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Cuttlefish and squid egg deposition patterns on artificial devices and trap-like gears: implications for offspring survival and population management

Konstantinos Ganias *, Rozalia Perri, and Anastasios Moditsis

Department of Zoology, School of Biology, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece

Corresponding author: tel +30 231 099 8760; e-mail: kganias@bio.auth.gr.

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We investigated the effect of trap-like gear deployment on the survival of European squid *Loligo vulgaris* and common cuttlefish *Sepia officinalis* eggs laid on various surfaces of these gears. In parallel, a detailed assessment of the two species' egg deposition patterns on such gears was performed with respect to both the fishing season and their preferences for artificial spawning substrates. Hemp ropes with floats were the most preferred spawning substrate for squid, whereas cuttlefish mostly deposited their egg clusters on the plastic mesh of rectangular pots. Almost no egg laying was observed on traps where netting frame was coated with antifouling paints (copper oxide or zinc pyrithione). A high proportion of squid egg mops and cuttlefish egg clusters were shown to either be lost or to die after a period of continuous operation (i.e. hauling and retrieval at frequent intervals), which exceeded egg incubation periods. It is thus advised that coastal fishers either completely avoid operating static gears, which act as artificial spawning substrates on the spawning fields or use gears with materials that are repellent for these animals to approach and lay their eggs, such as nets coated with antifouling substances.

Keywords: common cuttlefish, European squid, mitigation measures, offspring survival, substrate spawners, trap fishing

Introduction

Cephalopods are key ecosystem components of the world ocean (e.g. de la Chesnais *et al.*, 2019) whose populations are proliferating in response to large-scale processes such as global warming and finfish overexploitation (Doubleday *et al.*, 2016). Cephalopods are also important commercial resources, landings of which have been increasing rapidly in recent decades (Hunsicker *et al.*, 2010; Arkhipkin *et al.*, 2020). However, for most European countries, cephalopods are considered a minor resource and their fishing has been assigned a low priority, still not covered by the Common Fisheries Policy (Arkhipkin *et al.*, 2020). The only exception are some Mediterranean artisanal cephalopod fisheries (Quetglas *et al.*, 2015), which are managed nationally or regionally (Grati *et al.*, 2018; Arkhipkin *et al.*, 2020).

Two of the most commercially important cephalopods in the Mediterranean are the common cuttlefish *Sepia officinalis* and the European squid *Loligo vulgaris*. Cuttlefish and squid fisheries in the Mediterranean and the eastern Atlantic deploy both active (mainly bottom trawl) and static gears (Belcari *et al.*, 2002; Lefkaditou *et al.*, 2002; Pierce *et al.*, 2010). However, especially in south European fisheries, artisanal gears dominate, and both species are mainly caught by gillnets, trammel nets, and a great variety of highly selective gears, such as traps, lures, jigs, and spears (Pierce *et al.*, 2010). Small-scale fishery métiers targeting cuttlefish and squid are seasonal and take advantage of the species' spawning behaviour, i.e. migrating *en masse* from deep to shallower waters and thus becoming an exploitable resource for coastal fishers (Pierce *et al.*, 2010; Bloor *et al.*, 2013). Cuttlefish fishing takes place in winter and spring, usually from January to April (Belcari *et al.*, 2002; Tzanatos *et al.*, 2006), whereas squid are mainly fished during early autumn and winter (Lefkaditou *et al.*, 1998; Tsangridis *et al.*, 1998).

The above harvesting strategy can have severe impacts on the species' reproductive potential given that coastal fishers target mature breeding individuals while they are moving towards shore to reproduce (Blanc and Daguzan, 1998; Vila *et al.*, 2010; Ganias *et al.*, 2021a, b). Cuttlefish and squid are substrate spawners, laying their eggs near the shore, where they can remain exposed for prolonged periods to both abiotic (e.g. temperature changes, strong currents, ripples), and biotic (e.g. predation) risks (Bloor *et al.*, 2013; Cabanellas-Reboredo *et al.*, 2014; Martins *et al.*, 2018). Here, a second impact of artisanal coastal fisheries relates to the utilization of static fishing gears by these animals as artificial spawning substrates (Blanc and Daguzan, 1998; Melli *et al.*, 2014; Grati *et al.*, 2018). When cuttlefish and squid eggs are laid on the surfaces of these gears, e.g. on the netting frame, the ropes of the longline or even the floats, they are frequently exposed to ambient air conditions and thus to abrupt changes in humidity and temperature, plus the risk of being detached or even destroyed during the hauling process. Therefore, the need to investigate the effect of these gears' deployment on the viability of cephalopod eggs becomes imperative for the sustainable management and conservation of these resources.

The main aim of this study was to investigate the egg deposition patterns of squid and cuttlefish on trap-like fishing gears and the effect of the hauling process on embryonic survival. For that purpose, we used fishing pots of different shapes (rectangular vs. pyramidal) and netting material (plastic vs. fibre) in order to cover the widest possible range of pot types used in cephalopod—mainly cuttlefish—fisheries in European waters. A specially designed device consisting of various potential egg deposition surfaces was also used to examine possible species-specific preferences on the characteristics of the spawning substrates. In addition, we monitored the survival of squid egg mops and cuttlefish egg clusters deposited on the experimental gears during a period, which exceeded their incubation period under real fishing conditions, i.e. continuous operations at 2–4 d intervals. Finally, in an effort to reduce or even completely prevent egg deposition on these static gears we tested pots with nets coated with antifouling materials (copper oxide and zinc pyrithione), as potential repellents for squid and cuttlefish approaching to deposit their eggs.

Material and methods

Fieldwork consisted of monitoring the processes of laying and development of cuttlefish and squid eggs on specially designed artificial devices (ADs) as well as the occasional collection of some of these eggs for further study in the laboratory. A total of 25 sampling efforts were performed between February and July of 2020 by means of a coastal fishing boat in the area between Nea Michaniona and Angelochori, Thermaikos gulf (Figure 1). This area constitutes an important fishing ground for both squid and cuttlefish. Sampling was interrupted between 22 March and 4 May due to Corona virus disease 2019 (Covid-19) related mobility restrictions in Greece.

Four different types of ADs were used. Three of these types were trap-like fishing gears on which cuttlefish and squid eggs have been observed to be deposited. The first type, which was inspired by the Portuguese cephalopod fishery (PT-type; Figure 2a) was a rectangular pot with a metal frame (H: 40 cm, W: 50 cm, D: 60 cm) lined with a green plastic net (15-mm mesh opening). The two other ADs consisted of a truncated pyramid–shaped frame of galvanized steel, lined with Dyneema net (D-net; 25-mm mesh opening; *cuttletrap;* Figure 2b ; see detailed description in Ganias *et al.*, 2021a). The cuttletraps had two circular openings (42-cm diameter) placed diagonally on opposite vertical sides. A pilot study (Appendix 1) showed that, during prolonged stays at the sea bottom, netting traps undergo intense biofouling, which leads to net clogging in only a few months of continuous operation. For that reason, D-net in cuttletraps was coated with antifouling dyes: either with copper oxide (COPP type) or with zinc pyrithione (ZINC type). Because our purpose was only to examine egg deposition rates, all these fishing gears were inactivated either by using a 40-mm mesh at the rear side of PT pots, which allowed the catch to escape or by closing the openings of the cuttletraps with metal wires and cable ties.

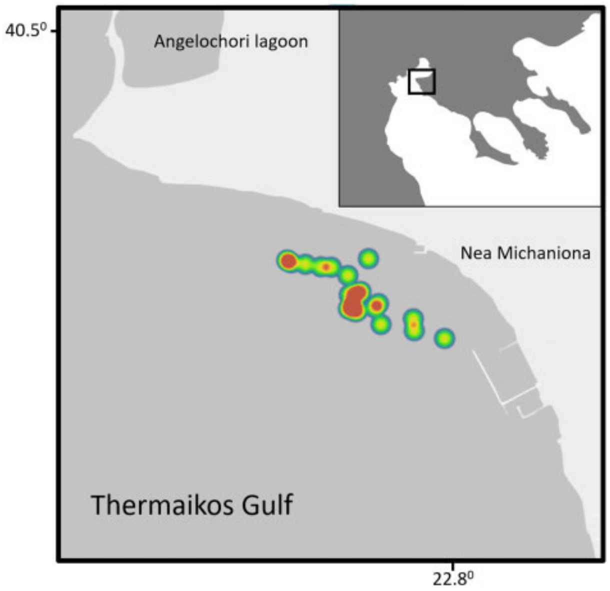

Figure 1. Map of the study area showing the spatial distribution and intensity (heat map) of sampling stations.

The fourth AD (EGG-type) was especially designed to offer various potential egg laying surfaces; therefore it consisted of the simple metal frame of the cuttletrap, with several additional items on it, to allure females (Figure 2c and d) including: (i) a netting frame (25 × 40 cm; uncoated D-net) attached to one of the vertical sides; (ii) two detachable funnel-shaped doors, each consisting of three metal rings (42-, 20-, and 18-cm diameter) attached to the openings; (c) a 3D web-like network made of six to eight elastic ropes (12 mm) with plastic hooks at the ends, hung diagonally and in an irregular manner along the frame bars (Figure 2d); (iv) five hemp ropes (5-mm thick, 1-m long) with a float to keep them vertically positioned in the water, regularly spaced along the upper horizontal side of the frame.

Two different strings of ADs were deployed simultaneously in the field. The first string consisted exclusively of 10 PT pots, whereas the second consisted of 3 ZINC, 2 COPP, and 5 EGG type ADs. During the second survey period, i.e. after cuttlefish eggs started being deposited, shrub branches were placed in five alternating pots of the PT string. The remaining pots of the string were occasionally baited with a female cuttlefish to examine their egg deposition activity on the pot net. In both strings, the ADs were attached to a longline at regular intervals of 20 m, which ensured that when one device was lifted, the next one remained at the bottom. The first and last devices in the string were connected to floats on the sea surface.

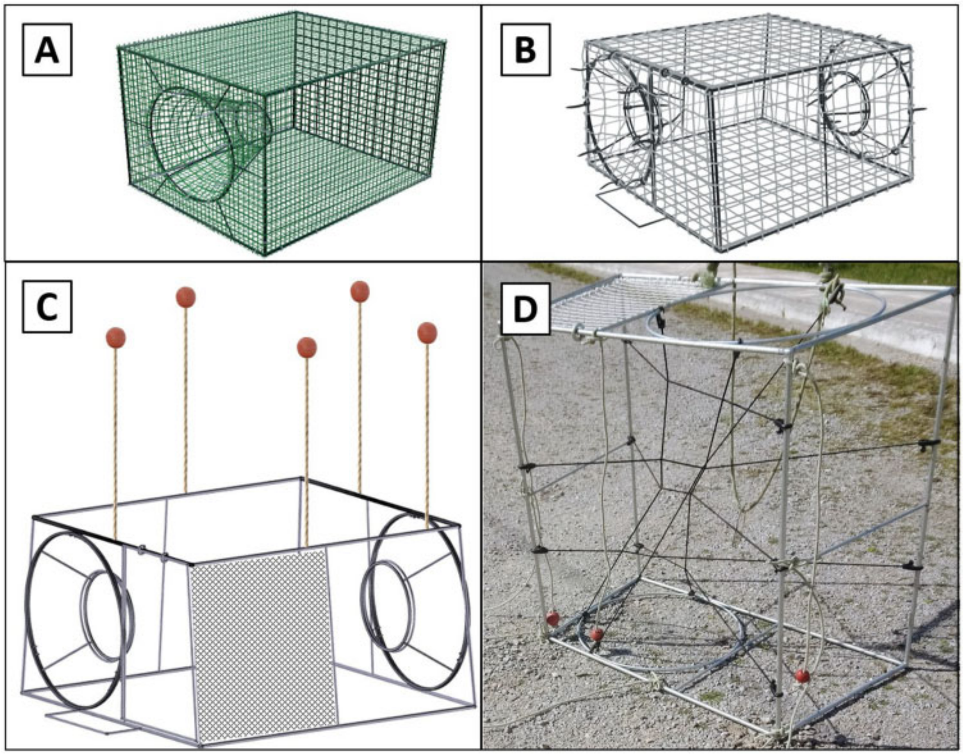

Figure 2. Side view of the PT pot (a) and of the cuttletrap (b). EGG type substrate showing the various artificial spawning surfaces including the vertical hemp ropes, the rectangular netting frame (c) and the weblike network made of elastic ropes (d).

Sampling was performed once or twice per week, depending on weather conditions. On each sampling occasion, geographical coordinates, soak time, sea temperature, bottom type and depth were recorded. Upon string retrieval, the prevalence and number of cuttlefish and squid eggs on each AD was recorded, including the part on which they were deposited, especially in EGG type device. The prevalence of eggs was also recorded on the snoods of the second string as part of the EGG ADs. Squid eggs always occurred in mops of 40–50 capsules, whereas cuttlefish eggs occurred either singly or in clusters. The latter were groups of 10–20 eggs of similar age, presumably deposited by the same female. In that respect, for squid our measurements concerned the number of mops, whereas for cuttlefish our measurements rather concerned the prevalence and number of individual eggs.

The development and mortality rates of squid egg mops and cuttlefish egg clusters were also monitored during the second sampling period *in situ*. This task was performed through identifying newly laid mops and clusters on specific parts of the ADs and subsequently following their development and status, i.e. whether they managed to hatch, or whether they died or were lost in the meantime. Egg development was assessed macroscopically according to the three-stage scale of Zatylny-Gaudin and Henry (2018) for cuttlefish and the four-stage scale of Feyjoo *et al.* (2016) for squid. Newly laid eggs could be identified by their external appearance (early developmental stage) and their first recording on the part of the device they were found.

Squid eggs mortality rates were further assessed in the laboratory. Three newly laid mops were detached with the utmost care from hemp ropes, placed in a portable cooler with sea water and transported to the lab within a few hours after sampling. The mops were placed in an aquarium (38 × 24 × 45 cm) with artificial sea water (30‰) and continuous ventilation (Figure 3a); temperature was maintained within the viability range of squid embryos (19–22°C). Each mop was initially suspended on a piece of plastic net (150 × 150 mm; 25-mm mesh opening) fixed horizontally to the upper layer of the water column, water column and there followed a 2-week incubation period of no treatment at all. After this control period, mops were removed from the water for ca. 5 min—i.e. the mean time that the ADs remained on-board during sampling—and this procedure was repeated every 2–5 d until all eggs were observed to have died. At every treatment, one capsule was removed from each mop and placed into a small vial with 10% neutral buffered formalin and retained for microscopic examination. The latter consisted of extracting all the eggs from the capsule, counting them and identifying their developmental stage based on Feyjoo *et al.* (2016). A fourth squid mop was split into two parts: the first part was subjected to the exact same conditions with the previous experiment (control) and the other part was placed into a second aquarium on a piece of net coated with copper oxide (Figure 3b).

Results

Egg deposition patterns in squid

In total, 46 distinct egg mops were recorded on ADs. Some of these mops were only recorded once, whereas others were recorded multiple times. This explains why the total number of ADs with mops ($n = 65$) and the total count of mop recordings ($n = 114$) during the survey were quite higher. These differences

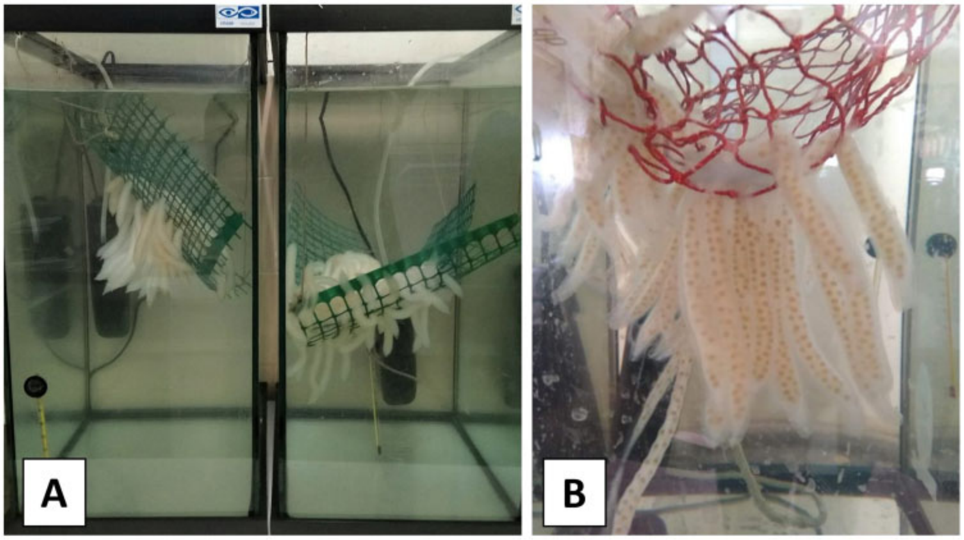

Figure 3. Laboratory kept squid egg mops placed on (a) a plastic mesh and (b) a fibre net coated with copper oxide.

were obviously due to large incubation periods for squid eggs and to devices with more than one mops on their surfaces. The monthly evolution of the number of devices with mops showed that squid eggs were mainly deposited during the first survey period, peaking in March, with a declining trend between May and July (Figure 4a).

Of all individual egg mops, 44 (96%) were recorded on EGG type ADs, and one on each of the COPP and ZINC types (Figure 4b). No squid eggs were recorded on PT type ADs. There were no statistical differences in the number of squid egg mops found on the five different EGG ADs used ($\chi^2 > 0.1$). Regarding the occurrence of squid eggs on the different parts of the EGG ADs the largest proportion was found on hemp ropes (39%). Field observations by means of scuba diving confirmed that these ropes floated in the water column while carrying the mops (Figure 5). The proportion of eggs deposited on the metal frame and the web-like surface of the EGG AD was 22% and 17%, respectively. A low amount of eggs was deposited on the doors (11%) and the snoods (9%) while almost no eggs were deposited on the netting frame (2%).

The proportion of hatched squid eggs was particularly low during the second period of the survey, since only 4 out of the 18 mops (22%) managed to remain attached to the ADs until hatching. Of the remaining mops, 6 (34%) were lost whilst 8 (44%) were found dead. It is presumed that those batches were lost either due to sampling procedure or because of harsh ocean conditions, e.g. strong currents. The hemp ropes were not only successful concerning the higher egg deposition rates but also concerning the egg survival rates since three-fourth of hatched mops were deposited on this type of surface; the other one was found on the metal frame of an EGG AD. These mops were first recorded on different sampling dates, between 20 and 29 May, but were finally scored as hatched on the same sampling date, on 18 June. Water temperature during this period ranged between 15°C and 20°C. Given the 10 d range in the date of first occurrence of these mops we considered the minimum laying-to-hatching duration, as the most reliable estimate of the incubation period for the given temperature range. Therefore, the incubation period of squid eggs in 15–20°C must be ca. 20 d.

Egg deposition patterns in cuttlefish

In most cases, cuttlefish eggs occurred singly and only on few occasions did we observe clusters of 10–20 eggs. This number is much less than oviduct capacity (150–200 eggs; Zatylny-Gaudin and Henry, 2018) meaning that cuttlefish spawning in the area might be characterized as highly intermittent with individual female laying around 100 egg batches or more (Rocha *et al.*, 2001). Only these clusters ($n = 9$) could be precisely monitored and thus utilized to track the development and the evolution of the status (i.e. hatched, dead, or lost) of cuttlefish eggs in the field. Because of this, the only parameter that could be directly compared with squid was the number of ADs with cuttlefish eggs ($n = 62$). The abundance of cuttlefish eggs was assessed using a rough estimate of their total number on each device.

None of the nine depositions that were monitored on PT ADs managed to hatch. However, four depositions were at stage 3 on the last date of our survey, on 29 July. These four depositions were first recorded on 7 July, suggesting that they remained on the ADs for 22 d. Of the remaining depositions, four were found dead, whereas two were lost.

The seasonal pattern of cuttlefish egg prevalence was different from squid, showing very low values until May and an abrupt increase between June and July (Figure 4b). The abundance of cuttlefish eggs showed a very similar pattern, being almost null till May and peaking in July. Concerning the preferred device, we again used the break-up of the number of ADs with cuttlefish eggs into the various device types. Most cuttlefish eggs (83%) were recorded on PT type ADs while their prevalence on the remaining devices was quite low, ranging from 13% in the COPP type, to only 2% and 3% in the ZINC and EGG types, respectively (Figure 4b).

In total, four PT pots were baited with a female cuttlefish: one on the 29 May and the other three on the 3 July. The first female remained in the pot for 23 h and the other three for ca. 48 h. In both trials, all pots of the string were free of cuttlefish eggs before the placement of the female. At retrieval, baited pots had 70–200 eggs each mainly laid on the plastic net. Simply based on observation, it was unclear whether the eggs were deposited from the inside or from the outside of the AD. Even though, given that the

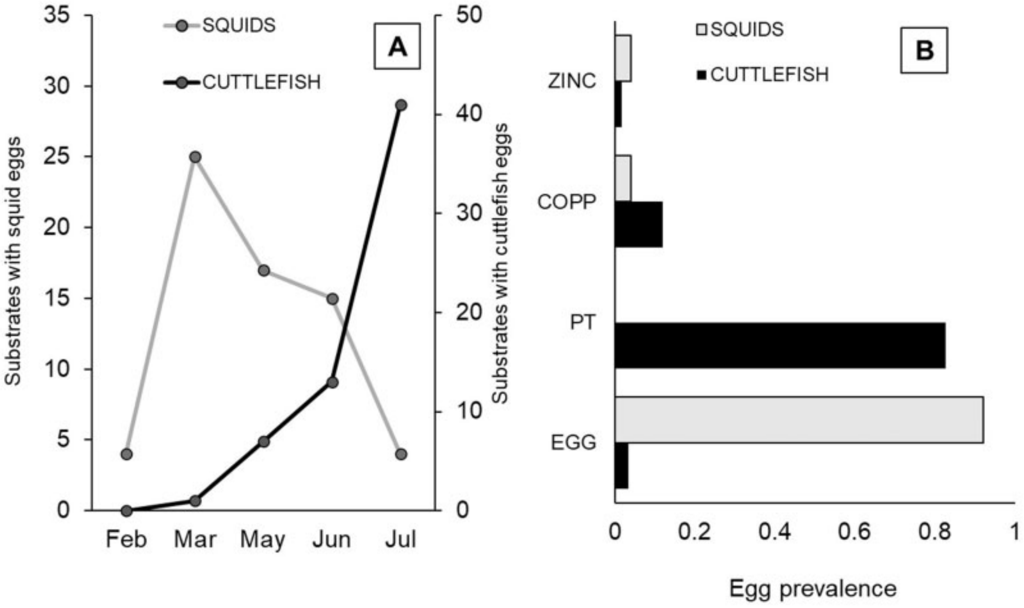

Figure 4. (a) seasonal variation in the number of artificial spawning substrates with squid egg mops and cuttlefish eggs; (b) breakdown of the prevalence of cuttlefish and squid eggs on the various substrate types.

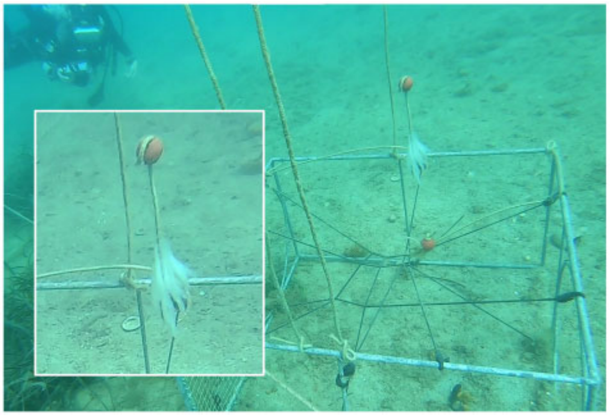

Figure 5. Photograph of an EGG type substrate with a squid egg mop attached to a hemp rope in the field.

remaining pots on the string had no eggs at all, it was presumed that the eggs were laid by the baited females from the inside of the device. The placement of shrub branches did not significantly affect the deposition of cuttlefish eggs compared with empty traps (Table 1).

Lab experiment

After the 2-week control period, 100% of squid embryos from all three mops were viable (Figure 6). In two mops, embryos remained alive 5 d after the onset of treatments, whereas in the third mop the survival rate was 80%. Thereafter, the fraction of viable eggs steadily declined at a similar rate between the three mops and all embryos were dead 19 d after the first treatment, that is, 33 d after the beginning of the experiment (Figure 6).

Concerning the second experiment, the embryos in the portion of the mop that was placed on the coated net died within just a few hours. The embryos became highly opaque which, however, allowed an accurate estimation of their number in each capsule (Figure 3b). The portion of the mop that was placed on the control net exhibited similar survival rates to the first experiment.

Table 1. Prevalence and mean intensity of cuttlefish eggs in PT pots of different state.

Pot status	Prevalence (%)	Intensity
With shrubs	22.9	16.0
Baited with cuttlefish	66.7	54.8
Empty	20.3	20.1

Discussion

Due to their reproductive life history, the survival of the common cuttlefish and the European squid populations depends directly on the availability of spawning substrates (Grati *et al.*, 2018). Artificial spawning substrates tested in this study were attractive for both species. Specifically, the egg deposition rate in squid peaked in March and declined thereafter whilst the prevalence of cuttlefish eggs was very low in February and March and increased abruptly during the second part of the survey, peaking in July. These patterns match previous reports on the spawning season of the two species in the eastern Mediterranean (e.g. Moreno *et al.*, 2005; Akyol, 2011). In addition to different spawning seasonality, the two species exhibited differences in their preferred AD types. These differences were solely attributed to the technical characteristics of the devices and not to regional or temporal effects since all AD types were operated simultaneously at the same sampling area. Specifically, most cuttlefish eggs were found on PT ADs, whereas squid mostly deposited their egg mops on EGG ADs. It is worth noting that only a few cuttlefish eggs were found on the EGG ADs, whereas no squid eggs were found on the PT ones. ADs with coated nets exhibited particularly low proportions of egg deposits; even in this case, the two species tended to deposit

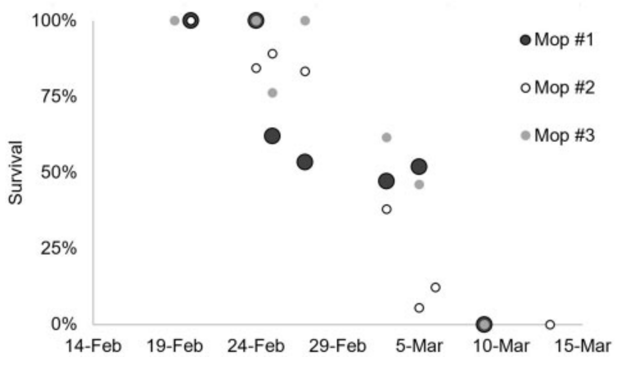

Figure 6. Change in the survival rate of the three laboratory-kept squid egg mops after the onset of the treatment period.

their eggs on different parts of their surfaces. Specifically, cuttlefish mostly deposited their eggs on the mesh, whereas squid used the metal frame, especially the upper parts.

Apparently, the observed differences in the preferred AD types are triggered by the egg-laying behaviour of the two species. Common cuttlefish lays their eggs separately in cases on structures fixed to the seabed including natural substrates, e.g. plants and sessile animals, and artificial structures, e.g. fishing pots, ropes and branches (Blanc and Daguzan, 1998; Bloor *et al.*, 2013). In their detailed description of the species' spawning habitat, Guerra *et al.* (2016) showed that the common cuttlefish mostly tends to spawn on hard bottom shoals covered by sea fans and sea worms. This explains why in artificial or laboratory conditions plastic nets are effective egg laying substrates for cuttlefish (Sykes *et al.*, 2006; Zatylny-Gaudin and Henry, 2018); obviously, a rigid plastic mesh resembles some of the species' physical spawning substrates like the densely branching, fan-like stems of corals or the ribbon-like leaves of seaweed. This type of mesh (green plastic with 15–25 mm opening) was used in the PT type AD explaining the high prevalence of cuttlefish eggs on this type. Conversely, the mesh of ZINC and COPP ADs exhibited low prevalence of cuttlefish eggs, presumably due to its elasticity or to its coating with zinc-pyrithione and copper oxide, respectively. The coatings' purpose was to reduce biofouling and thus keep the nets clean for longer operation periods. However, laboratory kept squid egg capsules placed on a mesh coated with copper oxide died in <1 d, suggesting that net coating might deter squid and cuttlefish from deposing their eggs.

Although Bloor *et al.* (2013) suggested that cuttlefish eggs can be attractive as spawning substrates for other females, this study showed that egg laying in the cuttlefish can also be a solitary process. In our string trials with a female spawner placed inside one of the pots, cuttlefish eggs only prevailed on the plastic net of this particular pot. This finding either suggests that some females were attracted by the baited females and, thus, ended up laying their eggs on the pot or that all the eggs were shed by the baited females. The latter option is more probable because spawning female cuttlefish mostly attract males and not other females (Watanuki *et al.*, 2000; Ganias *et al.*, 2021b). If this is indeed the case, these females deposited 70–200 eggs on the mesh of the pots within 23–48 h.

The study area is a major fishing ground for cuttlefish in the North Aegean, with trammel net catches exhibiting a seasonal peak between January and April (Ganias *et al.* 2021a). Given that during this period the largest females (older cohort) of the population, which are mainly targeted from this fishery are spawning capable (Laptikhovsky *et al.*, 2003, 2019; Önsoy and Salman, 2005), we assume that the observed lack of eggs on our artificial spawning devices is due to a seasonal shift in the spawning area of the population. During late spring and summer, i.e. the period when cuttlefish egg depositions prevailed on PT ADs, there is a well-documented demographic shift and only smaller females (younger cohort) occur in the population (Ganias *et al.*, 2021a). We may thus assume that our sampling area, which ranged between 6 and 11 m in depth only covered the spawning field of the younger cohort and not of the older cohort, which spawns first (see also: Laptikhovsky *et al.*, 2003; Önsoy and Salman, 2005). This assumption remains to be evaluated in a future study.

Squid attach their egg mops to rocks, debris and other hard objects on sandy to muddy bottoms (Jereb and Roper, 2010). Cabanellas-Reboredo *et al.* (2014) showed that *L. vulgaris* eggs were mostly recorded between depths of 18 and 50 m and on artificial spawning substrates located on sandy bottoms. Egg depositions were found less on rocky bottoms and only a few on phanerogam beds. According to Jereb and Roper (2010), females tend to lay egg capsules close to or on top of other egg masses, so that large mops of up to 40 000 eggs occur in nature. The individual contribution by a single female is limited to a thousand, ergo a mass of 6000 eggs suggests the contribution of six to seven females. Thus, in contrast to cuttlefish, squid deposit their egg capsules in a more social manner (Hanlon *et al.* 2002). This means squid egg mops need to be laid on physical or artificial substrates that are easily distinguishable by other females (Hanlon *et al.*, 2002), which explains why hemp lines with floats were the most successful spawning substrate for squid in this study. Similar artificial spawning substrates based on ropes vertically positioned in the water column have been used both for the European squid (Cabanellas-Reboredo *et al.*, 2014; Feyjoo *et al.*, 2016) and for other *Loligo* sp. (e.g. Hasaruddin *et al.* 2015).

The observed preference of both cuttlefish and squid for some of the artificial spawning devices used in this study, does not mean that the same species cannot show other preferences in other circumstances and/or geographical areas. For example, Grati *et al.* (2018) tested various artificial spawning substrates for the common cuttlefish in the Adriatic Sea showing that eggs were mostly deposited on floating ropes (50-cm long) attached to a longline set on the seafloor. Other efficient artificial spawning substrates for cuttlefish include hemp ropes placed inside cuttlefish traps (Melli *et al.*, 2014) and a seagrass-like device tested by Blanc and Daguzan (1998) in the northern Bay of Biscay. However, a similar device proved ineffective when tested in the Adriatic Sea and its failure was mainly due to its tendency to sink in the muddy sea bottom of the study area (Grati *et al.* 2018). We may thus postulate that that the inefficacy of hemp ropes to act as spawning substrates for cuttlefish in our study can be explained by the physical characteristics of the survey area. The seabed in this study was quite diverse, consisting of sand mixed with shell fragments and scattered clusters of *Posidonia oceanica* mats and/or *Cladocora caespitosa* colonies, while a great diversity of sedentary organisms including sponges, polychaetes, and ascidians also occurred. Another possible cause is that in contrast to other studies, the floating ropes in our EGG ADs were longer (100 cm) and tied to the upper panel of the metal frame, i.e. 40 cm above the seabed. This height might be preventive for cuttlefish that seek lower shelters to spawn but not for squid, which reproduce in a more social manner.

Implications for management

Due to their short-lived, fast-growing life cycle, cuttlefish and squid stocks are composed entirely of one or two overlapping generations (Arkhipkin, 1995; Moreno *et al.*, 2007; Bloor *et al.*, 2013). The lack of buffering from multiple overlapping generations leaves their populations vulnerable to variations in spawning and early life stage survival (Pierce and Guerra, 1994). Any effort in assessing the spatial and temporal extent of spawning activity and in enhancing offspring survival can thus be of vital importance for the conservation of these stocks.

Trap-like artificial spawning substrates similar to the ones used in this study can provide valuable insights on the spatiotemporal reproductive patterns of both species. Due to their simple design, low cost and ease of operation, they can be effectively used to assess the temporal and spatial range of spawning. This information can be utilized by scientists, fishery managers or even fishers themselves to map spawning fields and to subsequently protect them from degrading activities, including bottom fishing gears like dredges, beam trawls or otter trawls. Simple actions such as monitoring plans on the prevalence of cuttlefish or squid eggs on lightweight and cheap devices such as PT pots and EGG ADs delivered to professional or recreational fishermen might provide a good operational tool for the management of local cephalopod fisheries. A similar action is described by Grati *et al.* (2018) for the cuttlefish trap fishery in the Adriatic, where recreational fishermen collaborated well in providing logbooks of the coverage of artificial substrates with cuttlefish eggs.

Squid egg mops transferred to a lab aquarium were 100% viable 2 weeks after no treatment. However, when the mops started being taken out of the aquarium imitating the process of trap hauling and retrieval, i.e. for about 5 min every 2.6 d, their mortality suddenly increased and all the eggs died within 2 weeks. Amongst the mops that were monitored in the field 22% reached the hatching stage. This higher survival rate is likely due to the longer mean interval between field sampling, which was 5 d (double that of laboratory treatments). It may thus be deduced that under conditions of regular fishing operations, which may often reach daily retrieval intervals, the mortality of squid eggs would be much higher, even 100%. Concerning cuttlefish, 40% of egg clusters remained on PT pots for 22 d, until the end of fieldwork operations. Given that incubation time may exceed 30 d at 18–20°C (sea temperature in July) we may assume that the proportion of egg clutches that would have survived to hatching would be lower, had field effort been prolonged.

Based on these results the main advice that should be given to trap fishers is to avoid operating inside the spawning fields, given that the damage is already done by the time the eggs are attached to the gears. Similarly, advice of the type "if cuttlefish eggs are found attached to traps take care to minimize damage caused to these eggs when hauling and shooting gear" or "avoid cleaning or washing traps when cuttlefish eggs are found attached" [e.g. Southern Inshore Fisheries and Conservation Authority (2018)] is of little to no value at all since most of this spawn is destined to be lost either way. On the other hand, trap operations could become beneficial if used to map the spawning fields of cephalopods, as described previously.

An alternative way of minimizing egg deposition rates on fishing traps could be to use nets coated with antifouling paints. In this study, traps with nets coated with zinc pyrithione and copper oxide, two antifouling materials extensively used in finfish mariculture cages, displayed particularly low egg deposition rates compared with PT (for cuttlefish) and EGG (for squid) type ADs. Obviously, such biocides apart from preventing marine growth on man-made structures are also repulsive for larger animals like squid and cuttlefish to approach and deposit their eggs. These gears where inactivated during the survey, e.g. through closing their entrance doors; therefore, their fishing efficiency for cuttlefish or other organisms (e.g. fish, crabs and other crustaceans) could not be examined. Despite this, due to low biofouling rates and minimized clogging, net coating should overall be beneficial for the trap fishing process, increasing gear resilience and ease of operation due to lower weight, and minimizing cleaning time.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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References

Akyol, O. 2011. Preliminary results on the cuttlefish, *Sepia officinalis*, reproduction in Izmir Bay (Aegean Sea). Journal of Fisheries Science, 5: 122.

Arkhipkin, A. 1995. Age, growth and maturation of the European squid *Loligo vulgaris* (Myopsida, Loliginidae) on the west Saharan shelf. Journal of Marine Biological Association of the United Kingdom, 75: 593–604.

Arkhipkin, A. I., Hendrickson, L. C., Payá, I., Pierce, G. J., Roa-Ureta, R. H., Robin, J.-P., and Winter, A. 2020. Stock assessment and management of cephalopods: advances and challenges for short--lived fishery resources. ICES Journal of Marine Science, doi: 10.1093/icesjms/fsaa038.

Belcari, P., Sartor, P., Sanchez, P., Stract, a. B., Tsangridis, A., Leondarakis, P., and Lejkaditou, E. 2002. Exploitation patterns of the cuttlefish, *Sepia officinalis* (Cephalopoda, Sepiidae), in the Mediterranean sea. Bulletin of Marine Science, 71: 187–196.

Blanc, A., and Daguzan, J. 1998. Artificial surfaces for cuttlefish eggs (*Sepia officinalis* L.) in Morbihan Bay. France Fisheries Research, 38: 225–231.

Bloor, I. S. M., Attrill, M. J., and Jackson, E. L. 2013. A review of the factors influencing spawning, early life stage survival and recruitment variability in the common cuttlefish (*Sepia officinalis*). Advances in Marine Biology, 65: 1–65.

Cabanellas-Reboredo, M., Calvo-Manazza, M., Palmer, M., Hernández-Urcera, J., Garci, M. E., González, Á. F., Guerra, Á. *et al.* 2014. Using artificial devices for identifying spawning preferences of the

European squid: usefulness and limitations. Fisheries Research, 157: 70–77.

de la Chesnais, T., Fulton, E. A., Tracey, S. R., and Pecl, G. T. 2019. The ecological role of cephalopods and their representation in ecosystem models. Reviews in Fish Biology and Fisheries, 29: 313–334.

Doubleday, Z. A., Prowse, T. A. A., Arkhipkin, A., Pierce, G. J., Semmens, J., Steer, M., Leporati, S. C. *et al.* 2016. Global proliferation of cephalopods. Current Biology, 26: R406–R407.

Feyjoo, P., Cabanellas-Reboredo, M., Calvo-Manazza, M., Morales-Nín, B., Hernández-Urcera, J., Garci, M. E., González, Á. F. *et al.* 2016. New insights on the external features of egg capsules and embryo development in the squid *Loligo vulgaris*. Journal of Natural History, 50: 543–555.

Ganias, K., Christidis, G., Kompogianni, I. F., Simeonidou, X., Voultsiadou, E., and Antoniadou, C. 2021a. Fishing for cuttlefish with traps and trammel nets: a comparative study in Thermaikos Gulf, Aegean Sea. Fisheries Research, 234: 105783.

Ganias, K., Kompogianni, I. F., and Christidis, G. 2021b. How lure prevalence, survival rate and maturity status affect catchability in cuttlefish trap fishery. Fisheries Research, 238: 105919.

Grati, F., Fabi, G., Scarcella, G., Guicciardi, S., Penna, P., Scanu, M., Leoni, S. *et al.* 2018. Artificial spawning substrates and participatory research to foster cuttlefish stock recovery: a pilot study in the Adriatic Sea. PLoS One, 13: e0205877.

Guerra, Á., Hernández-Urcera, J., Garci, M. E., Sestelo, M., Regueira, M., Gilcoto, M., and González, Á. F. 2016. Spawning habitat selection by the common cuttlefish *Sepia officinalis* in the Cíes Islands (Northwest Spain). Fisheries Research, 183: 44–54.

Hanlon, R. T., Smale, M. J., and Sauer, W. H. H. 2002. The mating system of the squid *Loligo vulgaris* reynaudii (Cephalopoda, Mollusca) off South Africa: fighting, guarding, sneaking, mating and egg laying behavior. Bulletin of Marine Science, 71: 331–345.

Hasaruddin, H., Ibrahim, S., Hussin, W. M. R. W., Ahmad, W. M. A. W., and Muchlisin, Z. A. 2015. Artificial aggregating device for fish and squid eggs. AACL Bioflux, 8: 832–837.

Hunsicker, M. E., Essington, T. E., Watson, R., and Sumaila, U. R. 2010. The contribution of cephalopods to global marine fisheries: can we have our squid and eat them too? Fish and Fisheries, 11: 421–438.

Jereb, P., and Roper, C.F.E. (Eds). 2010. Cephalopods of the world. An annotated and illustrated catalogue of cephalopod species known to date. Volume 2. Myopsid and Oegopsid Squids. *In* FAO Species Catalogue for Fishery Purposes. No. 4, Vol. 2. Rome, FAO. 605 p. 10 colour plates.

Laptikhovsky, V., Barrett, C., Firmin, C., and Ouréns, R. 2019. Adaptations of the common cuttlefish *Sepia officinalis* L. to cold water reproduction in the english channel. Journal of Shellfish Research, 38: 629.

Laptikhovsky, V., Salman, A., Önsoy, B., and Katagan, T. 2003. Fecundity of the common cuttlefish, *Sepia officinalis* L. (Cephalopoda, Sepiidae): a new look at an old problem. Scientia Marina, 67: 279–284.

Lefkaditou, E., Sánchez, P., Tsangridis, A., and Adamidou, A. 1998. A preliminary investigation on how meteorological changes may affect beach-seine catches of *Loligo vulgaris* in the Thracian sea (eastern Mediterranean). South African Journal of Marine Science, 20: 453–461.

Lefkaditou, E. G., Kavadas, S., and Papaconstantinou, C. 2002. Cephalopod Fisheries Statistics in Greek Waters (NE Mediterranean). Working Group on Cephalopod Fisheries and Life History, Lisbon, Portugal.

Martins, C. P. P., Fernández-Álvarez, F., and Villanueva, R. 2018. Invertebrate predation on egg masses of the European cuttlefish, *Sepia officinalis*: an experimental approach. Estuarian, Coastal and Shelf Science, 200: 437–448.

Melli, V., Riginella, E., Nalon, M., and Mazzoldi, C. 2014. From trap to nursery. mitigating the impact of an artisanal fishery on cuttlefish offspring. PLoS One, 9: e90542.

Moreno, A., Azevedo, M., Pereira, J., and Pierce, G. J. 2007. Growth strategies in the squid *Loligo vulgaris* from Portuguese waters. Marine Biology Research, 3: 49–59.

Moreno, A., Pereira, J., and Cunha, M. 2005. Environmental influences on age and size at maturity of *Loligo vulgaris*. Aquatic Living Resources, 18: 377–384.

Önsoy, B., and Salman, A. 2005. Reproductive biology of the common cuttlefish *Sepia officinalis* L. (Sepiida: Cephalopoda) in the Aegean Sea. Turkish Journal of Veterinary and Animal Science, 29: 613–619.

Pierce, G. J., and Guerra, A. 1994. Stock assessment methods used for cephalopod fisheries. Fisheries Research, 21: 255–285. doi: 10.1016/0165-7836(94)90108-2

Pierce, G. J., Allcock, L., Bruno, I., Jereb, P., Lefkaditou, E., Malham, S., Moreno, A. *et al.* 2010. Cephalopod biology and fisheries in Europe. ICES Cooperative Research Report, 303: 175

Quetglas, A., Keller, S., and Massutí, E. 2015. Can Mediterranean cephalopod stocks be managed at MSY by 2020? The Balearic Islands as a case study. Fisheries Management and Ecology, 22: 349–358.

Rocha, F., Guerra, Á., and González, Á. F. 2001. A review of reproductive strategies in cephalopods. Biological Reviews, 76: 291–304.

Southern Inshore Fisheries and Conservation Authority. 2018. Southern IFCA Cuttlefish Traps Code of Practice. Available at: https://secure.toolkitfiles.co.uk/clients/25364/sitedata/files/Cuttlefishtrapcodeofpracti.pdf (last accessed 28 March 2021).

Sykes, A. V., Domingues, P. M., Correia, M., and Andrade, J. P. 2006. Cuttlefish culture - state of the art and future trends. Vie Milieu, 56: 129–137.

Tsangridis, A., Lefkaditou, E., and Adamidou, A. 1998. Analysis of catch and effort data of *Loligo vulgaris* in the W. Thracian Sea. Rapport Commission International Mer Mediterranee, 35: 494–495.

Tzanatos, E., Somarakis, S., Tserpes, G., and Koutsikopoulos, C. 2006. Identifying and classifying small-scale fisheries metiers in the Mediterranean: a case study in the Patraikos Gulf. Greece Fisheries Research, 81: 158–168.

Vila, Y., Silva, L., Torres, M. A., and Sobrino, I. 2010. Fishery, distribution pattern and biological aspects of the common European squid *Loligo vulgaris* in the Gulf of Cadiz. Fisheries Research, 106: 222–228.

Watanuki, N. A., Iwashita, T. O., and Kawamura, G. U. 2000. Cuttlefish spawning and visually mediated entry into basket traps. Fisheries Science, 66: 185–189.

Zatylny-Gaudin, C., and Henry, J. 2018. Egg-laying in the cuttlefish *Sepia officinalis*. Biological Resources of Water, Sajal Ray, IntechOpen, DOI: 10.5772/intechopen.71915. Available from: https://www.intechopen.com/books/biological-resources-of-water/egg-laying-in-the-cuttlefish-sepia-officinalis.

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Appendix 1. Biofouling rates in coated and uncoated netting traps

Due to long time periods that netting traps remain underwater they can be subject to biofouling i.e. the growth of unwanted organisms on their surfaces (nets, frames, and lines). The most common way to prevent or delay biofouling is to coat the submerged structures and net-cages with anti-fouling paints. A pilot study was carried out to test the effectiveness of coated netting

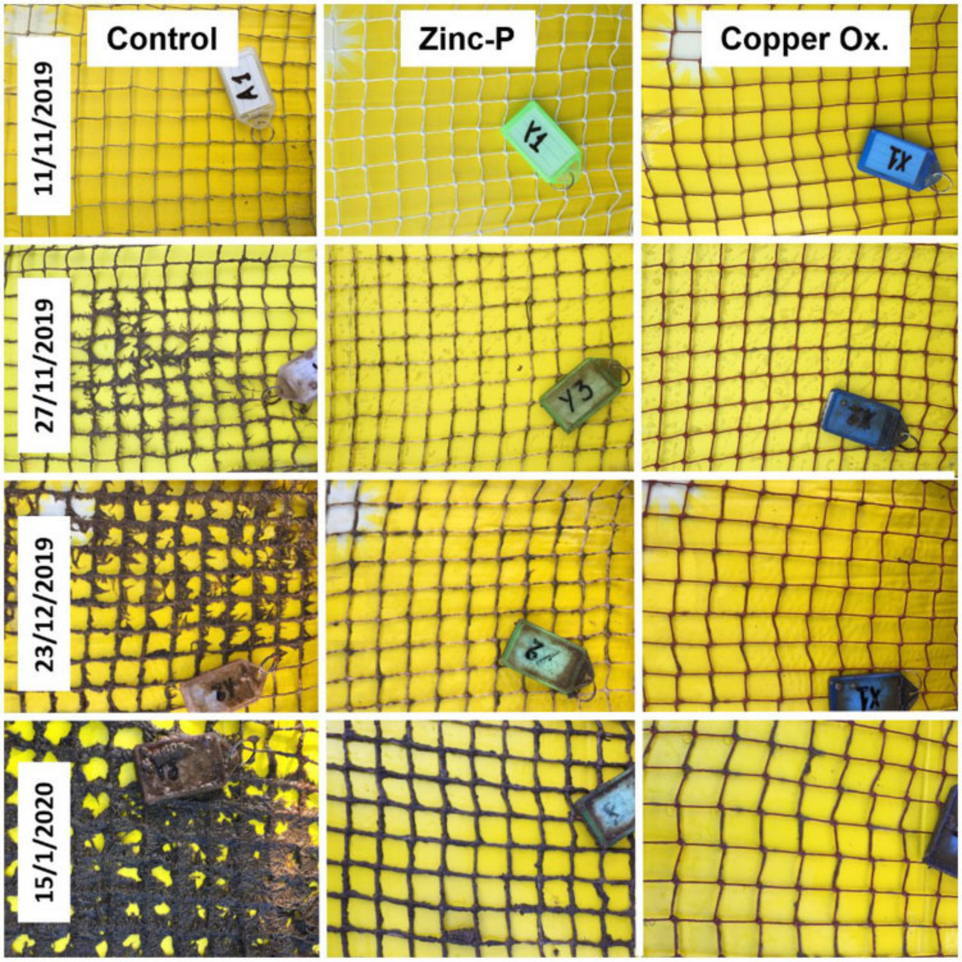

Figure A1. Change in the degree of fouling between the control nets and nets coated with zinc pyrithione and with copper oxide during the pilot study.

traps in relation to conventional traps with uncoated nets. Ten cuttletraps (pyramid-shaped netting traps) were used: five were covered with uncoated net (control traps) and five were covered with nets coated with copper-oxide (two traps) and zinc-pyrithione (three traps). Traps remained underwater, in the field (off northern Michaniona fishing harbour, Thermaikos gulf) from the beginning of October 2019 to mid-February 2020 and were monitored biweekly for the progression of biofouling on the nets. Biofouling was first evident in control traps ca. 1 month after the beginning of the survey and thereafter the settlement and growth of algal spores in *control nets* and their surface occupation on the nets' surface was continuous until the end of our survey (Figure A1). Four months after, mesh occlusion caused from biofouling was almost complete in control nets. In contrast, net coating caused either reduced marine growth rates in traps with zinc-pyrithione or almost no biofouling at all in traps with copper-oxide (Figure A1). More specifically, in nets coated with zinc-pyrithione the rate of biofouling was quite slow compared with control traps and their condition after 4 months underwater was very similar to that showed by control traps 1 month after the beginning of the survey. Traps coated with copper oxide remained completely clean until the end of the survey.