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Recruitment of Oysters by Different Collection Devices at a Longline Shellfish Farm in the Central Adriatic Sea

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Abstract: In 2020–2021, a trial to recruit flat oysters was implemented at a longline farm in the central Adriatic, whereby the efficiency recruitment (n. oyster/dm^2) of different suspended substrates was evaluated. Two lantern nets (50 cm diameter; 145 cm h) had different substrates composed of 8 mm wide wrinkled ribbon and empty oyster shells positioned in the upper levels of the lanterns. The tumbling evaluation and the presence of mud were also considered. The efficiency recruitment was similar between the wrinkled ribbon and the oyster shell. Recruitment was in the same proportion on the external rough part of the shells as on the internal smooth part of the shells. No significant differences were shown when comparing the different substrates in terms of recruitment efficiency.

Keywords: oyster collector; efficiency recruitment; oyster spat; cupped oyster; flat oyster; longline farming technique

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1. Introduction

Recently, it has been recognized that bivalves can improve the sustainability of the aquaculture sector, filling an important ecological role in "ecosystem goods and services". This concept is based not only on direct use, such as food sources through harvesting, but also on indirect use, which, in the case of oysters, includes habitat creation, recreational interest, water remediation, and carbon sequestration [1].

Globally, the cupped oyster (*Crassostrea gigas*) is the main farmed shellfish species, with 6060.566 million t being produced in 2020, corresponding to 7079.505 million USD in value [2]. Strategies to recruit this species mainly focus on seed self-sufficiency to support farming activities, with potential relevance for organic production protocols and natural banks. Contrastingly, the production of the European flat oyster (*Ostrea edulis*) in 2019, globally, amounted to 2746 million t, of which 79.5% were from aquaculture and 20.5% were from wild capture [3]. Wild capture has noticeably dropped over the last two decades as natural stocks are threatened by both overfishing and harmful parasites (*Bonamia* spp. and *Marteilia* spp.) [4–7]. At the same time, flat oyster farming had not developed, as wild seeds were rare following the depletion of natural stocks. Furthermore, recruitment techniques had not been fully assessed, and hatchery-produced spat showed low survival rates under farmed conditions.

Reliable methods for the reproduction and artificial breeding of oysters in hatcheries have been widely investigated [8–14]. While hatchery-produced spat is now available on the market, demand and quantity remain very limited, as survival rates during the nursery and pre-growing stages tends to be very low. The reproduction and recruitment in traditional ponds situated in marsh areas require improvement, even if always considered valuable for the environment [15,16]. The recruitment strategies for flat oysters have focused on restoring wild stocks, which could be a precondition for ensuring seed self-sufficiency for farming.

Both cupped and flat oysters have similar reproductive strategies. However, cupped oyster larvae have a longer planktonic stage (3–4 weeks) compared to flat oysters (about 2 weeks). At this stage, free larvae are released after incubating in the female pallial cavity; consequently, dispersal is shorter in flat oysters compared to cupped oysters. Furthermore, flat oyster larvae are heavier than cupped oyster larvae, swimming nearer to the seabed. At the end of the planktonic stage in the wild, the larvae of both species settle on a variety of natural substrates. Many trials have been carried out using different substrates to obtain oyster spats from the natural field. The most commonly used substrates to recruit oysters include empty bivalve shells (cultch), rope, coco rope, hemp rope, ribbon, Chinese hats (or coupelles), tubes, and tiles (sometimes covered with quicklime) [17–21]. Most of these substrates have been scientifically tested in hatchery or field conditions [7]. In particular, the flat oyster favors hard natural substrates [22], whereas the cupped one is also attracted to plate collectors [10,18,20].

Efficiency and yield must be distinguished when assessing recruitment results for different substrates [17,22]. According to Colsoul et al. [17], "recruitment efficiency" is the quantity of recruited spat that depends on the "strength of attraction" required for larvae to settle on the substrate used, as well as substrate preparation, timing of deployment, and immersion depth. "Recruitment yield" is the quantity of seed suitable for further farming after they detach from recruitment devices, with this phenomenon depending on recruitment efficiency and the ease of stripping recruited spat without causing damage. When analyzing "recruitment efficiency", both the concentration of larvae able to settle and availability of support must be considered. Consequently, recruitment can be "larvae-limited" or "substrate-limited", depending on stock depletion, suitability of substrate for recruitment, and availability of surfaces for larvae to settle, even when larvae are abundant in the environment [17].

In Italy, cupped oysters are reared on farms that use a longline system where they are cultured on ropes that remain suspended in the water from a longline composed of buoys; oysters are introduced on trays or in "poches", attached to the rope. Farming was initiated mainly using imported hatchery-produced spat, with an important rise in production occurring as the industry developed. In contrast, flat oysters are captured from the wild and represent a precious source of income for fishermen around the Adriatic Sea. However, in recent years, this industry has collapsed due to overfishing. Attempts to recruit flat oysters have had limited success, with it being unclear whether this issue has been due to low larval abundance or the unsuitability of recruitment structures and/or techniques. Furthermore, farming trials using hatchery-bought spat had very high mortality rates [23].

Thus, in 2020–2021, a trial was implemented to evaluate the recruitment efficiency of oysters on various collection devices suspended on longlines at a shellfish farm in the middle Adriatic Sea. This study was conducted in partnership with the Italian Fisheries Local Action Groups (FLAGs), particularly the FLAG Marche Centro and associated Centre for Innovation and Development of Fisheries and Aquaculture (CISP). The CISP partnership was created to contribute to the preservation and restoration of ecosystems and biodiversity, foster more sustainable food systems cleaner energy, and move towards a circular economy [5,7]. Thus, taking into account the most available and convenient substrata, the efficiency recruitments of the wrinkled ribbon and the oyster shells were assayed and compared using lantern nets attached to the longline system of a shellfish farm in the middle Adriatic Sea.

2. Materials and Methods

2.1. Area of Study and Water Temperature Monitoring

A recruitment trial was carried out at a longline shellfish farm in the central Adriatic region, which is located 5.5 km from the coast of Porto Recanati (43°26′42.76″ N–13°43′45.33″ E) (Figure 1). The site is characterized by an average sea depth of 13 m, a sandy sea floor, and continuous currents from the north (10–20 cm/s). Natural oyster banks are present in the area, where handcraft fishing was performed until 10–20 years ago.

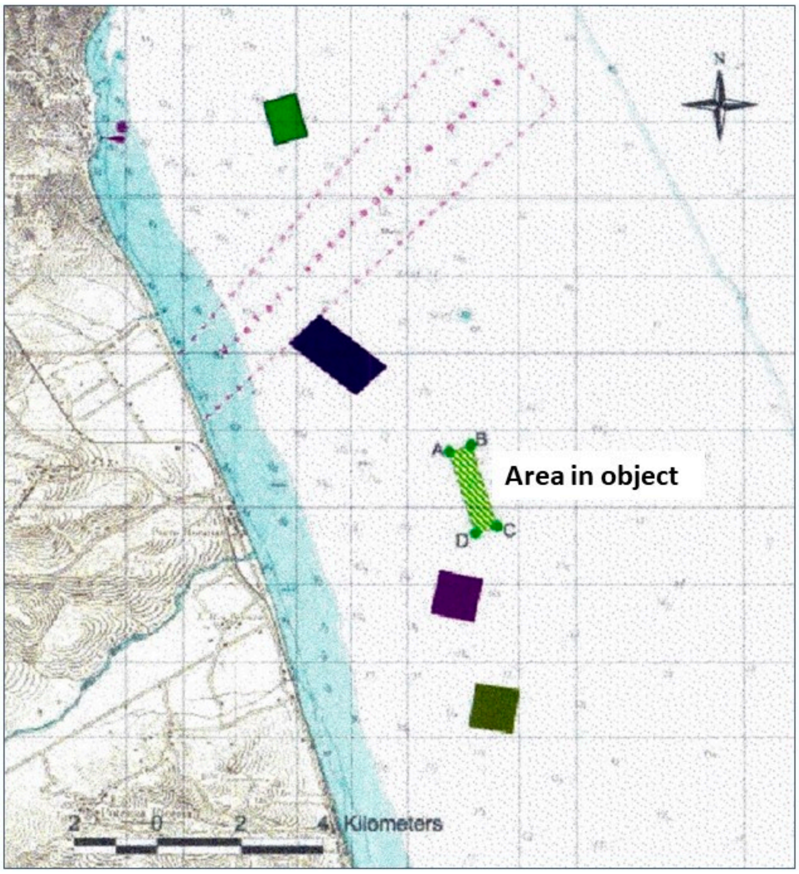

Figure 1. Map of the study area.

Cupped oysters were grown at the farm in the 5 years before implementing the trial. Flat oyster beds were considered to be present in the area. However, information on the consistency of these stocks was not available, and traditional fishing activity of this species had stopped in the last 5 years because of stock depletion.

Water temperature was regularly monitored using a portable instrument (mod. 30 YSI, Yellow Springs, OH, USA).

2.2. Types of Collectors Assayed

The two devices (net lanterns) used to recruit spat were suspended 4 m below the surface of the water. This depth was chosen for convenience, considering the opportunity to interact with the longline farming system. Each lantern had five levels (Figure 2; diameter: 50 cm; 9 × 9 mm mesh size; 145 cm total height), which were used to hold the tested substrate. The surface area of each substrate was similar. The number and length of the ribbons introduced on each level were similar. The surface was calculated as n° of ribbons (22) × ribbon length (4 m) × ribbon width (8 mm) × 2 sides.

The top of both lanterns had a 3 kg ballast to prevent the recruited oysters from tumbling, due to the effects of wave and current action. Tumbling is the movement oysters undergo to balance the lanterns; it is proportional to the distance between the point where the lantern is tied on the headline and the level of the lantern. It tends to increase with the increasing distance from the headline [24].

Both lanterns had the two upper levels containing the same wrinkled ribbon (8 mm wide) filling the entire volume of the compartment, but they differed on the other levels.

In Lantern 1, the third level contained empty cupped oyster shells of intermediate size (each shell of 66–85 g total weight, corresponding to size N°3 standard commercial size in France), filling 1/3 of the compartment volume. The fourth level contained flat oyster shells, filling 1/10 of the compartment volume. The fifth (bottom) level was empty.

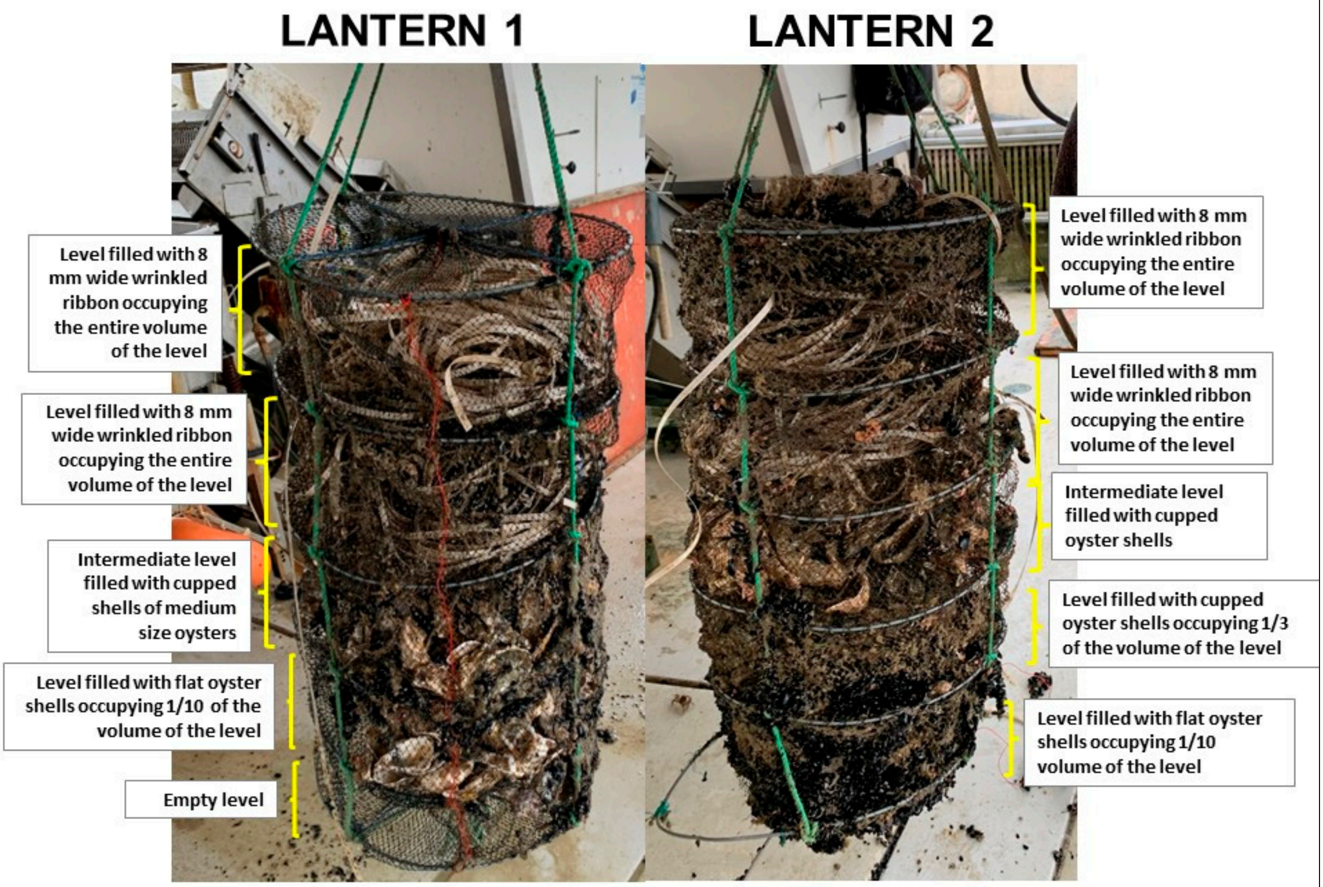

Figure 2. Image showing the two lanterns used in the oyster recruitment trials.

In Lantern 2, the third level contained empty cupped shells, obtained from large-sized oysters (each shell of 110–150 g total weight, corresponding to size N°1 standard commercial size in France), filling 2/3 of the compartment volume. The fourth level contained empty cupped oyster shells (size N°3), occupying 1/3 of the compartment volume. The fifth (bottom) level contained flat oyster shells, filling 1/10 of the compartment volume (Figure 2).

In order to show significant differences in spat density for the substrate, a statistical analysis was used with start time as a factor, considering the fact that each lantern net provided one set of samples that experienced common conditions, with Time × Substrate as the interaction effect.

Lanterns 1 and 2 were suspended in the water column in early (1 August) and late (27 August) August 2020 to intercept young oysters in the water column from two different time points and were harvested in March 2021. Both lanterns were previously suspended for one week at the longline farm and were dried for 48 h before being used for the test.

2.3. Sampling Activities

Starting October 2020, the structures were checked for their general conditions, with surveys being carried out by a diver. The lanterns were checked once a month to remove any fouling in situ until harvest (March 2021). During March 2021, the lanterns were lifted from the sea and were opened on a boat to count all recruited spat in each level/compartment separately (i.e., for each type of collector: rough ribbon, oyster shells of different sizes). The seeds were counted in each compartment before removing them from the substrate. Recruitment efficiency was expressed as the number of oysters/dm^2. The rate of filling in the lantern compartment was expressed in %. Tumbling cannot be measured because it depends on variable and unknown meteorological conditions. It was reported using a theoretical scale with a score from 0 (minimum) to 5 (maximum). Tumbling effect was evaluated as the expected tumbling between different levels of the lantern, due to the distance from the attachment point of the lantern to the headline and the total weight of the lantern; these are the physical parameters that determine balancing amplitude.

The presence of mud and pseudo-feces was also recorded with a score from 0 (minimum) to 5 (maximum). After counting, the seeds were removed from each substrate to assess the ease of detaching them and potential damage. Oyster size was measured for 120 individuals from the spat removed from the ribbons and from *C. gigas* shells in both lanterns. The visual inspection was achieved by two independent observers. The size, stripes, and color were the main factors used to discriminate cupped oysters from flat oysters (Figure 3).

Figure 3. Cupped oyster (*Crassostrea gigas*) (center and left) and flat oyster (*Ostrea edulis)* (right) species evaluated in this study.

The sampled individuals were weighed using an electronic scale (mod. CP224S, Sartorius, Gottingen, Germany). Maximum shell length was measured with a caliper.

2.4. Statistics

Size frequency distribution was reported in order to show differences between individuals from the two structures and substrates. The shell length frequency (%) of oysters recruited on shells and ribbon was graphically represented using Excel 16.0. In order to show significant differences in terms of substrate and start time of the trial, a Student's *t*-test was performed using the General Model Procedure of SPSS 25 (IBM Corp., 2017).

3. Results

3.1. Temperature Monitoring

The water temperature between August 2020 and March 2021 showed a clear decline from the start to the end of the experiment, when 10.11 °C was recorded (Figure 4).

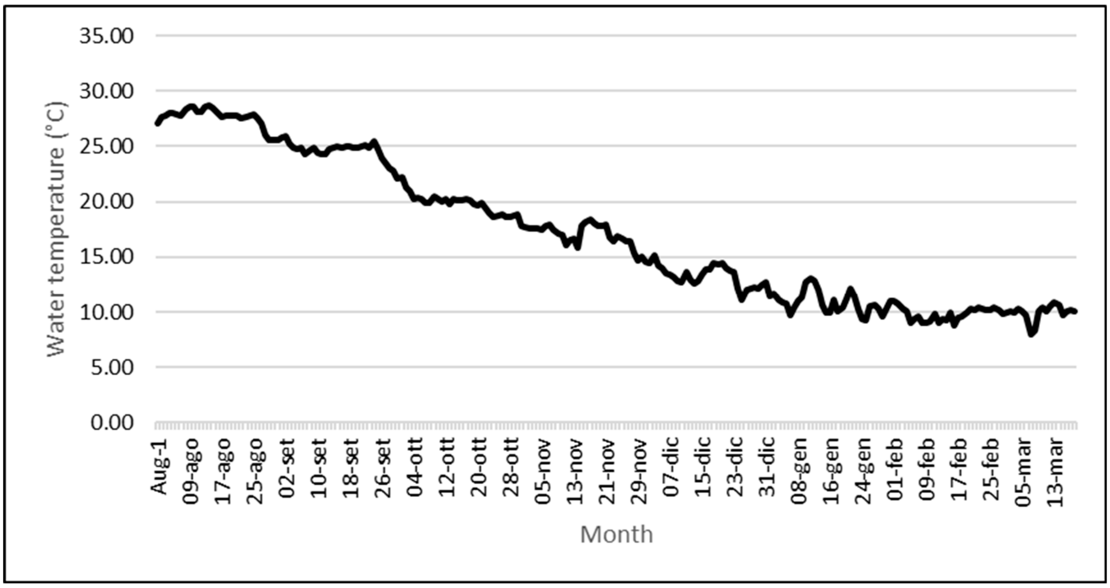

Figure 4. Water temperature during the study at the longline farm located 5.5 km from the coast of Porto Recanati, Italy in the Adriatic Sea (Mediterranean).

3.2. Recruitment Efficiency

The oyster spat removed from the four substrates were of various sizes. The average weight of the collected seed was about 2 g [Supplemental Materials], with no significant difference between ribbon and shells as substrate, as shown by the p level for Student's t analyses. At this weight, it was difficult to distinguish cupped oyster seeds from flat oyster seeds. Based on the visual evaluation, flat oyster seeds were estimated to represent 20% of the total. For both lanterns, the recruitment efficiency of the different substrates is reported in Table 1. Of note, the number of seeds is based on the count before oysters were removed from the substrate. Data on ribbons, cupped oyster shells, and flat oyster shells were assessed for both lanterns.

Table 1. Recruitment efficiency (N° oyster/dm^2) at each level/substrate in the two lanterns (counts were made before spat removal from substrate).

	Total Available Surface (dm^2)	% Filling of the Compartment	Expected Tumbling	Recruitment Efficiency (N° Oyster/dm^2)
Lantern 1:				
Ribbon	140	100%	0	0.62–0.60
Cupped oyster shells N°3	95	33%	1–2	0.18
Flat oyster shells	35	10%	4–5	0.18
Lantern 2:				
Ribbon	140	100%	0	0.19–0.59
Cupped oyster shells N°1	235	66%	0–1	0.71
Cupped oyster shells N°3	95	33%	1–2	0.66
Flat oyster shells	35	10%	4–5	0.24

The average abundance of spat was similar for the wrinkled ribbon and cupped oyster shells. In contrast, flat oyster shells had the lowest recruitment in both lanterns. Recruitment on oyster shells was similar on the external rough part of the shells and on the internal smooth part of the shells. Fouling was limited; mud was mainly detected in compartments with the big shells of cupped oysters, which had deep cavities but lower tumbling values. In comparison, the wrinkled ribbon substrate was completely free of mud (Table 2).

Table 2. Recruitment efficiency (n° oyster/dm^2) based on substrate, independent of lanterns (counts made before spat removal from substrate), and relationship with tumbling and the presence of mud.

	N° Spat/dm^2	Tumbling Evaluation	Presence of Mud
Wrinkled ribbon	0.53	0	0
Cupped oyster shells N°1	0.52	0–1	2–3
Cupped oyster shells N°3		1–2	1–2
Flat oyster shells	0.21	4–5	0–1

Oysters found on the wrinkled ribbon were retrieved easily (i.e., no detachment issues). A mark was visible on the oysters following detachment from the substrate; however, this disappeared with growth. Oysters that recruit on shells tend to be very difficult to remove without damaging them, with 50% being damaged and successively discarded. Larger oysters were recovered from the wrinkled ribbon, while smaller oysters were recovered from the cupped oyster shells (Figure 5). Recruitment density in relation to substrate did not show differences by substrate, as the statistical parameters show in Table 3.

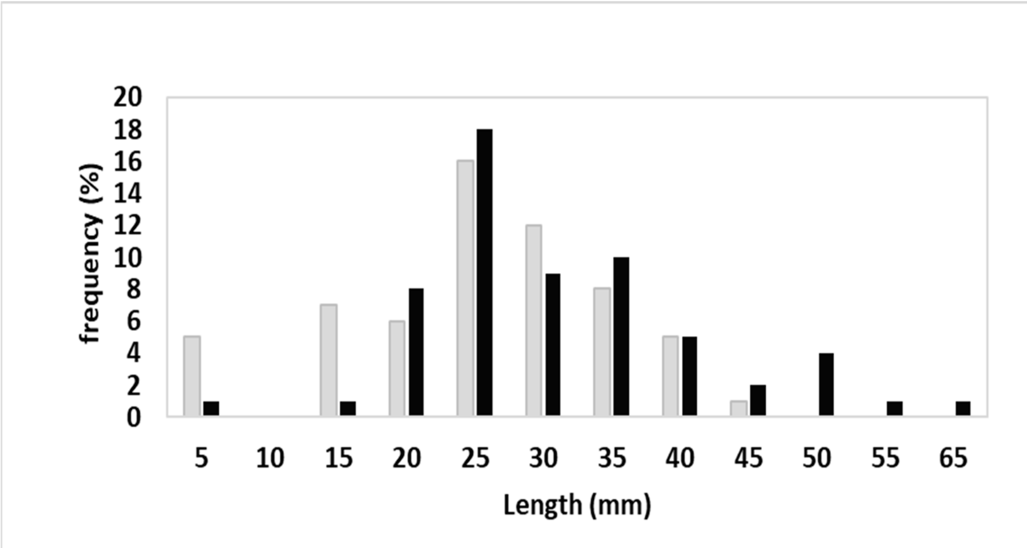

Figure 5. Shell length frequency of undamaged oysters recovered from shells (grey bar) and wrinkled ribbon (black bar).

Table 3. Statistical test performed to show differences between shells and ribbon and their abilities to garner more oyster per area. Time is referred to as the start time: early (1 August 2020) and late (27 August 2020).

	Df	Sum Square	Mean Square	F Value	Pr (>F)
Start Time	1	0.00125	0.001250	0.0205	0.8932
Substrate	2	0.11255	0.056275	0.9212	0.4687
Residuals	117	0.24435	0.061088	-	-

4. Discussion

The current study tested different substrates under similar conditions of preparation placed in suspended lanterns attached to the longline system of a shellfish farm. Both oyster species (cupped and flat oyster species) were detected on the tested substrates. The number of factors that affect recruitment clearly demonstrate the complexity of assessing suitable and cost-effective approaches to farming oysters [25]. This finding also shows the importance of standardizing studies to compare different recruitment techniques of wild oyster stock consistently, including the concentrations of planktonic larvae. This was related to spat availability, also a function of larval production as a result of broodstock reproduction and successful spat recruitment. Substrates adequately overlapped to create interspaces, as oyster shells or tapes have been investigated to attract more juveniles [26].

Our study showed that the position of the tested substrate in the lanterns affected tumbling. For instance, tumbling was higher in the lower levels or in levels where the total weight of the used material was lower. In a review dedicated to oyster quality traits [24], the tumbling effect is described in relation to tide levels, which are responsible for causing floating in the suspended lanterns attached at the top to longlines. Although no tide level occurred in our study, it is known that this condition affects the optimal growth of oysters. Mud accumulation was inversely proportional to tumbling. We also found that the encumbrance of the used material affected the ability of mud and pseudo-feces to accumulate on it.

The current study showed that recruitment on wrinkled ribbons and *C. gigas* shells produced good results, despite excessive mud on big *C. gigas* shells. Previous studies also showed that empty shells were more effective than other substrates. For instance, in coastal waters off Holland, sacks containing four times of bivalve shells were found to be more effective at collecting spat compared to "Chinese hats", "Vexar mesh nets", or PVC tubes [19]. In our case, we also noted that minimal sediment accumulated when using smaller shells. Furthermore, densities of supports in a single lantern appeared to be a disadvantage.

Although not statistically shown, the recruitment of oysters on flat oyster shells was not suitable compared to cupped oyster shells, which might have been associated with the former having a lower density in the lanterns, higher tumbling in the lower compartment, and shell characteristics (shape/size) of flat oyster shells.

In our trial, the rough surface of the external part of empty shells did not enhance recruitment, with the smooth and rough parts of the shells having similar levels of recruitment. Supports used within the last year or briefly immersed in seawater beforehand are sometimes more attractive than new material. In the current study, while recruitment efficiency was similar for wrinkled ribbons and cupped oyster shells, removal yield from shells was lower. Some authors [27] have recorded strong recruitment of the mangrove oyster on halves of plastic soda bottles due to the biofilm that developed immediately after the collectors were immersed in seawater with the inner face upwards. The efficiency of low-cost recycled materials to collect spat has also been assessed along the coast of the Amazon (Brazil). Spat was more easily removed from PET bottles compared to PVC, on which the lower valve frequently broke [18]. This phenomenon was probably attributed to biofilm formation, which enhances recruitment efficiency. However, fouling could also impede recruitment over long periods [26,28]. Thus, it is important to prepare the supports in a way that improves recruitment efficiency. Conversely, Devakie and Ali [28] found that smooth plastic surfaces needed to be covered with a rough surface coated with tissue extracts or a biofilm to promote recruitment. In our study, none of the collector devices were sanitized before immersion, and all were prepared in the same way to allow a superficial biofilm to form immediately. Some authors [17] clean and sterilize the substrate before use to evaluate the substrate without any biofilm growing on them. In contrast, our trial aimed to maximize the attractiveness of the substrate to oysters, which partly depends on the presence of biofilm, to quantify the potential of the tested techniques in a realistic setting. All the substrates used in this trial had been used in recruitment trails in previous years. Based on experience, previously used ribbons were considered to be more attractive than new ones coming directly from the factory.

In our study, the two lanterns were put in water in late summer (August), about two months after the first mature and milky oysters of both species were observed. The wide range in sizes documented for the recruited spat of both species in March provided clear evidence that recruitment occurs over a protracted period. This finding reflects the fact that the reproduction period for both species extends over multiple months (May to September). One experiment showed that *O. edulis* larvae are active and are not homogeneously distributed in the water column [26]. Because the breeding season begins in May, recruitment on lanterns might be even higher if lanterns were placed in the sea in spring (May). In general, globally, the abundance of *O. edulis* and *C. gigas* larvae, including peaks, varies with both location and year [7]. Furthermore, most spat tend to appear on collectors deployed 1–2 weeks after larval abundance peaks. Importantly, heavy fouling might prevent larval settlement on collectors deployed too early with respect to breeding season [19].

On the east coast of Croatia (opposite from our study site in Italy) in the Adriatic, native and non-native oysters have been detected during May [29], with populations distributing differently in relation to depth. In particular, flat oysters have been recorded in the subtidal zone, whereas cupped oysters have been found in the tidal zone and in much higher abundance compared to the former. Thus, flat oysters could be recruited more efficiently by placing lanterns closer to the seabed, where they are more likely to settle. In our study, spat was more easily removed from wrinkled ribbons compared to shells. In the southeast of Spain, assessing the recruitment of oysters by installing spat collectors consisting of plastic mesh bags and attached to a rope fixed to a concrete mooring and vertically held to a submerged buoy, Lunetta et al. [30] was not able to collect oyster spat belonging to *O. edulis* but only to the *O. stentina* species. According to the authors, this lack of recruitment of flat oysters was not due to the kind of collector or substrate used (bags containing onion bag pieces aiming to provide settlement for oyster larvae), but to the low presence of breeding adults in the lagoon investigated (Mar Menor).

As observed by van den Brink et al. [19], detaching oysters from on-bottom cultivation is often difficult and is overcome by the seed being allowed to grow to an advanced stage on supporting shells, after which detachment is easier. This technique is commonly performed in the "on-bottom" farming practiced in the Northern Europe; the "cultch" is laid on the seabed.

Overall, oysters can be easily detached from ribbons, plastic bottles, and "Chinese hats", with these approaches having the potential to be optimized, allowing for spat self-sufficiency for farming activities [31]. It would also be interesting to find natural substrates that have the same characteristics as these that are easy to use as substrates. In contrast to high biofouling impacting the recruitment of oyster stocks on the Atlantic coast, biofouling was very limited in the Adriatic. For instance, no barnacles were detected in the current study. Only *Calyptraea* sp. represented a possible competitor; however, the densities of this species were limited compared to oyster spat. Until recruitment techniques have been fully assessed, as has been done in the Arcachon basin in the South of France, spat self-sufficiency, as in the case of mussels, does not appear to be a target that can be easily achieved any time soon [32]. As mentioned above, positioning the recruitment supports for longer periods according to planktonic larvae monitoring should increase the effectiveness of the entire strategy. The fact that recruitment in the Adriatic Sea concerns both species raises the problem of separating them before farming or carrying out restoration strategies.

The present study could support criteria for future trials performed using homogeneous batches of shells as substrate. In this study, recruitment efficiency of oysters was investigated in correspondence with a longline shellfish farm, in the area in concession to the shellfish farm. In this way, no oyster wild stocks were removed from protected areas. Enhancing the recruitment potentialities, seed for the diversification of farming should be available and could lead to a higher survival rate compared to hatchery-produced spat [33]. Therefore, applying "good practices" for the growth and management of natural oyster (bivalve) seabeds is essential. Issues that concern operators and researchers include the over-exploitation of the natural banks of flat oysters, a decrease in benthic ecosystems, and a lack of efficient management of marine protected areas. The flat oyster (*Ostrea edulis*) is also considered important for the preservation and restoration of natural habitats [25,26]. This species has been declared as "threatened and declining" by the Convention for the Protection of the Marine Environment of the Northeast Atlantic (OSPAR) and the European Union (UN) Marine Strategy Framework Directive [19]. In both cases, it would contribute to create employment in a sustainable way. In implementing restoration strategies, it seems important to bear in mind that due to the dispersion of larvae across long distances during their pelagic phase, a basin scale approach is needed, according to the European Maritime Spatial Planning (MSP) Platform which is the tool to manage the use of seas and oceans coherently and to ensure that human activities take place in an efficient, safe, and sustainable way (Directive 2014/89/EU).

5. Conclusions

The current study demonstrated that the efficiency recruitment was similar between the wrinkled ribbon and the oyster shells and in the same proportion on the external rough part of the shells and on the internal smooth part of the shells. Considering the increase in the production costs, which are seriously worrying all the operators of shellfish farms, our results suggest that the optimization of the use of collection devices must allow for high attractivity and easy removal of the oysters recruited.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su15118685/s1, Oyster spat weight sampling.

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