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Analysis of marine bivalve spat settlement on experimental polyethylene collectors with different immersion periods

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ABSTRACT

This study presents results on the diversity of marine bivalve community based on analyses of experimental polyethylene collectors with different immersion durations. The collectors were deployed in the Boka Kotorska Bay between June and December 2017 and from June 2018 to May 2019 at two existing shellfish farms located in Orahovac and Kamenari. The most abundant taxa identified were: *Mytilus galloprovincialis*, *Talochlamys multistriata*, *Modiolarca* sp., *Limaria hians*, and *Ostrea edulis*. Collectors immersed for six months recorded higher numbers of attached individuals and greater taxa diversity than those immersed for twelve months. This suggests that aligning collector deployment with the species' reproductive cycles is important, and that longer immersion periods offer no benefit for optimal settlement and survival.

Keywords: marine bivalves, experimental polyethylene collectors, settlement, diversity

INTRODUCTION

Shellfish farming in Boka Kotorska Bay dates back to the early 1960s. In its initial stages, bivalve spat were collected using bundles of branches and traditional *pergolari* – net-like structures traditionally used for mussel cultivation. The ecological characteristics of the Bay support a high diversity of bivalves, with 55 edible species recorded to date (Mandić, 2008; Mandić M. *et al*., 2016). Among these, *Mytilus galloprovincialis* and *Ostrea edulis* are farmed commercially. Research on molluscan diversity along the Montenegrin coast has been more comprehensive within the Bay than in the open sea (Petović *et al*., 2017; Gvozdenović *et al*., 2022). Based on published literature and recent studies, the marine bivalve fauna in Montenegro comprises 165 taxa (Gvozdenović *et al*., 2022). In addition to the two commercially farmed species, other valuable bivalves such as *Mimachlamys varia*, *Pecten jacobaeus*, and *Limaria hians* are also present in the Bay (Stjepčević, 1967; Gvozdenović *et al*., 2022).

Collecting wild bivalve spat from local bivalve populations can reduce production costs and mitigate the risks associated with introduction of non-indigenous species or facilitating habitat invasions that may occur when bivalve spat is imported from allochthonous source habitats (Marčeta *et al*., 2022). Historically, a variety of collector types were used, as bivalves spat showed no consistent preference for specific materials. These included different type of natural and artificial substrates (Evans *et al*., 1973; Motoda, 1977; Naidu & Scaplen, 1979; Pena *et al*., 1996; Mandić M. *et al*., 2016; Bratoš Cetinić, 2024). Since the 1960s, collectors based on Japanese designs, typically made from onion or vegetable bags filled with fishing nylon, have been widely used (Wilson, 1994; Peharda & Onofri, 2000; Acarli *et al*., 2011; Popović Perković *et al*., 2021; Marčeta *et al*., 2022).

The timing of collector deployment and the duration of immersion are critical factors affecting bivalve spat settlement success. Immersion length strongly influences both the abundance and diversity of collected species, largely depending on the timing of spawning events (Thouzeau, 1989; Marčeta *et al*., 2022; Theodorou *et al*., 2025). Extended immersion may reduce species diversity, as some individuals metamorphose, transition to a benthic lifestyle, or detach from collectors.

This study introduces an experimental collector design inspired by the Japanese model for collecting bivalve spat. These collectors are made entirely of artificial materials, polyethylene bags filled with fishing line. The increased internal volume provides a larger effective surface area for bivalve spat settlement compared with previously used collector types.

Using this collector design may yield several advantages, including higher bivalve spat collection due to increased settlement area, improved handling during operations, and enhanced protection of juvenile stages from predation due to the durability of synthetic materials.

The primary aim of this research was to evaluate the effectiveness of these experimental collectors for marine bivalve spat collection and to assess how immersion duration influences attachment rates, species diversity, and overall spat bivalve abundance.

MATERIALS AND METHODS

Study area

The Boka Kotorska Bay is a semi-enclosed basin located along the Montenegrin coast of the Adriatic Sea. It consists of four interconnected sub-bays: Kotor, Risan, Tivat, and Herceg Novi, covering a total water surface area of approximately 87.3 km² (Figure 1). Due to its geomorphological configuration and restricted connection to the open sea, the bay exhibits unique hydrographic and hydrodynamic characteristics (Stjepčević & Žunić, 1964; Magaš, 2002; Drakulović *et al*., 2016).

During both winter (January) and summer (August), stronger sea currents are observed in the surface layer. In the Verige Strait, the highest current velocities (exceeding 20 cm/s) occur during periods of maximum freshwater inflow. In the inner parts of the bay, current intensity generally decreases in winter, except in areas influenced by the Ljuta River. In the central regions, the dominant flow direction is outflow, with localized circular currents formed by the irregular coastline (Bellafiore *et al*., 2011). At depths of around 20 m, weaker inflow currents prevail along the bay margins, functioning as compensatory bottom flows that balance the strong outflow surface currents and tidal influences (Mandić S. *et al*., 2016). During periods of minimal freshwater inflow, surface currents of lower intensity (~10 cm/s) are directed southeastflow, while bottom currents remain inflow-directed but are less pronounced than in winter (Bellafiore *et al*., 2011).

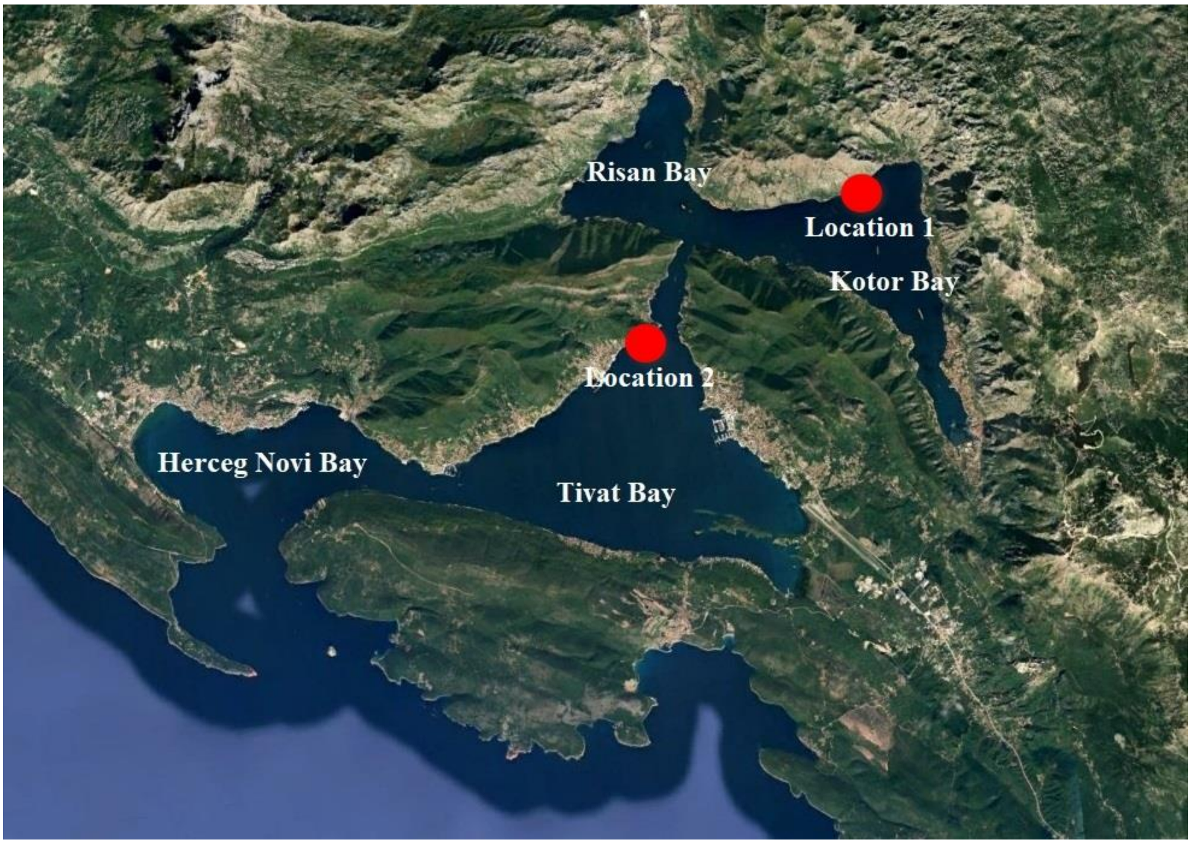

Figure 1. Boka Kotorska Bay with location of sampling (red dots)

Freshwater inflow into the bay is highly variable and strongly seasonal. Seven rivers discharge into the Bay of Kotor, of which the Škurda and Široka Rivers are perennial, while the others flow mainly during late autumn, winter, and early spring. The predominantly calcareous and porous geological composition of the surrounding terrain supports numerous freshwater springs, whose discharge rates decline significantly during summer (Radović, 1964; Bortoluzzi *et al*., 2016). In the hinterland, particularly in the Crkvica area, precipitation is extremely high, and water, due to the karstic terrain, infiltrates and re-emerges as submarine springs, significantly affecting the color, salinity, and density of bay waters (Magaš, 2002).

During periods of peak freshwater inflow, surface waters (0–2 m depth) in the inner Kotor and Risan bays show markedly reduced salinity. In contrast, salinity reductions are less pronounced in the outer parts of the bay, with minimum recorded values of 12.68 PSU in Tivat Bay and 18 PSU in Herceg Novi (Regner *et al*., 1998; Mandić S. *et al*., 2016). Seasonal variation in river discharge leads to substantial salinity fluctuations throughout the year. In summer, when freshwater inflow is minimal and evaporation is high, maximum surface salinity reaches 35.46 PSU in the Bay of Kotor, 37.39 PSU in Tivat Bay, and 37.67 PSU in Herceg Novi. Salinity variation in bottom layers is much smaller: in the Bay of Kotor, the difference between maximum and minimum surface salinity is 32.22 PSU, while in the bottom layer it is only 10.39 PSU (Regner *et al*., 1998; Mandić S. *et al*., 2016).

Organic matter produced through primary production forms the basis of the marine food web, but imbalances in key nutrients, particularly phosphorus and nitrogen, can destabilize ecosystems. Excessive nutrient inputs lead to eutrophication, while insufficient levels result in oligotrophication.

In Boka Kotorska Bay, intensive coastal urbanization, inadequate spatial planning, and increased nutrient loads from marine outfalls have degraded coastal habitats. Freshwater inflows from karstic streams and submarine springs further influence environmental conditions, especially in the inner Bay of Kotor. As a result, the bay is generally considered mesotrophic, although conditions vary seasonally: winter peaks in nutrient concentrations and chlorophyll *a* may cause short-term eutrophication, whereas summer conditions often shift toward oligotrophy (Krivokapić *et al*., 2011; Drakulović *et al*., 2016).

Experimental collectors

Experimental polyethylene collectors were deployed at two shellfish farms: in Orahovac in the Bay of Kotor (Location 1) and in Kamenari in Tivat Bay (Location 2) (Figure 1). The bivalve collectors were constructed from vegetable bags filled with another vegetable bag, fishing line, and used *pergolari* (tubular nets commonly used for mussel cultivation). Each collector measured 45 × 45 cm, with a mesh size of 5 mm (Figure 2).

At each location, collectors were attached to a rope at 1-m intervals and deployed at depths ranging from 2 m below the water surface to 2 m above the seabed. The rope with collectors was secured to the outer line of the floating farm, and a stone anchor was placed at the bottom to keep the rope and bags in a vertical position. After the immersion period, the collectors were retrieved, stored in containers, and preserved in 70% ethanol until further processing.

At Location 1, the first group of 14 collectors was installed and monitored from June to December 2017. At Location 2, two groups of collectors were deployed. The first group, similar to Location 1, was installed from June to December 2017, while the second group, consisting of nine collectors, was deployed from July 2018 to May 2019.

The determination of bivalve species was done in the laboratory using: Poppe & Goto (2000), Riedl (2002) and Milišić (2007) and counted. The collected data were processed using Microsoft Excel (Microsoft Corporation, 2007).

The Kruskal-Wallis test was used to assess whether there were significant differences in the number of bivalves spat settled on collectors with different immersion periods, while Mann-Whitney test was used to assess whether there were significant differences in bivalve spat abundance among years and sampling locations.

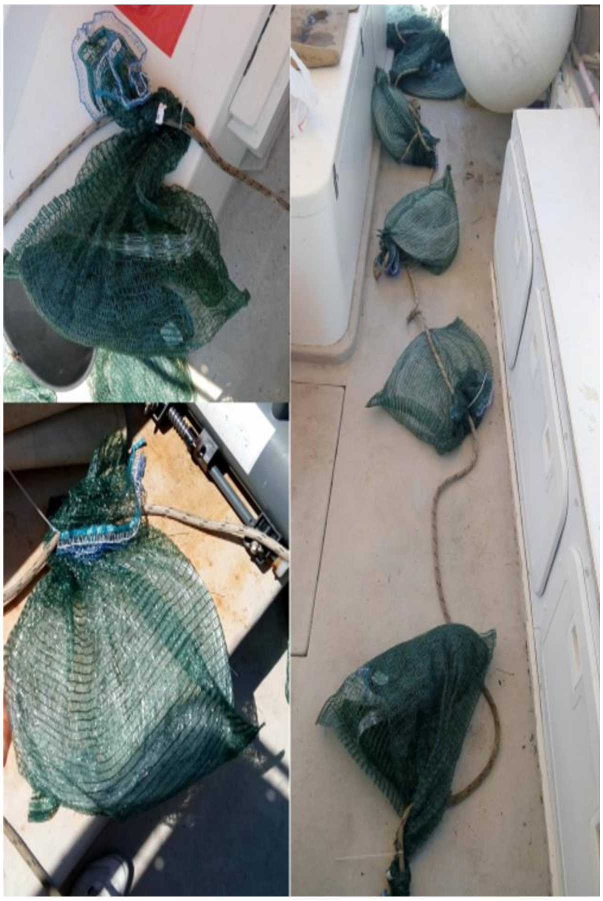

Figure 2. Experimental polyethylene collectors

RESULTS

Analysis of the first group of collectors (immersion for 6 months) revealed 14 bivalve taxa at Location 1 and 15 taxa at Location 2, with a total of 1,129 and 960 individuals, respectively. The second group (immersion for 12 months) of nine collectors at Location 2, recorded 11 bivalve taxa, with a total of 516 individuals. The species identified from both immersion periods are listed in Table 1.

Numerous specimens of *Mytilus galloprovincialis*, *Talochlamys multistriata*, *Modiolarca* sp., *Limaria hians*, and *Ostrea edulis* were recorded on the collectors. After six months of immersion at Location 1, the most abundant taxa were *Mytilus galloprovincialis*, *Talochlamys multistriata*, and *Modiolarca* sp. At Location 2, the dominant taxa after the same immersion period were *Anomia ephippium*, *Talochlamys multistriata*, and *Modiolarca* sp. (Table 1).

For collectors immersed for twelve months at Location 2, the most numerous taxa were *Mytilus galloprovincialis* and *Limaria hians*, followed by a slightly smaller number of *Talochlamys multistriata* (Table 1).

The Kruskal-Wallis test showed that the abundance of organisms on the collectors did not differ significantly across immersion periods (KW-H = 2.28; $p > 0.05$).

The Mann-Whitney U test indicated no statistically significant differences in bivalve spat abundance between sampling locations ($Z = –1.12$, $p > 0.05$), but significant differences were observed among years ($Z = 2.20$, $p < 0.05$).

Table 1. Diversity and abundance of bivalve taxa on experimental collectors according to different immersion periods (Orahovac – Location 1; Kamenari – Location 2)

Taxa	Immersion for 6 months (Orahovac)	Immersion for 6 months (Kamenari)	Immersion for 12 months (Kamenari)
Acanthocardia sp.	15	12	1
Anomia ephippium Linnaeus, 1758	47	125	3
Aequipecten opercularis (Linnaeus, 1758)	20	22	/
Flexopecten flexuosus (Poli, 1795)	/	/	1
Flexopecten glaber (Linnaeus, 1758)	29	38	/
Pecten jacobaeus (Linnaeus, 1758)	43	12	/
Talochlamys multistriata (Poli, 1795)	224	308	92
Hiatella rugosa (Linnaeus, 1767)	28	11	26
Limaria hians (Gmelin, 1791)	43	107	159
Modiolarca sp.	200	140	8
Mytilus galloprovincialis Lamarck, 1819	410	99	176
Ostrea edulis Linnaeus, 1758	15	52	41
Pictada radiata (Leach, 1814)	6	23	7
Pinna nobilis Linnaeus, 1758	43	9	2
Polititapes aureus (Gmelin, 1791)	/	1	/
Ruditapes decussatus (Linnaeus, 1758)	6	1	/

DISCUSSION

Throughout the history of mariculture, various methods have been employed for the cultivation of marine bivalves and the collection of spat. Traditionally, bundles of branches and used *pergolari* were utilized for this purpose. However, during the 1980s and 1990s, collectors made from vegetable bags became widely adopted due to their high efficiency in bivalve spat collection (Brand *et al*., 1980; Marguš *et al*., 1993; Knuckey, 1995; Pena *et al*., 1996; Peharda & Onofri, 2000; Acarli *et al*., 2011). In this study, we used this type of collector to achieve the most effective collection of bivalve spat during the experimental period. Research conducted in neighboring countries also indicates that this collector type is efficient and suitable for capturing a wide range of bivalve taxa (Tsotsios *et al*., 2016; Papa *et al*., 2021; Marčeta *et al*., 2022).

Determining the optimal periods for deploying spat collectors is largely conditioned by the reproductive timing of marine bivalves, as spawning events define the intervals when the highest concentrations of competent larvae are present in the water column and available for settlement. Equally important are the duration of immersion and the precise timing of collector retrieval, as these factors critically influence larval attachment, early post-settlement survival, and the overall quantitative and qualitative composition of the resulting spat assemblage. Therefore, aligning deployment and retrieval procedures with species-specific spawning patterns and prevailing environmental conditions is essential for obtaining methodologically robust and ecologically representative settlement data (Evans *et al*., 1973; Motoda, 1977; Pena *et al*., 1996; Mendo *et al*., 2015).

Our results showed that collectors immersed for six months resulted in higher numbers of bivalve spat at both study sites: 1,129 individuals at Location 1 and 960 individuals at Location 2. In contrast, collectors left in immersion for twelve months at Location 2 showed a reduced number of individuals (516) and lower taxonomic diversity (11 taxa). One likely explanation for this decline is biofouling, the accumulation of other organisms on collector surfaces, which reduces water flow and nutrient availability.

Statistical analysis revealed significant differences between the years in which bivalve spat collectors were deployed, highlighting the influence of temporal factors on bivalve spat settlement. These findings are consistent with previous studies and emphasize the importance of optimizing collector immersion duration to improve the settlement and retention of spat from different bivalve species, which is critical for successful aquaculture practices. Several studies have shown that prolonged immersion promotes extensive fouling, thereby decreasing the available space for bivalve spat attachment (Avendaño *et al*., 2007; Carroll & Peterson, 2013; Marčeta *et al*., 2022).

The use of this type of experimental collector for bivalve spat settlement worldwide has shown consistently positive results, as the mesh structure allows uninterrupted water flow carrying essential nutrients while also providing protection from predators. In this study, a total of 16 different species were collected. Studies throughout the Mediterranean report varying levels of species diversity using similar collectors: 17 species in the northern Adriatic (Brijuni) (Bakran-Petricioli *et al*., 2023), 28 taxa in the northwestern Adriatic (21 taxa at Pellestrina and 22 at Caleri) (Marčeta *et al*., 2022), and 15 species in the Aegean Sea (Izmir Bay) (Acarli *et al*., 2011).

The experimental collectors used in this research were deployed following a period of heavy rainfall, which resulted in increased nutrient input from land. At the same time, a moderate rise in temperature combined with spring circulation created optimal conditions for growth and development. The natural spawning period of marine bivalves overlapped with the deployment period, which explains the dominance of species such as *Mytilus galloprovincialis*, *Talochlamys multistriata*, *Modiolarca* sp., *Limaria hians*, and *Ostrea edulis* recorded on the experimental collectors (Stjepčević, 1967; Nikolić *et al*., 2023; 2024).

Extended immersion periods also intensify spatial and nutritional competition among organisms, further limiting bivalve spat settlement and growth (Yamamoto, 1964; Gruffydd & Beaumont, 1972; Gruffydd *et al*., 1975; Minchin, 1976; Paul, 1978; Naidu & Scaplen, 1979; Brand *et al*., 1980; Fegan, 1983; Mason, 1983; Thouzeau, 1989; Chauvaud *et al*., 1996; Marčeta *et al*., 2022; Theodorou *et al*., 2025). In addition, various crustaceans, common fouling organisms can significantly reduce the survival and attachment of bivalves spat (Lefcheck *et al*., 2014). The results obtained from experimental collectors immersed for twelve months corroborate these findings: the reduced number of individuals is likely attributable to increased competition for limited space and nutrient resources.

The decline in bivalve spat abundance on long-immersed collectors may also be linked to metamorphosis and the subsequent transition to later life stages. Marguš *et al*. (1993) suggested that a four-month immersion period is optimal, as it minimizes fouling and prevents colonization by undesirable organisms. Studies from the northwestern Adriatic, Ionian, and Aegean Seas similarly indicate that prolonged immersion (beyond 12 weeks) leads to increased biofouling, a higher presence of invasive species, and greater risk of bivalve spat loss or reduced quality. To ensure optimal growth and support further cultivation of collected species, it is therefore recommended to shorten immersion duration in accordance with the seasonal reproductive cycles of target bivalves (Theodorou *et al*., 2025; Marčeta *et al*., 2022; Papa *et al*., 2021). In our study, the experimental collectors immersed for six months were deployed in June, coinciding with the spawning season of several bivalve taxa such as clams, scallops, mussels, and oysters (Stjepčević, 1967; Marguš *et al*., 1993; Nikolić *et al*., 2023; 2024). This timing allowed for effective bivalve spat settlement and post-metamorphic attachment.

CONCLUSION

Based on the obtained research data, it can be concluded that understanding the ecology of marine bivalve species is essential for optimizing spat collection. Knowledge of the spawning periods of target species enables the precise timing of collector deployment, thereby maximizing spat settlement while minimizing colonization by non-target bivalves and fouling organisms.

Although the Kruskal-Wallis test did not indicate a statistically significant difference, a slight decrease in the abundance of both bivalve spat individuals and various species was observed in group of collectors immersed for 12 months compared to the group of collectors immersed for 6 month. Prolonged immersion leads to increased fouling, reduced water flow, and the loss of bivalve spat due to metamorphosis and transition to other life stages. Therefore, to achieve the most effective collection of bivalve spat, it is crucial to align the deployment period of collectors with the reproductive cycles of the target species and to maintain immersion conditions that support optimal settlement and survival.

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Analiza prihvata mlađi školjkaša na eksperimentalnim polietilenskim kolektorima sa različitom dužinom imerzije

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SAŽETAK

Ova studija prikazuje rezultate istraživanja raznovrsnosti zajednica morskih školjki zasnovana na analizama eksperimentalnih polietilenskih kolektora sa različitim periodima imerzije Kolektori su bili postavljeni u Bokokotorskom zalivu od juna do decembra 2017. godine i od juna 2018. do maja 2019. godine na dva postojeća uzgajališta školjki, u Orahovcu i Kamenarima. Najzastupljenije taksonomske grupe su bile: *Mytilus galloprovincialis, Talochlamys multistriata, Modiolarca* sp., *Limaria hians* i *Ostrea edulis*. Kolektori koji su bili u imerziji šest mjeseci zabilježili su veći broj jedinki i veću taksonomsku raznovrsnost u poređenju sa kolektorima nakon dvanaest mjeseci imerzije. Ovi rezultati ukazuju da je važno uskladiti postavljanje kolektora sa reproduktivnim ciklusima vrsta, i da duže vrijeme imerzije ne doprinosi optimalnom naseljavanju i preživljavanju.

Ključne riječi: morske školjke, eksperimentalni polietilenski kolektori, naseljavanje, diverzitet