specimens. Egg cases were attributed to nursehound according to the dichotomous key proposed by Mancusi *et al.* (2021). In the case of fertilized eggs, the developmental stage of the embryo was assigned based on Micarelli *et al*. (2016).

For further underwater observation of the spawning areas, two Poseidon (Shenzhen Quanyou Company, China) R.O.V.s were used. They were equipped with LED lamps, camera and depth gauge and they were able to operate up to 120 m depth. R.O.V.s were driven remotely by a joystick connected to a mobile phone, which made it possible to follow the movements in real time and determine the vehicles' depth (Micarelli and Reinero, 2021). R.O.V.s were used in 2018 and 2019 covering areas between 1000 and 2000 m^2 each year, for a total of approximately 78 hours of survey (Table 2). In order to use the R.O.V.s, vertical monitoring transects were organized defining a distance, from both sides, of about 50 m from the anchor point indicated as *zero point* (coordinates reported in Table 1) for a total straight line of 100 m horizontally and 50 m maximum in depth to completely cover the gorgonian meadows on the precipitous rocky ridges.

In addition, a B.R.U.V.S. was used only in 2024 as an auxiliary method to diving activities to record the presence of the nursehound and other shark species around the areas. It was used for two days and during two dives for a total of approximately 90 minutes only around Argentarola Island (Table 2). Once tied to the boat with a rope, the B.R.U.V.S. was put on the seabed above biogenic rocks at 30-40 m depth close to gorgonians where eggs were observed. A high-definition underwater camera (GoPro Hero 8) was mounted on a steel surface with a small closed and perforated canister containing

Table 2. Monitoring effort of diving, R.O.V.s and B.R.U.V.S. during the study period. Effort in days and minutes is reported in the brackets.

Year	Diving	R.O.V.s	B.R.U.V.S.
2018	No	Yes (6 days, 2.800 minutes)	No
2019	Yes (3 days, 700 minutes)	Yes (4 days, 1.870 minutes)	No
2020	Yes (2 days, 450 minutes)	No	No
2021	Yes (2 days, 400 minutes)	No	No
2023	Yes (4 days, 400 minutes)	No	No
2024	Yes (4 days, 400 minutes)	No	Yes (2 days, 90 minutes)

the bait composed of small cephalopods and fish, typical prey of *Scyliorhinus* spp. (Reinero *et al*., 2023, 2024).

RESULTS AND DISCUSSION

Observations were carried out periodically over 22 days (552 hours) during which 66 eggs were spotted in *Paramuricea clavata* meadows (Table 1). They were observed in all the sampling areas, except Isolotto, Punta Avvoltore, and Punta Torre Ciana where activities were carried out for 13 hours in each spot.

All the eggs were recorded through diving activities from 2019 to 2024, while in 2018 only one empty egg was observed in Capo D'Uomo using R.O.V.s. This discrepancy could be caused by the limited movements of the instrument in red gorgonian meadows. On the contrary, divers could get closer to red gorgonians and observe them from multiple sides with the help of an underwater light. This could have enhanced their sighting capabilities.

Of these 66 eggs, 21 were empty, 11 hosted embryos with a development stage of around 3-5 months, whereas for the remaining 34 it was not possible to determine whether they hosted embryos because of biofouling around the eggs (Table 1, Fig. 2). Eggs were spotted between 25.3 and 50 m depth during the entire study period (Table 1).

For each monitoring day their number ranged between one and ten in Argentarola Island in eight days of survey (six in 2020; one and five in 2021; two and five in 2023; seven, ten, and seven in 2024), ten in Grotta del Corallo in one day, eight in Cala Grande in one day, one in Capo D'Uomo in one day, three in Cala della Maddalena in one day, and two in Punta Scaletta in one day. Eggs were spotted in Argentarola Island (n=42), Grotta del Corallo (n=10), Cala Grande (n=8), Cala della Maddalena (n=3), Punta Scaletta (n=2), and Capo D'Uomo (n=1) (Table 1). All eggs were found attached exclusively to *P. clavata* branches.

Only two different nursehound individuals were observed during diving activities inside small caves close to *P. clavata* meadows around Argentarola Island in 2023 and 2024 at 38 m and 39.1 m depth, respectively. Divers were able to get close to these two nursehound specimens and noticed the apparent absence of claspers in the pelvic area, suggesting they were probably females. However, it was not possible to confirm the sex because sharks were partially hidden in the caves. Through a visual estimate of size, they were both adults, certainly reaching 80 cm in TL. In both cases, water temperature was 16 °C. No additional specimens were spotted using B.R.U.V.S., probably due to the short exposure time during data collection.

The absence of eggs spotted in Isolotto, Punta Avvoltore, and Punta Torre Ciana could indicate that these sites do not represent spawning areas. Conversely, as partly assumed by Micarelli and Reinero (2021), three possible spawning areas could be Argentarola Island, Grotta del Corallo, and Cala Grande sites, where the highest number of eggs was observed during the entire study period. Of particular interest, the Argentarola Island site appears to be a potential nursery area. Indeed, a higher number of egg cases was spotted compared to the other sites, and two different adult specimens, probably females, were observed in 2023 and 2024, suggesting that this habitat is repeatedly used across the years (Heupel *et al*., 2007). Based on definition proposed by Ellis *et al*. (2012), the finding of juveniles is required to confirm Argentarola Island as a nursery ground. Further investigations will be performed in this area to confirm this hypothesis.

In Cala della Maddalena, Capo D'Uomo and Punta Scaletta, the observation of few eggs suggests these areas are not spawning sites, although the sampling period coincides with the peak breeding season occurring in spring and summer (Serena, 2005). The possibility of deeper spawning areas around these spots, not investigated in this study, could not be excluded. Indeed, further data could be collected in these areas through more in-depth investigation with R.O.V.s.

Many of observed eggs were empty, which suggests either that the juveniles had just been hatched or that the eggs had not been fertilized, and several others showed embryos about to be hatched (Micarelli and Reinero, 2021). Unfortunately, the biofouling around 34 eggs did

Fig. 2. Egg with embryo inside **(A)**; empty egg **(B)**; and egg with evident biofouling around **(C)**. These eggs were spotted around Argentarola Island during the study period.

not give us the possibility to verify the presence of the embryo inside. It has been demonstrated by Thomason *et al.* (1994) that macrofouling occurred after a long time (11 months) from deposition in *Scyliorhinus canicula* egg cases. Considering the incubation period (7-9 months) and hypothesizing a similar phenomenon for *S. stellaris*, biofouled egg cases could have been laid much earlier than the non-biofouled ones. Further studies are required to better investigate this aspect.

The depth range (25.3-50 m) at which eggs were observed agrees with the information reported by Micarelli and Reinero (2021). Apparently, a decrease in the number of eggs was never observed during the six years of data collection and this could suggest that water temperature range (16-21 °C) at which eggs were observed was optimal for the development of the embryos (Micarelli *et al*., 2016). However, scuba divers in the past, before 2018, attested a greater number of spawning areas between 30 m and 40 m depth in these sites (Micarelli and Reinero, 2021). The increase of water temperatures in the Mediterranean could induce females to lay eggs in deeper *P. clavata* meadows down to about 50-70 m depth (Micarelli and Reinero, 2021) and this aspect will be investigated in the future.

In conclusion, diving activities allowed to identify nursehound spawning areas and one potential nursery area along the Argentario peninsula. While SCUBA diving proved crucial for spawning areas observations, the use of R.O.V.s and B.R.U.V.S. could be useful in the future to improve investigations on spatial distribution patterns, relative abundance, population estimates and behaviors of the nursehound along the Tuscany coast (Leonetti *et al*., 2024). Given the evident decline of the nursehound in the Mediterranean, its vulnerable status (Finucci *et al*., 2021), and its higher vulnerability to population depletion than the lesser spotted dogfish *S. canicula* (Micarelli and Reinero, 2021), identification of spawning and nursery areas is crucial for the conservation of this species. The monitoring and identification of spawning and nursery areas will continue in order to contribute to the establishment of protection plans and conservation measures for this species within this area.

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