Deliverable 5.2.6 Protocol for the sustainable management and protection of a shared fish stock

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INTRODUCTION

The ARGOS Working Package 5"Knowledge-based decision-making process", led by Zadar County, intends to improve the sectorial capacities and know-how to change the behaviours of fishery and aquaculture operators towards shared environmental sustainability and responsible actions. To this goal, the WP foresees a set of actions at local and cross-border level with a high level of involvement of fisheries and aquaculture operators both in exchanging experiences and knowledge and in testing pilot actions for a common approach to the management of shared fish stocks and best practices for improving sustainable behaviours in fisheries and aquaculture practices. The project Action 5.2 is, in fact, dedicated to the improvement of fishermen behaviours by the definition and testing of sustainable protocols for the management and protection of a shared fish stock.
Within this framework, Marche Region (PP3) and the Department of Environmental and Life Sciences of the Polytechnic University of Ancona defined and tested - with an interactive engagement of local fishermen from the Marche Region pilot area - a pilot action for the development of a transferable "Protocol for the sustainable management and protection of a shared fish stock" targeting *Sepia officinalis.*

THE ARGOS PILOT ACTION TOWARDS A SUSTAINABLE MANAGEMENT OF CUTTLEFISH

Background

The common cuttlefish (*Sepia officinalis)* has always represented an important resource in the fishery history of the Mediterranean Sea. To date, it is one of the most abundant cephalopod species captured by trawl fishing activities in the Adriatic Sea, especially along its northern and central part extending from the waters off the Friuli Venezia Giulia up to the area off the Abruzzo coast. As a short-lived (1-2 years) and semelparous spawner, the common cuttlefish lays the eggs in separate batches during the single spawning season at the end of which both males and females die. Furthermore, most cuttlefish have low fecundity (300–3000 eggs per female), producing large benthic eggs and larvae with limited dispersal potential. Considering its reproductive strategy and short life cycle, population stability primarily depends on successful recruitment which in turn requires sufficient breeding rates, appropriate habitats for eggs laying and embryo survival. All these requirements are strongly influenced by several anthropic and environmental factors including eggs disruption during fishing gear cleaning, reduction of natural habitat for the eggs laying, temperature, salinity, light intensity, and pollution.

Despite their relevance in the seafood market, cephalopods are the molluscan class that has received the least consideration regarding monitoring and management. Introducing monitoring and eggs loss reduction measures will ensure adequate annual recruitment and stock conservation.

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Goals of the Pilot Action

The pilot action is aimed to define a protocol for sustainable management and protection of a shared stock such as that of cuttlefish.

This protocol address to three main objectives:

1) to develop a program for annual and spatial monitoring of cuttlefish eggs quality.
2) to reduce cuttlefish eggs losses by developing an innovative in-land system.
3) to reduce cuttlefish eggs losses by testing and applying sustainable "good practices" already used by few fishermen.

The Pilot Action Target Groups

The pilot action targeted the following actors of the local and cross-border fisheries sector:

⇒ Fishermen and fish farmers
⇒ Local, Regional and National Public Authorities
⇒ Regional and Local Development Agencies
⇒ General public
⇒ Universities and Research Institutes
⇒ Education and Training Organizations
⇒ Protected Areas and Natural Heritage Management Bodies Associations

Fishermen involvement and pilot area

The local fishing community was an active part of the pilot action as the fishermen have been involved in different phases of the activity itself. Fishermen from 5 different sites of Marche coasts have been involved: Fano, Senigallia, Ancona, Numana e San Benedetto.

Figure 1: geographical maps indicating the 5 port and fishermen involved in the pilot action

Pilot action Description and structure

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This pilot activity is aimed to define a protocol for sustainable management and protection of a shared stock such as that of cuttlefish. This protocol consists of three main actions:

1) Development of a protocol for assessing and monitoring the quality of cuttlefish egg.
2) Development of a "collection, recovery and releasing" protocol for the reduction of embryos losses due to the inappropriate fishing gears activities.
3) Testing alternative sustainable "good practices" for the reduction of cuttlefish eggs losses.

1. Development of a Protocol for assessing and monitoring the quality of cuttlefish egg.

A pilot study has been carried out to evaluate the health status of cuttlefish embryos collected from different sites along the Marche region in relation to the presence of pollutants (such as microplastics).

<u>Samples collection protocol</u>

Cuttlefish eggs were collected, in collaboration with local fishermen, from the fishing gears where trapped females had released their eggs. Samplings were performed within June 2021 from 4 different areas/sites off the coast of the Marche region:

- ⇒ site A, from an area off the coast of Senigallia (AN);
- ⇒ site B, off the coast of Ancona (AN);
- ⇒ site C, off the coast of Numana (AN)
- ⇒ and site D, off the coast of San Benedetto del Tronto.

Eggs were collected from each sampling site and successively embryos were sampled. Only the latest embryonic stages (28-29) were selected following the identification criteria suggested by Boletzky et al. (2016) based on morphological features (such as eyes color and position, arms structure and chromatophores distribution) and the mantle length. Each embryo selected was weighed and the mantle length was recorded. Yolk samples and whole embryos were collected separately in 3 pools of 10 embryos and 3 pools of 10 yolks weighed and stored in glass tubes at -20 °C for microplastic detection analysis. 10 embryos (between 28-29 stage) per site were also sampled and kept in formaldehyde/glutaraldehyde solution (NaH_2PO_4–H_2O + NaOH + Formaldehyde (36.5%) + Glutaraldehyde (25%) + H_2O) at 4°C for 24 h and successively washed in 70% ethanol three times (15 minutes each) and stored in the same ethanol solution at 4°C to perform histological analysis.

Results obtained

Considering the dorsal mantle length of embryos between the 28 and 29 stages, values from site A, site C and site D were significantly lower ($p \leq 0.0001$, $p \leq 0.0001$ and $p \leq 0.01$ respectively) than those from site B. Embryo' average weight appeared the highest in site B respect to the other sampling sites, however the differences among embryos weight were not significant ($p > 0.05$) due to the high variability of samples analyzed. The comparison between hatchling weight and mantle length highlighted significantly lower values ($p \leq 0.001$ and $p \leq 0.01$ respectively) in animals from site C compared to hatchlings from site B (Figures 2 and 3).

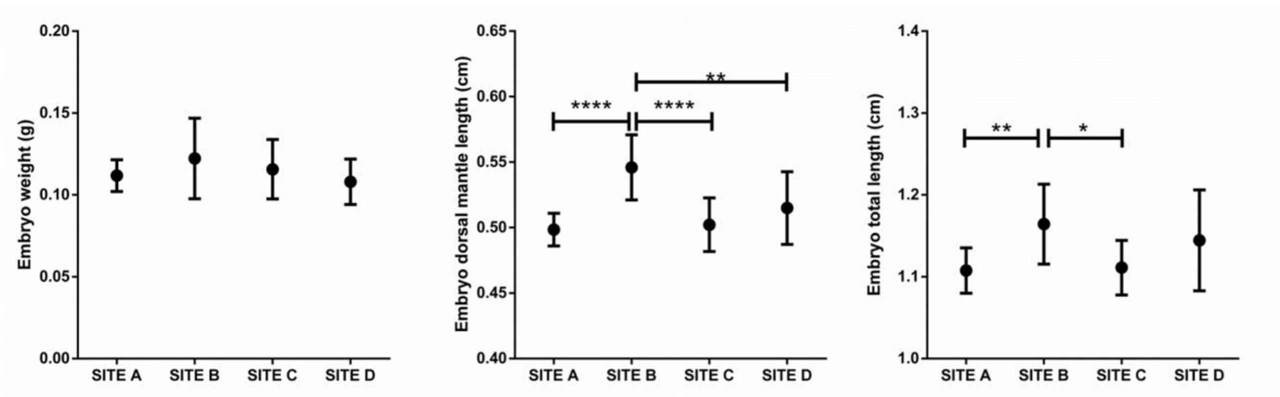

Figure 2. Embryo weight, Dorsal mantle length and weight of Sepia officinalis embryos among sampling sites expressed as mean values ± SD. Site A = Senigallia; site B = Ancona; site C = Numana; site D = San Benedetto del Tronto.*= $p \leq 0.05$; **= $p \leq 0.01$; ***=$p \leq 0.001$; ****=$p \leq 0.0001$.

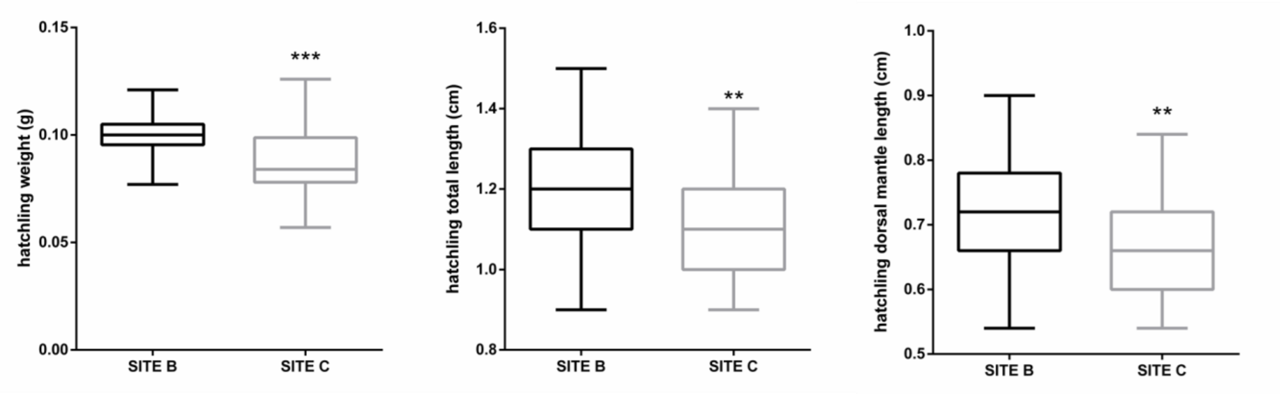

Figure 3. Boxplot (min. to max.) of hatchlings' weight, total length and dorsal mantle length from site B and site C; the line inside boxes represented the mean value. Site A = Senigallia; site B = Ancona; site C = Numana; site D = San Benedetto del Tronto. *= p ≤ 0.05; **= p ≤ 0.01; ***=p ≤ 0.001; ****=p ≤ 0.0001.

The histological analysis did not highlight evident structural or morphological differences among embryos from different sampling sites and between hatchlings from sites B and C. Moreover, the three stains performed showed that all the internal organs and tissues of adult specimens are formed or sketched at the same developmental level in all samples analyzed

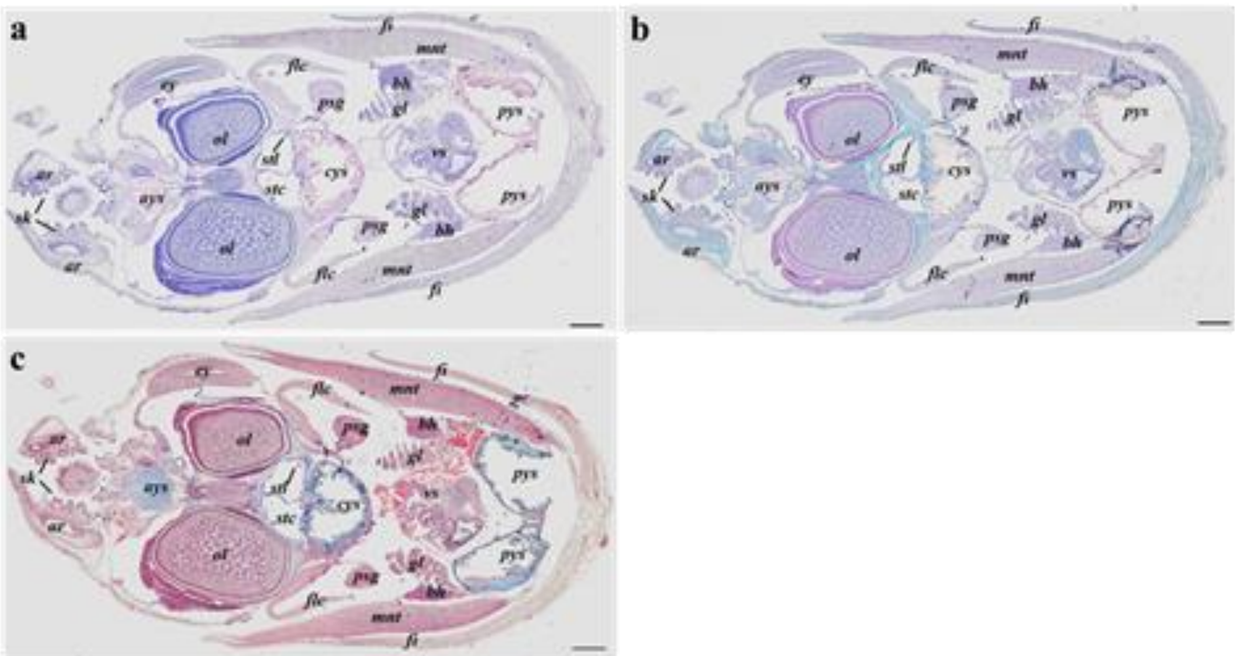

Figure 4. Example of longitudinal sections of *S. officinalis* representative of both embryos at the 28-29 development stages and hatchlings from all sampling sites, stained with Haematoxylin eosin stain (a); Alcian blue stain (b); Masson's

trichrome stain (c). ar, arms; ays, anterior yolk sac; bh, branchial heart; cys, central yolk sac; ey, eye; fi, fins; flc, funnel-locking cartilage apparatus; gl, gills; mnt, mantel; ol, optical lobe; psg, posterior salivary gland; pys, posterior yolk sac; sk, suckers; stc, statocyst; stl, statolith; vs, visceral sac. Scale bar: 500µm

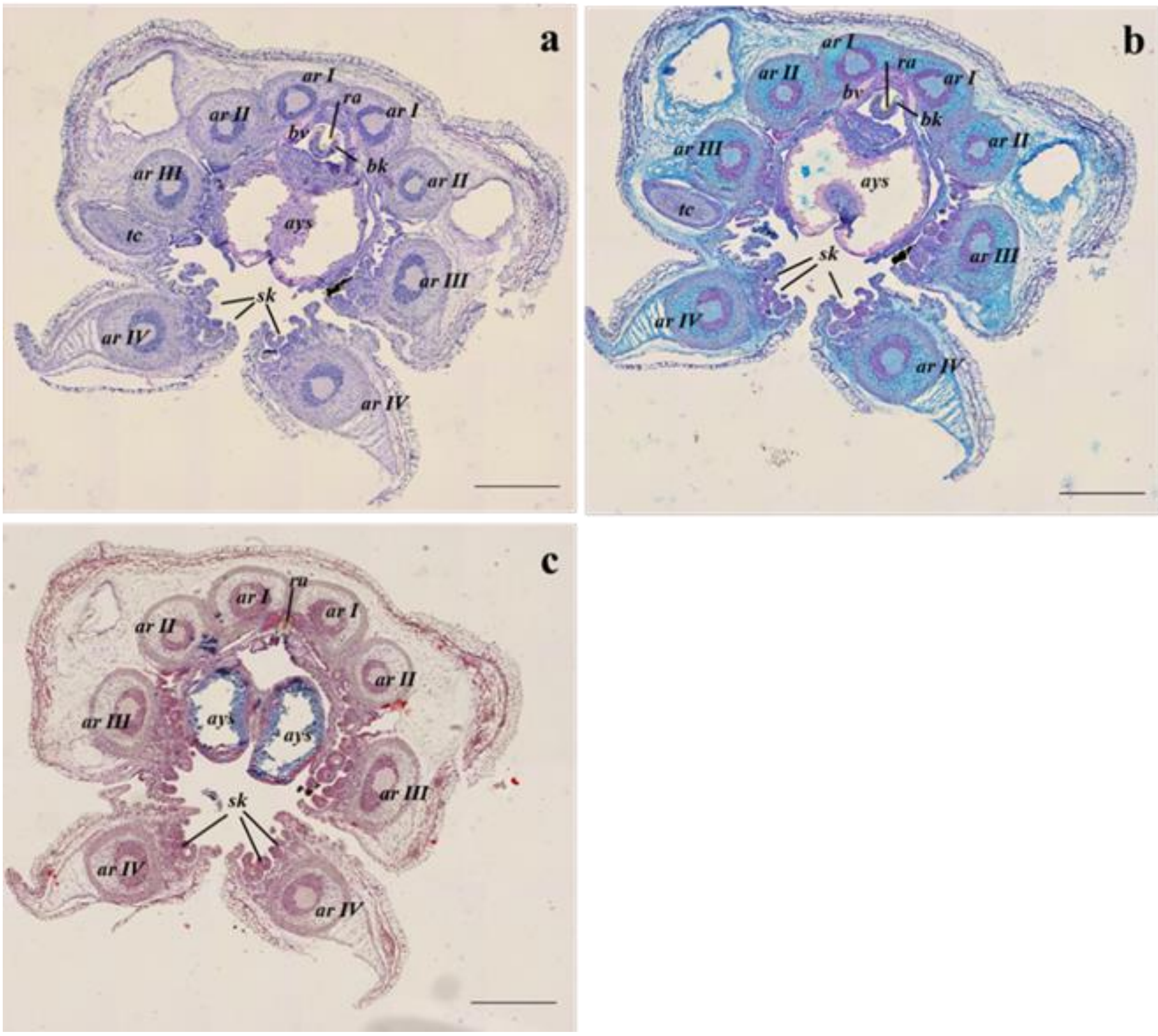

Figure 5. Example of transversal sections of the anterior part at the buccal mass level of *S. officinalis* representative of both embryos at the 28-29 development stages and hatchlings from all sampling sites, stained with (**a**) Haematoxylin-eosin stain; (**b**) Alcian blue stain; (**c**) Masson's trichrome stain. **ar** I-IV, arm I-IV; **ays**, anterior yolk sac; **bk**, beak; **bv**, blood vessel; **ra**, radula; **sk**, suckers; **tc**, tentacle. Scale bar: 500µm.

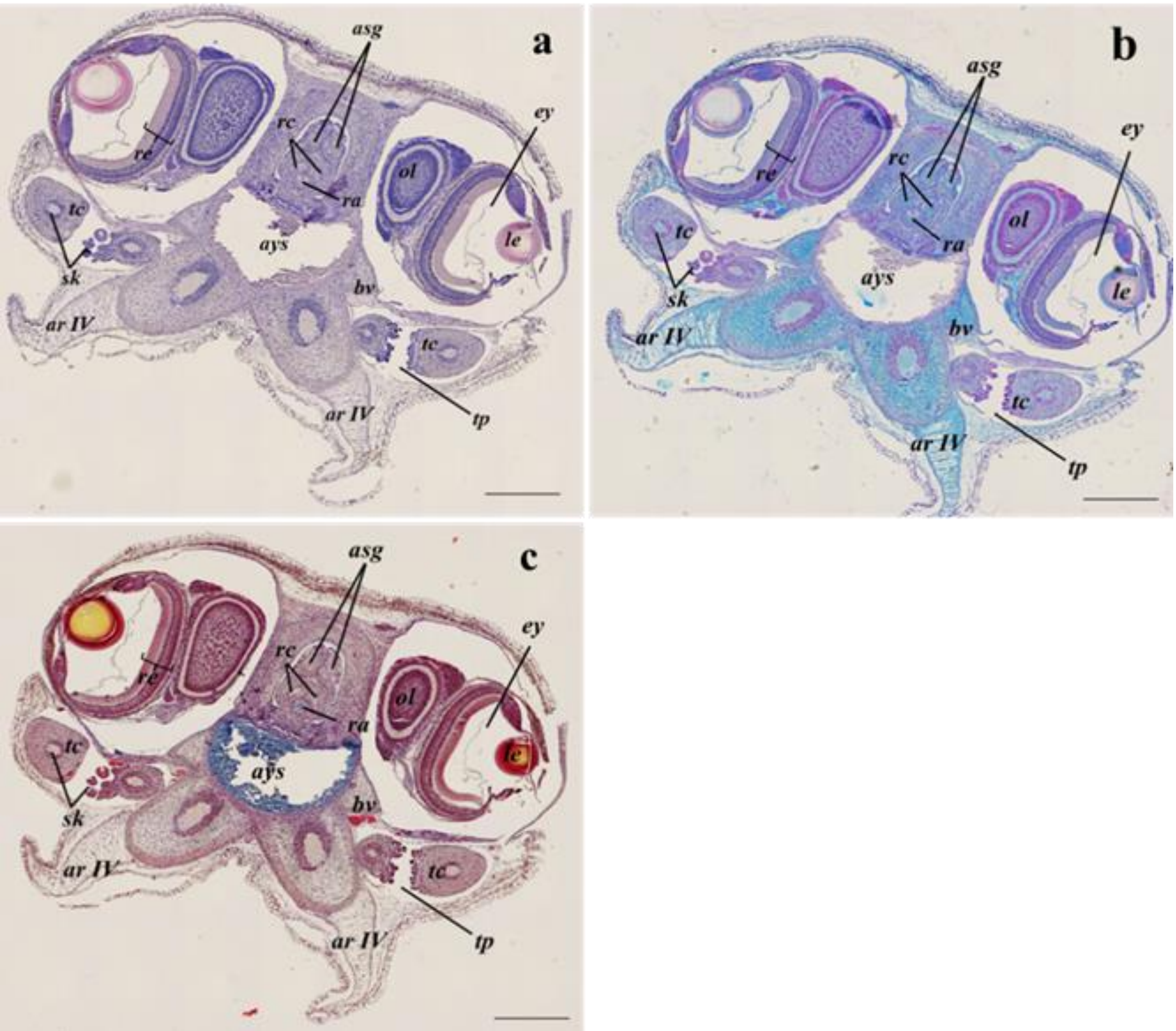

Figure 6. Example of transversal sections of the anterior part at the visual system level of *S. officinalis* representative of both embryos at the 28-29 development stages and hatchlings, from all sampling sites, stained with Haematoxylin-eosin stain (a); Alcian blue stain (b); Masson's trichrome stain (c). ar IV, arm IV; asg, anterior salivary gland; ays, anterior yolk sac; bv, blood vessel; ey, eye; le, lens; ol, optical lobe; ra, radula; rc, radula cartilage; re, retina; sk, sucker; tc, tentacle; tp, tentacular pocket. Scale bar: 500µm

MPs were detected and characterised in embryos and yolk samples. Raman microspectroscopy analysis identified a different number of MPs in yolk and embryos samples among the sampling sites, all MPs size was < 5 µm. In all sampling sites considered, a higher number of MPs were found in yolk samples compared to embryo pools (Table 1). Site B (Ancona) was the only site with no MPs detected in embryos and the site with the lowest number of MPs observed. The two sampling sites with the highest number of MPs per sample's weight in both yolks and embryos were sites A and C.

Site	#MPs/yolk	MPs/yolk (MPs/g)	#MPs/embryo	MPs/embryo (MPs/g)
A	0.45 ± 0.07^{a}	14.17 ± 2.33^{a}	0.2 ± 0.03^{a}	2.35 ± 0.18^{a}
B	0.15 ± 0.07^{b}	4.99 ± 2.13^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
C	0.40 ± 0.01^{a}	18.64 ± 0.18^{a}	0.25 ± 0.07^{a}	2.35 ± 0.67^{a}
D	0.25 ± 0.07^{ab}	10.59 ± 0.78^{c}	0.05 ± 0.07^{b}	0.62 ± 0.88^{ab}

Table 1. Number of microplastics detected in *S. officinalis* yolk and embryo pools. Site A = Senigallia; site B = Ancona; site C = Numana; site D = San Benedetto del Tronto; **# MPs/yolk**, average number of MPs in each yolk; **MPs/yolk**, average number of MPs per g of **MPs/embryo,** average number of MPs in each embryo; **MPs/embryo**, average number of MPs per g of embryo. Values are reported as mean ± standard deviation. Different letters indicate a statistically significant difference ($p < 0.05$).

The sites with the highest number of MPs were Site A and C which both represented 37% of the total amount (Figure 7).

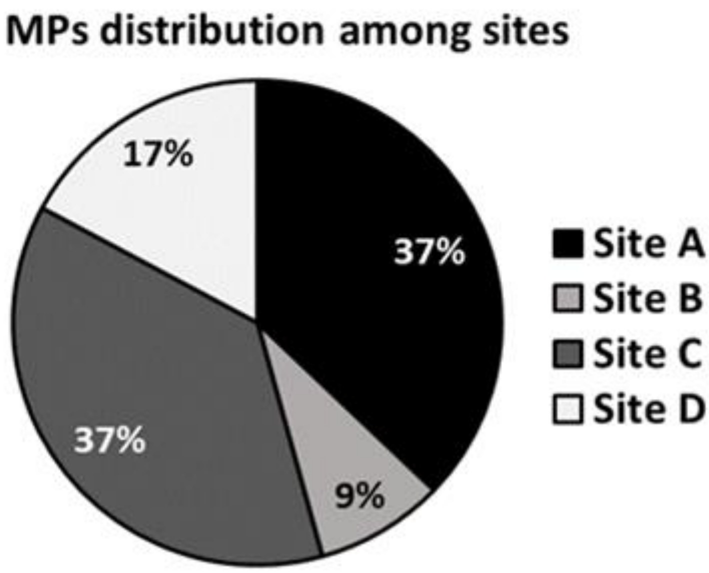

Figure 7. Total microplastics percentage detected in each sampling site. Site A = Senigallia; site B = Ancona; site C = Numana; site D = San Benedetto del Tronto.

Only two MP types were detected among all the samples observed: fragments, corresponding to most MPs (74%), and spheres (26%) (Figure 8a). MP fragments and spheres were observed only in sites A and C, while in sites B and site D only MP fragments were detected (figure 8b).

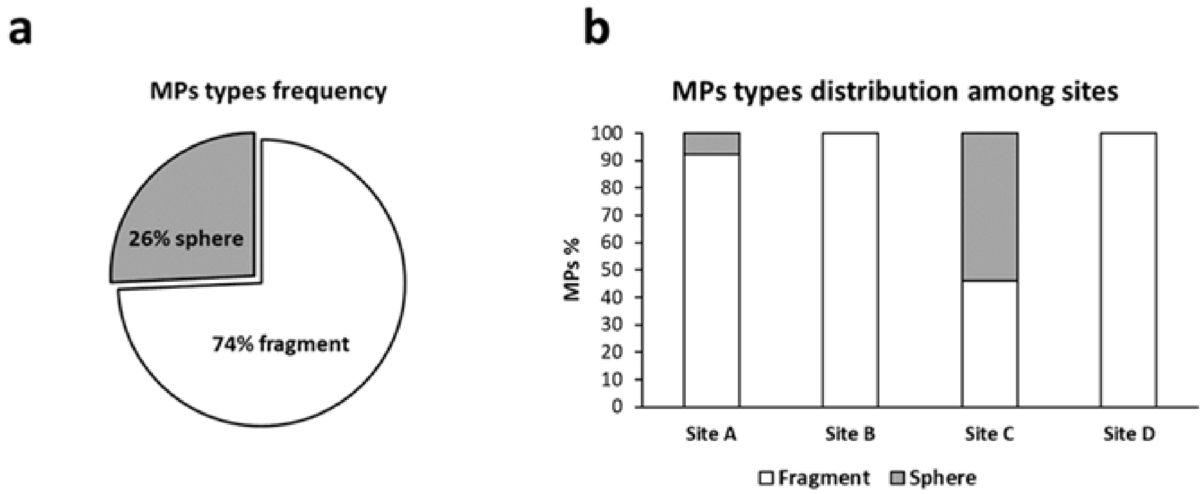

Figure 8. Percentage frequency of microplastic types (**a**) and types distribution among sampling sites (**b**). Site A = Senigallia; site B = Ancona; site C = Numana; site D = San Benedetto del Tronto.

The most frequent polymer identified was PVC which accounts for more than half of MPs observed (52%) followed by PP and CA (both 15%) (Figure 9a). The site with the highest number of polymers is site A while in site B only PP was observed (Figure 9b).

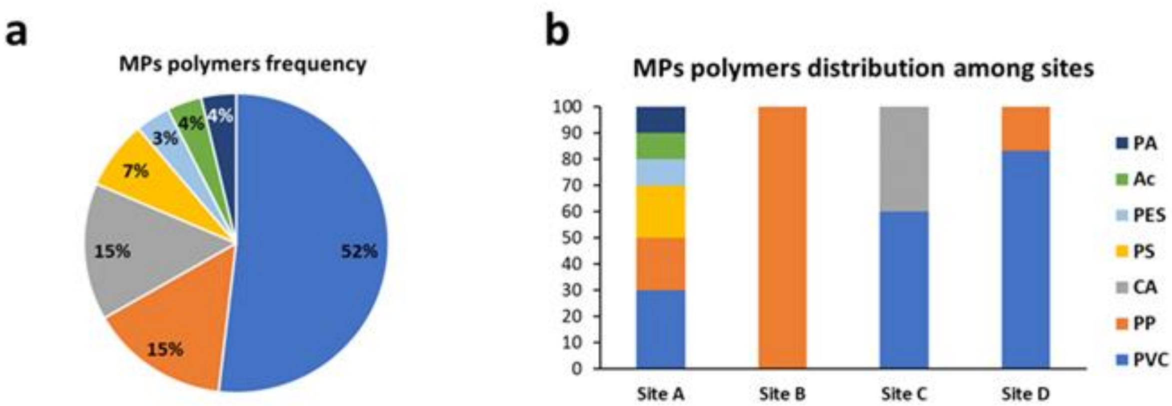

Figure 9. (a) Percentage frequency of microplastic polymers and their distribution **(b)** among sampling sites. **PVC**, Polyvinyl chloride; **PP**, Polypropylene; **CA**, Cellulose acetate; **PS**, Polystyrene; **PES**, Polyethersulfone; **AC**, Acrylic; **PA**, Polyamide. Site A = Senigallia; site B = Ancona; site C = Numana; site D = San Benedetto del Tronto.

Regarding the MP colors, a high variety of colors was registered. The most frequent colors were black, blue, transparent and light blue representing 23, 17, 11 and 11% respectively (Figure 10a). No evident trend of colors distribution was observed among the sampling sites, the site with the highest number of colors detected was site A (Figure 10b).

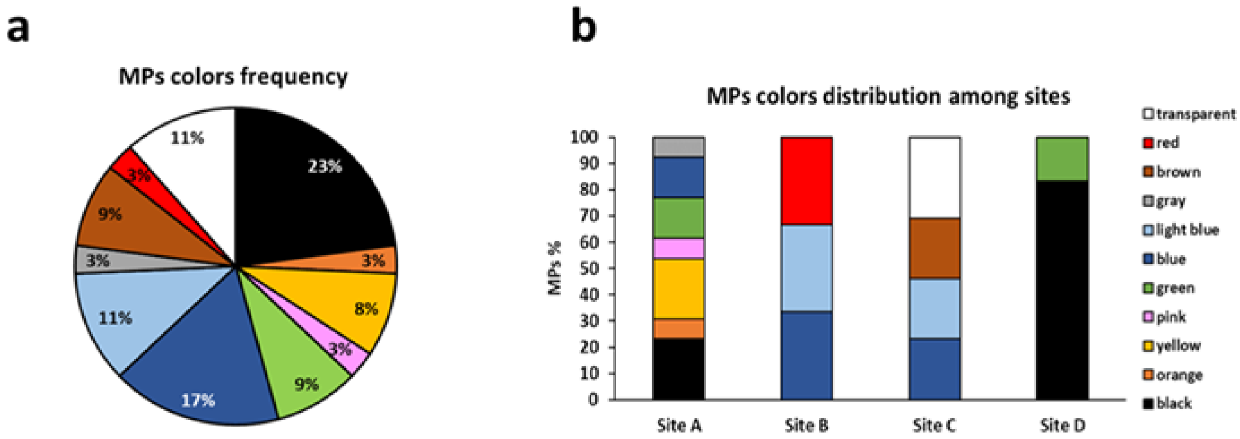

Figure 10. Percentage frequency of MP colors (**a**) and color distribution among sampling sites (**b**).

<u>Conclusions</u>

In this study, the presence of MPs in *Sepia officinalis* embryos from different sites of the central Adriatic Sea was observed for the first time. These preliminary results could suggest that the presence of MPs does not impact the embryo development and organogenesis. However, regardless of the pathway through which MPs enter the egg, a better understanding of their relationship with embryo and hatchling health and growth is required since these species will experience chronic exposure to MPs from the embryonic development to the adult phase. In this light, an annual monitoring program, including biometric, histological and molecular data, should be developed in order to assess the health status of cuttlefish embryos and the presence of MPs or other pollutants inside the yolk. This monitoring program should be extended to other sites of Italian and Croatian coasts in order to obtain a clear and complete assessment of the health status of the Adriatic cuttlefish stock.

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2.Development of a "collection, recovery and releasing" protocol for the reduction of embryos loss due to the inappropriate fishing gears activities.

Fishermen from Ancona e Numana sites have been involved in the collection of cuttlefish embryos during the cleaning operations of the fishing equipment. To this purpose, an operative protocol for the collection of the embryos has been developed: fishermen called us when they came back from their fishing activities. Each fisherman called us on different days or in different hours of the same day depending on their activity's programs and on weather and sea conditions. Initially, together with the fishermen, we collected the embryos from their fishing gear, teaching them not to damage the eggs and to keep them correctly.

Figure 11: A fisherman involved in the cuttlefish eggs collection

Figure 12: example of cuttlefish eggs collected by fishermen

Once learned, the fishermen collected the eggs themselves. At the moment of egg collection, fishermen informed us on the quantity and quality of both eggs and adults collected and on any changes they monitored regarding the sea (water temperature, variation of sea currents, presence of alien species or other).

For short transport (5-10 minutes) a plastic bucket (25 litres) with a lid containing sea water was used.

Figure 13: example of plastic bucket used for eggs collection

Once collected, the eggs were transferred in the on-land system. An innovative on-land system has been designed and developed and realised in the UNIVPM facilities. This innovative plant consists of 2 different units:

A special tank for the maintenance of the embryos collected at different developmental stages till their hatching and 3 tanks for the maintenance of the juveniles till their release at sea.

Figure 15: tank for the maintenance of the embryos collected in different developmental stages till their hatching

Figure 16: tanks for the maintenance of the juveniles till their release at sea.

A 97% hatching rate was obtained for embryos maintained in the on-land system. After the hatching, juveniles were transferred in maintenance tanks till their release on the sea.

Fishermen have been constantly updated on the progress of the pilot plant with updating meetings, and they have been involved in the release of hatched juveniles.

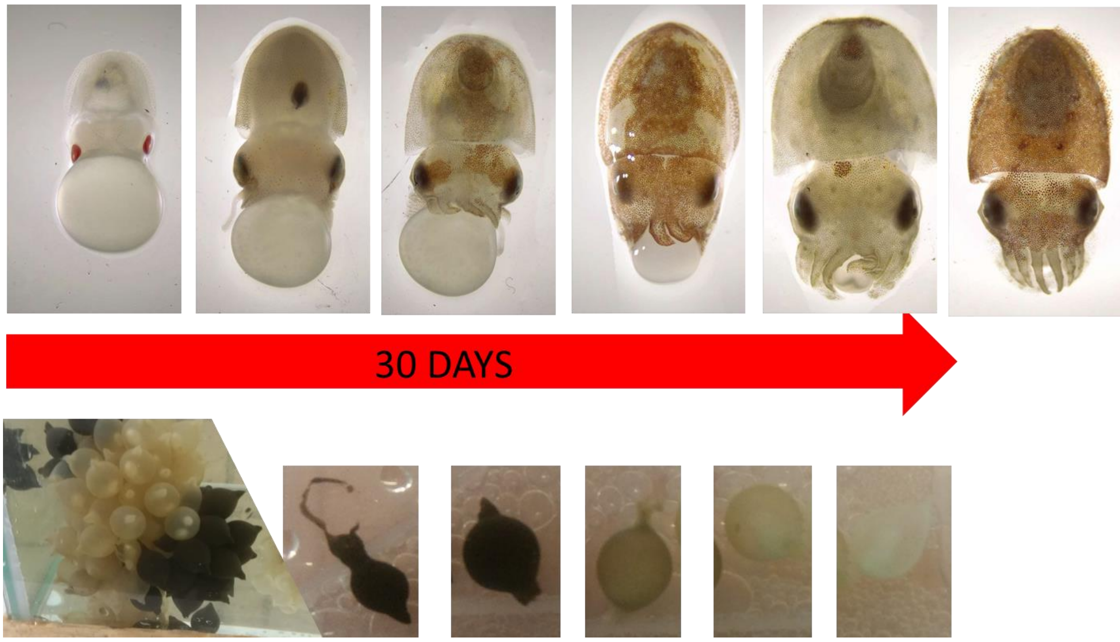

Figure 17: microphotographs representing cuttlefish embryos at different developmental stages

3.Testing alternative sustainable “good practices” for the reduction of cuttlefish eggs losses.

This activity was based on optimising and testing "sustainable" good practices currently implemented by the fishermen involved, which were collected during the interviews. From these first interviews various practices emerged, of which the most interesting were selected and tested. -The first practice tested was an “old” strategy which consists of adding laurel branches inside the traps. This natural support, mimicking algae, induces the caught cuttlefish to lay their eggs on it and not on the net of the trap. Fishermen use laurel because it is an easily available, not expensive terrestrial plant which can resist for a long time in sea water. When a fisherman recovers the traps, he releases the laurel branches into the sea, thus reducing the loss of eggs. Starting from this strategy we evaluated its “sustainability” by analysing it from a scientific point of view.

Figure 18 example of eggs laid on laurel branches

Several laurel branches full of laid eggs were collected and transferred in ad-hoc thanks to assess embryo development in comparison to eggs collected at the same time in the same site but from the net of the fishing gear. Preliminary data revealed that embryos laid on laurel branches showed higher mortality during all the developmental period (30 days) and lower hatching rate.

Figure 19 example of eggs laid on laurel branches maintained in rearing condition

This negative result could be ascribable to the fact that laurel is known to be poisonous for several animals and its effects on cuttlefish is still unexplored. In addition, the closed system represented by the rearing tank can have exacerbated this effect. In conclusion, the use of "terrestrial" branches, as alternative and sustainable support for egg laying, should be a good practice to apply, but the selection of the best terrestrial tree to use is still to be performed. During the next breeding season, different terrestrial branches will be tested in terms of resistance in the marine environment, ability to be recognized by cuttlefish as a good substrate for spawning and safety for hosted embryos.

-The second practice tested was the use of an "accessory" small net to attach to the trap in order to provide cuttlefish an alternative substrate for eggs lying. When a fisherman recovers the traps, he releases the net with eggs into the sea, thus reducing the loss of eggs. This practice was immediately eliminated because clearly the net left free in the sea represents a danger to other marine animals and a source of pollution. Anyway, the use of an "accessory and biodegradable" support for eggs lying to be added to traps, could be promising.

-The last “good practice” tested was the development of use of an artisanal hatchery in the sea, consisting of a trap for sea snails (like “cogullo”) attached to the quay of the port where to put the eggs recovered during the cleaning operations of fishing gears. The success rate of these embryos was still to be evaluated. In order to assess the suitability of this action, in the next breeding season an ad hoc experiment will be performed.

-However, the most effective good practice that was tested was the "educational" one. Going to the port upon landing, interviewing the fishermen, starting a collaboration with them and exchanging information and explaining to them that the eggs laid on the gear can be recovered and must not be destroyed, were the best strategies. Some fishermen have changed the way they clean their fishing gears and started collecting eggs spontaneously. In this light, a brief documentary has been produced (duration 15 minutes) to present all the activities carried out in this pilot action and on cuttlefish fisheries. The fishermen involved in the development of the pilot action were also the main actors of the documentary, recounting their decades of experience in cuttlefish fishing, how this has changed over the years and proposing sustainable solutions for improving the stock.

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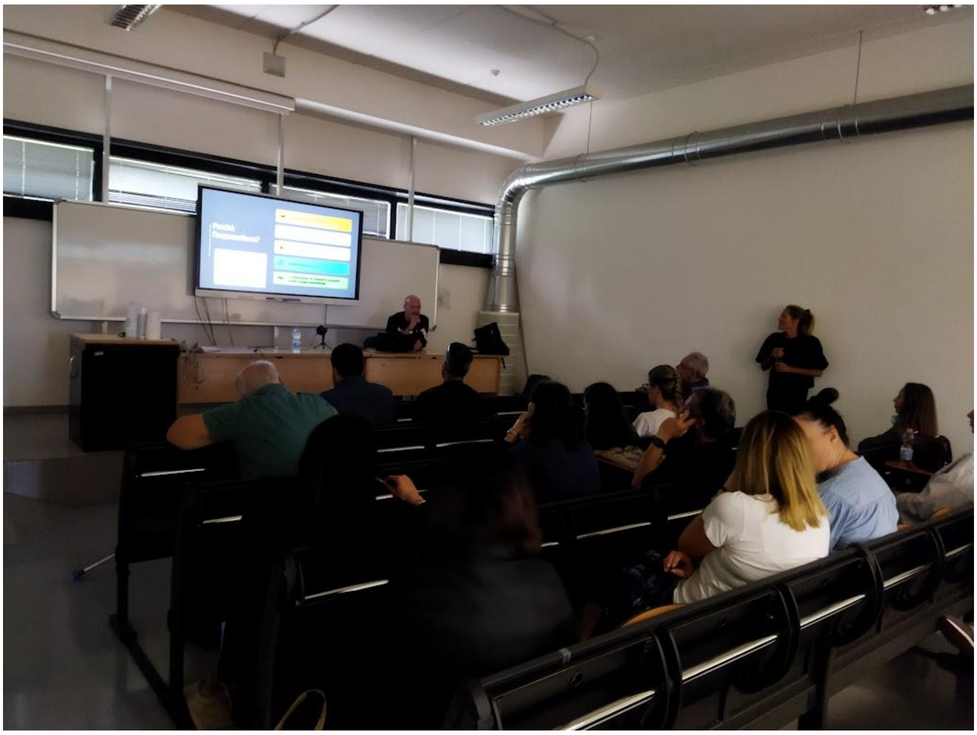
The documentary will be presented soon during an ad hoc meeting scheduled in May 2023. It will be presented to schools, sector operators and ordinary citizens. A preview of this documentary has been presented during the 1st Cross-border training lab and exchange of experiences between Croatian and Italian operators in Italy (11TH October).



FINAL CONSIDERATION AND PROTOCOL TRANSFERABILITY AT CROSS-BORDER LEVEL

Concluding, this pilot action aimed to define a protocol for sustainable management and protection of a shared stock such as that of cuttlefish, starting from the collaboration of local fishermen.

⇒ The activities and measures proposed here are absolutely replicable and transferable to other Italian and Croatian sites.

⇒ The protocol proposed for the assessment and monitoring of the eggs quality represents an important element for monitoring the cuttlefish stock in the Adriatic. The results of this extended monitoring on all the Adriatic coasts (Italian and Croatian) will allow a complete picture of the whole cuttlefish stock and will help its management.

⇒ -The in-land system can be replicated in any other part of both the Italian and Croatian coasts and if used fully it can represent an excellent support for the restocking of cuttlefish in the Adriatic

⇒ -The involvement of fishermen, their availability and support led to realise a documentary which could be used to reach several targets (consumer, school, fishermen…) both Italian and Croatian.