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Abstract: The high variability in natural recruitment of Pectinidae is a common feature of many marine invertebrates with a pelagic larval stage, but may negatively affect aquaculture activities. Detailed information on settlement patterns and spat availability is required to reduce costs and labor. In this regard, we attempted to establish the precise immersion time and the deployment dates for spat collectors in the Taranto Gulf (Mediterranean Sea, Italy). The first experiment was carried out from June to October 2013, deploying collectors every 15 days and retrieving them after 4, 6, 8, and 10 weeks. Results from the first experiment allowed us to select 8 weeks as the best immersion time for spat collection. The second experiment was carried out from June 2013 to July 2014 when we deployed spat collectors every 15 days and recovered them after 8 weeks to detect the favorable periods to place the collectors in water to obtain the highest scallop spat harvest rate. Mimachlamys varia was the most abundant pectinid (greater than 90%), whose recruits were collected during most of the year studied, followed by Flexopecten glaber with the highest rates in July (87%) and Pecten jacobaeus, which never exceeded 17% of collected spat. M. varia had a long recruitment period (from October to early June), F. glaber showed a high number of spat during autumn months and from June to July while P. jacobaeus showed a restricted period of spawning. Our experiments provide useful insights into strategies for establishing scallop aquaculture in order to promote these mollusks as alternative candidates for aquaculture farming in the area.

Keywords: scallops; collectors; spat recruitment; Ionian Sea; Mediterranean Sea

1. Introduction

The need to feed a growing global population, and growing demand for seafood puts pressure on natural resources, is challenging the sustainability of marine and inland fisheries [1]. Aquaculture holds the key to mitigating the use of these resources and satisfying the increasing request for seafood, providing healthy food for the growing population in an environmentally responsible manner [2,3].

Within the aquaculture sector, molluskan culture is viewed as highly sustainable and is defined as a green industry. As a matter of fact, compared with other cultured species, bivalve culture occupies a low position in the food chain in that they exploit phytoplankton, thus obviating the need for external feed inputs and making a lower ecological impact on coastal ecosystems. [4]. Moreover, the deposition of bivalve feces under the farming plants contribute to the mineralization of organic matter settled on the sea beds [5]. For this reason, shellfish aquaculture does not result in additional nutrient loading, but rather, constitutes a fundamental link between water column nutrients and bottom communities.

The high prices for bivalves have accelerated the expansion of the bivalve aquaculture industry in various countries [6].

In 2018, mollusks production (17.3 million tons) represented 56.3% of the production of marine and coastal aquaculture of the world [6]. Considering the importance of the



Citation: Papa, L.; Prato, E.; Biandolino, F.; Parlapiano, I.; Fanelli, G. Strategies for Successful Scallops Spat Collection on Artificial Collectors in the Taranto Gulf (Mediterranean Sea). *Water* **2021**, *13*, 462. https://doi.org/10.3390/ w13040462

Academic Editor: Akintunde O. Babatunde Received: 19 January 2021 Accepted: 4 February 2021 Published: 10 February 2021

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sector, and the great interest in its sustainability, the aquaculture of bivalve species is strongly encouraged in the European Union. Shellfish species are a valuable food source in the socioeconomic context of the EU, but it is evident that their culture is still limited and scarcely diversified. In fact, European shellfish production is mainly based on oysters and mussels, that together represent 93% of the total European cultivated mollusk production, while clams, cockles, ark shells, and scallops cover a little European market share (about 16% in 2001) [7]. France is by far the leading producer of oysters (\pm 85,000 tons in 2011), Spain of mussels (\pm 209,000 tons in the same year) [7]. Italian aquaculture represents 11.4% and 10.7% of the EU production in terms of the total volume and value, respectively [8]. The Italian shellfish market is based only on the culture of two bivalve species: *Ruditapes philippinarum*, with 94.2% in volume and 91.6% in value, and *Mytilus galloprovincialis* which supply two-thirds of the community aquaculture production [9].

This evident lack of diversification stimulates the development of new aquaculture species, adding value to the productive sector through the production of high market value species. The advantage is to reduce the pressure on over-exploited fisheries, reduce the dependence of the EU on imports, and explore new segments and advantageous products for the EU market.

As a potential candidate for aquaculture purposes, scallops may offer interesting prospects for rearing, considering not only that their market value but also that its use in aquaculture might contribute to a reduction in the negative impacts of currently used fishing gears (called "rapidi") on the sea bottom and benthic communities [10].

Recently, the consumption of marine bivalve mollusks of the family Pectinidae, commonly known as scallops, has significantly increased, representing an important part of the European seafood market [11]. Scallops are popular as fresh seafood, but are also well suited to be frozen; thus, their rearing may be profitable and able to provide a good product for human consumption with high market value.

The main bottleneck of scallops' culture is a reliable, plentiful, and inexpensive supply of seed [12]. The deployment of collectors is a widespread technique for obtaining wild scallops spat and developing the aquaculture of different pectinid species all around the world [13–16].

However, the natural availability of seed is influenced by different factors such as site, depth, temperature, food availability, and occurrence of competitors and predators, which could affect scallop settlement [17]. Therefore, in the past 20 years, most studies on the collection of scallop spat have been focused on the selection of areas suitable for their harvesting and on the identification of the date of optimal collector deployment, which is related to the scallop spawning period and the immersion time of collectors [15–20].

The breeding season of scallops shows a latitudinal trend with acute seasonality and synchronism at higher latitudes, becoming longer and less synchronized as latitudes decrease [21].

The natural recruitment might guarantee continuous availability of scallop spat, thereby overcoming the problem of high variability of seed as a main constraint for aquaculture activities. Collectors are only effective for a short period and must be deployed for a timespan useful to become attractive for spat attachment [21–27]. Therefore, the perspective of commercial bivalve culture requires the knowledge of recruitment timing.

A previous study determined the natural availability of scallops spat in the Mar Grande of Taranto (Ionian Sea, Mediterranean Sea), identifying the best collection site and confirming the efficiency of traditional collectors [28].

This paper attempts to answer the following questions to maximize the collection of wild scallops on artificial collectors in the Taranto Gulf (Mediterranean Sea): how long should the collectors be immersed and what is the appropriate deployment timing of collectors?

2. Materials and Methods

2.1. Study Area and Sampling Design

The study was carried out in the Mar Grande of Taranto $(40^{\circ}26'73'' \text{ N}, 17^{\circ}13'50'' \text{ E})$ chosen according to information obtained from our previous work [28].

Mar Grande represents an important aquaculture area where many mussel farm plants are localized. It is a semi-enclosed basin in the northern side of the Gulf of Taranto (Mediterranean Sea) extending about 36 km² with a maximum depth of 42 m (Figure 1). Water temperature ranges between 14 °C and 28 °C and the average salinity is 38 psu remaining rather constant throughout the year [29].



Figure 1. Location of the study sites in the Gulf of Taranto (Ionian Sea, Mediterranean Sea).

The employed collectors were made by a Netlon outer bag measuring 64×36 cm, with 0.3×0.3 mm mesh, filled with a thermoplastic and folded net measuring 100×50 cm with 5×5 mm mesh. A rope was used to close the bags, which were randomly suspended on an anchor rope attached to a long-line mussel plant.

The presence of pediveligers at deeper waters suggests the deployment of collectors near the bottom to ensure high recruitment of scallops [30]. For this reason, collectors were placed at 9–12 m depth. The installation and removal of the bags were done by scuba divers.

2.2. Analysis of Samples

Once collected, the collectors were analyzed with the naked eye, collecting all the recruits present in the bag and on the net. All scallop spats from each sample were counted and measured for shell height (SH) (the distance from the umbo to the opposite shell margin). Individuals smaller than 10 mm SH were measured using an ocular micrometer $(\pm 0.01 \text{ mm})$ while larger individuals were measured using an electronic caliper $(\pm 0.1 \text{ mm})$.

2.3. Assessment of the Best Immersion Time

In the first experiment, 84 collectors were deployed from July 2013 to October 2013 to determine the appropriate immersion time (Tm). Four series of collectors (three replicates for each immersion time), were deployed every fortnight at 7 different dates (Dt) and were recovered by scuba divers after 4, 6, 8, and 10 weeks.

2.4. Assessment of the Appropriate Deployment Timing of Collectors and Settlement Pattern

In the second experiment, carried out from October 2013 to July 2014, another 20 series of collectors were deployed every fortnight to determine the appropriate deployment timing and to determine the frequency of occurrence (%) of each Pectinidae species found.

Each series included 3 collectors as replicates, except for July 2014 when only 1 replicate was available due to a loss of the remaining bags. Collectors were retrieved after 8 weeks, according to the results of Experiment 1. In July 2014, all 8-week-old collectors were inspected (i.e., from June 2013 to July 2014) to establish the appropriate deployment timing.

2.5. Species Identification

Although the aim of this work was not to identify the Pectinidae species, this information has been obtained for spat recruited in 20 additional series of 8-week-old collectors deployed at the same deployment dates as in Experiment 2 and reared until they reached a size appropriate for species identification.

2.6. Data Analysis

We determined spat abundance of individuals on each collector (individuals per bag⁻¹). The interactions between immersion time (Tm 4, 6, 8, and 10 weeks) and deployment time (Dt) (from June 2013 to October 2013) were tested using two-way ANOVA, with Tm (fixed, 4 levels, and orthogonal) and Dt (fixed, 7 levels, and orthogonal). The square route transformation [Sqrt (X + 1)] was applied to spat abundance per collector. Heterogeneity of variances was checked with Cochran's test. Post-hoc comparisons of means (at $\alpha = 0.05$) were performed through the Student-Newman-Keuls (SNK) tests [31].

The differences among all deployment times of collectors were tested using one-way ANOVA and the multiple comparisons analysis [32] was used to test the differences of spat recruitment among deployment dates.

Cohort analysis (decomposition of size-frequency distributions into Gaussian components) was performed with Bhatthacharya's method [33], using FiSat II software [34] for all 8-week-old collectors.

3. Results

3.1. Assessment of the Best Immersion Time

Scallop spat showed the highest abundance on the collectors deployed in late August (with recruit abundance ranging from 432 ± 175 to 563 ± 81 individuals bag⁻¹(mean + sd, n = 3), after 8 and 10 weeks, respectively). Then the spat abundance decreased to minimum values on the collectors deployed at the last date of October with mean values ranged from 12 ± 5 (after 4 weeks), 19 ± 9 (after 6 weeks), 50 ± 14 (after 8 weeks), to 68 ± 17 (after 10 weeks) ind. bag⁻¹ (mean + sd, n = 3) (Figure 2).

An abundance of recruits was influenced by immersion time (Tm) and deployment time (Dt) (ANOVA, p < 0.001) (Table 1). Post-hoc analysis (SNK) showed no significant differences among immersion time (Tm) of collectors in late August and at beginning of October (p > 0.001), while significant differences were observed for other deployment times (Dt) (p < 0.001) (Figure 2).



Figure 2. Immersion time. Spat abundances of the three scallop species found on collectors retrieved after 4, 6, 8, and 10 weeks. At each deployment time different letters indicate significant differences (Post-hoc analysis, SNK; p < 0.05).

Source	SS	DF	MS	F	p
Tm	208.645	3	69.5483	18.72	0.000
Dt	2321.7549	6	386.9592	104.13	0.000
TmxDt	360.3146	18	20.0175	5.39	0.000
Residual	208.105	56	3.7162		
TOTAL	3098.8196	83			
Dt TmxDt Residual TOTAL	2321.7549 360.3146 208.105 3098.8196	6 18 56 83	386.9592 20.0175 3.7162	104.13 5.39	0.000

Table 1. Two-way ANOVA to test the effect of immersion time and deployment time on spat abundances.

Tm = immersion time; Dt deployment time; SS= Sum of Square; DF= degree of freedom; Mean square; F-value.

In general, spat collected from the 4-week-old collectors were significantly lower than those recruited on collectors immersed for longer immersion times (6, 8, and 10 weeks). In five of the seven cases, we found no significant differences in scallop recruit abundance among 6-, 8-, and 10-week-old collectors (Figure 2) Furthermore, spat recruited on the 8-week-old collectors were large enough to be easily manipulated in further culture phases. Therefore, an 8-week period was chosen as the best immersion time to collect the greatest number of scallop spat.

3.2. Assessment of the Appropriate Deployment Timing of Collectors and Settlement Pattern

In the study area, scallop spat continuously recruited along the year (Figure 3) but their abundance on the collectors immersed for 8 weeks showed significant differences among deployment dates (ANOVA, F = 21.40; p < 0.0001) (Table 2).

The highest abundances of scallop spat were observed on the collectors deployed in late August 2013 with 432 \pm 175 ind. bag⁻¹ (mean \pm sd, n = 3), at the beginning of April 2014 with 630 \pm 151 ind. bag⁻¹ (mean \pm sd, n = 3), and in July 2014 with spat abundance of 750 and 1099 ind. bag⁻¹ (1 replicate out of 3). During the autumn and winter months, scallop recruitment was considerably lower (Figure 3).



Figure 3. Deployment time. Scallop spat abundances (Individuals/bag), of the three scallop species found, on 8-week-old collectors immersed from June 2013 to July 2014.

Table 2. Results of ANOVA, used to test for differences of the spat abundances at all deployment dates (Dt) of collectors immersed for 8 weeks.

Source	SS	DF	MS	F	p
Dt	20434083	25	817363	21.4	0.0000
Residual	1986351	52	38199		
IOTAL	22420435	11			

Dt = deployment timing; SS= Sum of Square; DF= degree of freedom; Mean square; F-value.

3.3. Species Composition and Occurrence

Starting from late October 2013, through the rearing of harvested spats, we managed to identify the different species recruited in the bags and this allowed us to evaluate the temporal distribution of scallop species.

The most abundant pectinid settled on the collectors was *Mimachlamys varia*, whose recruits were collected during most of the year studied, followed by *Flexopecten glaber* and *Pecten jacobaeus*, which recruited exclusively in warmer months (Figure 4).

At 8 different deployment dates, from January 2014 to April 2014, *Mimachlamys varia* showed the highest abundance, with values that ranged from 150 ind. bag⁻¹, in late January 2014 (representing 94.9% of total Pectinidae) to 586 ind. bag⁻¹ in April 2014 (92.1% of total Pectinidae). The highest abundance of *Flexopecten glaber* was observed in late July with 974 ind. bag⁻¹, representing the frequency of occurrence of about 88.6% of total Pectinidae. The lowest abundance rates were recorded for *Pecten jacobaeus*, with the highest abundance was found in late July, with 126 ind. bag⁻¹ and the frequency of occurrence of 11.4% of total Pectinidae.



Figure 4. Species occurrence of the three identified scallop species found on collectors within the period from October 2013 to July 2014.

3.4. Modal Analysis of Spat Size Frequency Distributions

In general, SH ranged from 0.5 to 8 mm and 0.5 to 15 mm in spring and summer, respectively. The minimum shell size was 0.5 mm, recorded on almost all collectors, while the maximum shell size (15 mm) was found on the collectors immersed on 9 August 2013, 2 October 2013, and 14 July 2014 (Figure 5).

The size distribution of recruits showed a multimodal pattern, especially from June to September 2013, with a minimum of 3 to a maximum of 6 cohorts. The highest number of cohorts was observed on 11 July 2013 with six modes: the C1 mode, with the smallest value of a mean shell size $(1.03 \pm 0.6 \text{ mm})$, representing the majority of recorded individuals (49%); followed by C2 ($2.93 \pm 0.5 \text{ mm}$; 12.7% of total), C3 ($4.44 \pm 0.4 \text{ mm}$; 11% of total), C4 ($6.5 \pm 0.7 \text{ mm}$; 15% of total), C5 ($8.04 \pm 0.4 \text{ mm}$; 7.3% of total) and C6 ($9.41 \pm 0.7 \text{ mm}$; 4.8% of total), with a maximum shell size of 11 mm (Figure 5a). New cohorts were found at each sampling time. From October 2013 to May 2014, the size distribution showed a different pattern with a minimum of 1 cohort to a maximum of 3 cohorts in mid of November 2013 and May 2014.

In late October and on 14 November 2013 the cohorts were represented by *M. varia* (52%) and *F. glaber* (48%) (Figure 5b). In late November, the C1 cohort was characterized by *M. varia* (85%), *F. glaber* (5%), and *P. jacobeus* (10%); the latter showed a slight increase to 18% in early December (Figure 5b). From late December to early April 2014, *M. varia* spat represented the major proportion of Pectinidae (92–97.5%). *F. glaber* recruitment started in late April, representing 14–15% of total Pectinidae and a marked increase in this proportion was registered in following months. From late June to July 2014, a multimodal pattern similar to the previous year was observed, with *F. glaber* spat being the most abundant (Figure 5c).



(a)

Figure 5. Cont.



(b)

Figure 5. Cont.



(c)

Figure 5. (a). Modal analysis of size frequency distributions (from 18 March 2014 to 22 July 2014) of Pectinidae recruited on artificial collectors in the Gulf of Taranto. In (b,c), modal analysis of size frequency distributions, the pie charts represent the frequency of occurrence of each Pectinidae at each sampling date.

4. Discussion

Scallop species such as the smooth scallop *Flexopecten glaber*, the black scallop *Mimachlamys varia*, and the Saint James scallop *Pecten jacobaeus* are the major representative species widely distributed in the Mediterranean [27]. *Pecten jacobeus* have a flat upper brownish shell and a convex whitish lower shell with 15–18 accentuated ribs, and a maximum shell length of 15 cm. They are hermaphrodites, achieving sexual maturity at 5–6 cm of shell length [35]. *F. glaber* can be distinguished on the basis of the number of radial ribs on the shell surface (9–13). In the Northern Adriatic Sea, *F. glaber* reaches sexual maturity after 9–10 months and at 22.4–25.1 mm shell height [10], while in the Atlantic Ocean *M. varia* reaches the age of sexual maturity within the first year at an 18 mm shell height [36].

They are a high-value seafood [28,37], for which the market demand is so strong that existing supplies are inadequate. [38]. For this reason, natural stocks have been seriously overfished and are in danger of extinction [38]. Aquaculture plays a key role in the supply of these resources, and this industry covers the existing market gap, so the methodologies and recruitment strategies for successful scallops spat culture are needed.

The results of this study respond to important issues for maximizing the collection of wild scallops on artificial collectors in the Taranto Gulf (Mediterranean Sea).

Once immersed, collectors are rapidly colonized both inside and outside by Pectinidae and other organisms, including competitors and predators [39–42]. Hence, scallop spat abundances on collectors could be a trade-off among different factors; first of all, the natural spat availability which occurs after the spawning period [43]; second, the appropriate deployment time of collectors to ensure an suitable substrate on which scallop spat can settle; and, at last, mortality of larvae due to competition and/or predation.

Artificial collectors immersed for 6 and 8 weeks recruited higher numbers of scallop spat than the 4- and 10-week-old collectors; probably the lack of available space in the bags and predation might justify the decline in the effectiveness of the collectors immersed for 10 weeks, while the lowest recruitment observed on the 4-week-old collectors might be explained by a too-short period of time immersion to capture a high number of scallop spat and also because the development of a biofilm requires a longer period of time [44].

Since no significant differences were observed in spat abundance on the 6- and 8-weekold collectors at 6 of 7 deployment times tested, the optimal immersion time providing the highest scallop spat abundance was 8 weeks. In addition, after 2 months recruited individuals are large enough that they can be easily handled and sorted for the purpose of aquaculture practices.

The spat abundance recorded during the study period ranged from 26.3 ± 11.8 (mean \pm sd, n = 3) to 1099 ind. bag⁻¹ (no sd) after 8 weeks. These abundances were higher than those found in other Mediterranean areas, but lower than those recorded in other world aquaculture sites. In fact, other studies [45] reported 0.25–99 ind. bag⁻¹ for *Aequipecten opercularis*, 0–101 ind. bag⁻¹ for *M. varia*, and 0–9.5 ind. bag⁻¹ for *P. jacobaeus* while, for *A. tehuelchus* in the Gulf of San Matias, Argentina, abundances were 400–1600 per spat collector⁻¹ [18]; recruited *Argopecten ventricosus* individuals ranged from 400 to 2550 recruits collector⁻¹ in the Bahia of California [20].

The continuous presence of scallop spat found in the Mar Grande and the great abundance of it suggests high reproductive activity of the scallops at this site. To date, no information exists about the reproductive cycle of wild pectinid populations in this area. Since this location is already an important shellfish farming region of the Mediterranean Sea, the findings of strong scallop recruitment, using artificial collectors, might be useful to differentiate the local aquaculture production.

The second experiment allowed us to identify two favorable periods for successful spat collection. The most important occurred from July to September and the second from March to April. According to this study, the larval period lasts between 16 to 25 days [46], and we can suppose that the spawning occurred from June to August and from February to April.

In previous studies in the northern Adriatic Sea, three spawning periods in May, August, and December were registered for *P. jacobeus* [22] and their recruits were collected in shallow waters (2–20 m depth) [23,47]; in the Atlantic Ocean, two main spawning periods (i.e., May–June and at the end of summer) are usually reported for *P. maximus* [48]. Other authors identified different spawning periods for the described species. In fact, in the Atlantic Ocean, *Pecten maximus* showed two peaks of spawning, one between April and May and another between August and September; *M. varia* showed spawning peaks between May and June and between September and November; and *A. opercularis* spawned throughout the year with three peaks (February and March, June–July, and September–October) [24].

On the contrary, in the Mediterranean Sea, the spawning period of *A. opercularis* occurs throughout the year with two different peaks (i.e., in April and December). *M. varia* and *P. jacobaeus* have restricted reproductive and spawning cycles, with the spawning periods in April and June for the first species and between February and May for the latter [45].

Our findings provide additional information about the recruitment period of scallop species. In particular, *M. varia* has a continuous recruitment period throughout the year (from October to June), and presents the main peak of abundance in spring; also, *F. glaber* has a long recruitment period although it occurs with a low frequency (0.4–16.7%) in January–May and high frequencies in August–September (51.5–88.6%) and November–December (47.2–48.3% of total scallops); *P. jacobaeus* spats occurred with a low frequency (0.3–2.0%) in January–March and showed a high frequency in July (11.4%).

5. Conclusions

The easiness of seed collection, the fast growth [28], and attractive market value make scallops a very interesting product for the diversification of aquaculture in order to enhance the productivity and profitability of this sector. Although natural spat collection on artificial collectors is considered the best economical practice, aquaculture facilities incur major costs for installing hundreds or thousands of spat collectors. The optimal timing of spat collector deployment [17] and the appropriate immersion time of the collectors are important prerequisites for the success of Pectinidae farming because they could reduce initial investment costs.

Our data suggested that the collectors deployed 2 months before spawning provided the highest abundance of spat. In addition, spats harvested after such a period have a size sufficiently large to facilitate and to reduce costs of the subsequent stages of the farming process. In this Mediterranean region, the recruitment of scallops is continuous throughout the year with peaks of *M. varia* in April–June and *F. glaber* in July–September.

To date, aquaculture diversification continues to be constrained by several limitations (technologies for effective husbandry, regulations, profitability, sustainability, community acceptance).

Our findings provide important data to support scallop aquaculture in this southern region of the Mediterranean Sea. Our findings provide important data to develop scallop aquaculture in this southern region of the Mediterranean Sea. Further studies will be focused on evaluating interannual variability in the recruitment pattern of the scallops to understand whether these species can concretely represent a way to diversify the productions. At the same time, by completing the collection of data with the physicalchemical variables of the water column, the influence of climate change on these resources, potentially so important for Mediterranean aquaculture, can also be verified.

Author Contributions: Conceptualization, L.P., E.P., G.F.; Methodology, L.P., E.P., G.F.; investigation, F.B., I.P.; L.P.; data curation, E.P., L.P., G.F., F.B., I.P.; writing—original draft preparation, E.P., I.P., G.F.; L.P. and I.P. writing—review and editing, L.P., E.P., G.F.; supervision, E.P. and G.F.; project administration G.F. and E.P.; All authors have read and agreed to the published version of the manuscript.

Funding: This study was carried out within the framework of the REPAIR project, supported by FEP Apulia Region 2007–2013 (Code: 17/OPI/010).

Institutional Review Board Statement: Not applicable" for studies not involving humans or animals.

Informed Consent Statement: "Not applicable" for studies not involving humans.

Data Availability Statement: All data generated or analysed during this study are included in this published article.

Conflicts of Interest: The authors declare no conflict of interest.

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