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FAN MUSSEL *PINNA NOBILIS* LINNEAUS, 1758 SPAT COLLECTION ON "CHRISTMAS TREE" ROPE IN THE MALI STON BAY AREA (SOUTH ADRIATIC SEA)

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During November 2006 on a 500 m long experimentally placed "Christmas tree" rope collector for juvenile mussels (*Mytilus galloprovincialis* Lamarck, 1819) placed the previous year a significant number of juvenile fan mussels (*Pina nobilis* Linnaeus, 1758) were observed. Live juvenile individuals (N=322) of *P. nobilis* with shell length from 17 to 93 mm were found in two sampling events. All juvenile fan mussels were found exclusively on deeper parts of the mussel rope. Observed population density was from 6 to 43 individuals per meter of the rope collector. The 120 smallest juveniles of *P. nobilis* were separated for the purpose of monitoring the growth rate in suspension, and the remaining juveniles were placed in the natural environment.

Key words: fan mussel spat, "Christmas tree" rope, the Bay of Mali Ston

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Tijekom studenog 2006., na 500 m prethodne godine eksperimentalno postavljenog kolektora "Christmas tree" za mlađ dagnji (*Mytilus galloprovincialis* Lamarck, 1819), opažen je značajan broj mlađi periske (*Pina nobilis* Linnaeus, 1758). U dva prikupljanja pronađene su žive mlade jedinke (N=322) *P. nobilis* duljine ljušture od 17 do 93 mm. Sve mlade periske pronađene su isključivo na dubljem dijelu užeta. Opažena gustoća populacije bila je od 6 do 43 jedinki po metru kolektorskog užeta. Najmanjih 120 jedinki periske odvojeno je za monitoring rasta u suspenziji, a ostala mlađ je položena u prirodni okoliš.

Ključne riječi: mlađ periske, kolektor "Christmas tree", Malostonski zaljev

INTRODUCTION

The eastern coast of the Adriatic Sea has a very long tradition of bivalve production, through both exploitation of wild populations and farming. One of the largest of farming sites is the Mali Ston Bay, famous for the production of the European flat oyster *Ostrea edulis* Linnaeus, 1758. The bay has a centuries-old history of breeding of this shellfish known by the local name "Malostonska kamenica". In October 2020 the European Commission declared the European flat oyster from tMali Ston Bay authentic

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and unique to the European Union (ANONYMOUS, 2020). Along with oysters, Mediterranean mussels *Mytilus galloprovincialis* Lamarck, 1819 have been intensively cultivated in the bay since the Second World War (BENOVIĆ, 1997).

The spat of the Mediterranean mussel along the eastern coast of the Adriatic area has in the past been collected on nylon (polyurethane, plastic) ropes with a diameter of 25 mm placed on the surface of the sea. At the end of December of 2005 and the beginning of January of 2006 in order to modernize mussel farming, the shellfish association "Croatian Oyster" from Ston set up a new type of collector for collecting juvenile mussels, the so-called "Christmas tree" 'rope on its concession site in the central part of Mali Ston Bay. It is a solid core polyurethane (plastic) rope with a diameter of 12 mm and plastic threads around it that increase the total diameter of the rope to 34-36 mm. This type of collector material multiplies the area for settling younger mussels compared to classic ropes, because each thread becomes the surface for the settling of juvenile mussels. Five hundred meters of this type of collector have been set up to collect juvenile mussels. A year later, in November of 2006, along with the mussel spat, a significant number of juvenile fan mussels *Pinna nobilis* were observed, with shell length up to 10 centimeters, which has never been recorded on traditional collectors.

The species *P. nobilis* is an endemic bivalve in the Mediterranean (POPPE & GOTO, 2000), and widespread along the eastern coast of the Adriatic Sea. Maximum length of the longest axis of the shell is up to 120 cm. It is the largest shellfish living in this sea (ZAVODNIK *et al.*, 1991). The fan mussel prefers sandy-muddy bottoms and seagrass meadows. It is attached to the substrate with strong bisus threads with about third of the shell dug in (ZAVODNIK, 1967; TEMPLADO *et al.*, 2004). The depth distribution of the fan mussel is from 0.5 to 60 m. ROUANET *et al.* (2015) suggest that *P. nobilis* can reach ages of up to 45 years. Due to its size, shells are an ideal substrate for numerous epibiotic flora and fauna (Algae, Spongiae, Cnidaria, Mollusca, Polychaeta, Crustacea, Echinodermata, Bryozoa, Tunicata). Shells of dead *P. nobilis* can serve as a shelter for some small coastal fish that can also lay eggs inside them. Inside the shells of living individuals, fish of the genus *Gobius* have also been observed (ZAVODNIK, 1967).

Due to human activities, *P. nobilis* has become an endangered species and it is strictly protected by law throughout the Mediterranean. In Croatia, it is under strict protection under the 1977 law (Official Gazette, 23/1977). In the past, mother-of-pearl from fan mussel was used to make jewelry, bisus threads for fine gloves and fabric, and today this shellfish is illegally hunted, partly for consumption in gastronomy, and mostly for the souvenir value of shells. The survival of *P. nobilis* is also threatened by pollution and habitat degradation, illegal trawling, boat anchoring and coastal construction (BA-sso *et al.*, 2015). In the Adriatic Sea during 2018 and 2019 areas with dense populations of *P. nobilis*, with approximately 9 to 13 individuals/100m² were observed (ČIŽMEK *et al.*, 2020).

In the early fall of 2016, mass mortality of up to 100% of individuals of *P. nobilis* caused by a combination of parasites and bacteria (Vázquez-Luis *et al.*, 2017) was observed in the western Mediterranean off the coast of Spain. Soon it spread to the eastern Mediterranean (KATSANEVAKIS *et al.*, 2019; CABANELLAS-REBOREDO *et al.* 2019).

The wave of mortality of *P. nobilis* in 2019 quickly spread along the east coast from south to north, and in some localities mortality was 36% up to 100% (Čižmek *et al.*, 2020). According to ŠARIĆ *et al.* (2020) it is caused by the combination of the parasite *Haplosporidium pinnae* and bacteria *Mycobacterium sp.* while PEHARDA *et al.* (2022) indicate the

possible impact of environmental factors on long term stress on fan mussels prior to the mass mortality events.

During 2019 and 2020 the rich settlements of *P. nobilis* in the Mali Ston Bay also disappeared. According to information from the field (local farmers), today there are probably no more living specimens of *P. nobilis* in the whole of the bay, which is somewhat confirmed by the collectors for collecting juveniles set up within the national program for the conservation of *P. nobilis* in the Adriatic Sea. On the collectors inspected at the end of 2021 no settled juvenile *P. nobilis* was found (BRATOŠ-CETINIĆ, unpublished data). Before the mass mortality, recruitment of *Pinna nobilis* on artificial structures was reported by several authors (CABANELLAS-REBOREDO *et al.*, 2009; ACARLI *et al.*, 2011a; KERSTING & GARCIA-MARCH, 2017). Spat settlement on artificial structures of some other species within the genus *Pinna* has been reported by CENDEJAS *et al.* (1985), BUTLER (1987), NARVÁEZ *et al.* (2000) and BEER & SOUTHGATE (2006).

MATERIALS AND METHODS

Farmers of the Croatian Oyster Association experimentally placed a 500 m long floating "Christmas tree" rope to collect juvenile mussels in the central part of the Mali Ston Bay at the end of 2005. A year later juvenile fan mussels up to 10 centimeters long were spotted. "Christmas tree" rope was checked for samples in detail on two occasions, November 19 and 28, 2006. Juvenile *P. nobilis* individuals on populated parts of ropes were randomly counted in one meter sections. They were then carefully removed individually from the ropes, care being taken not to break or damage them in any way. Parts of the shells of fan mussel individuals crushed by juvenile mussels settled on the "Christmas tree" were also observed (Fig. 1).

Each juvenile fan mussel was measured in antero-posterior length using vernier calipers. After that, 120 of the smallest shellfish were transferred to specially designed experimental cages hung on floating parks owned by cooperatives. In the cages, the growth rate of *P. nobilis* in suspension in three layers of seawater (surface, middle and



Fig. 1. Fan mussel Pinna nobilis spat on the "Christmas tree" collector.



Fig. 2. Measuring of juvenile Pinna nobilis individuals.

bottom) was monitored for two years (KožuL *et al.*, 2012). The remaining juvenile fan mussels picked up from the "Christmas tree" rope were placed in a natural environment on the sandy seabed in the part of the bay where the association has a concession for shellfish farming (Fig. 2).

RESULTS AND DISCUSSION

The population density of juvenile *P. nobilis* was significantly higher on the parts of the "Christmas tree" rope that sank under the weight of juvenile mussels to a depth of two to three meters. Juvenile *P. nobilis* was not observed in the shallower parts of the collector that remained closer to the seawater surface. The entire the Mali Ston Bay is under strong influence of freshwater coming from underwater springs and the Neretva River. Throughout the whole year, surface freshwater results in large fluctuations in temperature and salinity, which can probably be tied to the absence of spat on surface portions of the collector rope. According to BENOVIĆ *et al.* (2003) surface temperature in the inner parts of the bay can fluctuate largely, from summer 26 °C to 0°C in the winter. Salinity also greatly oscillates, from 15 to over 30 PSU. Close to the locality with the "Christmas tree" rope, surface salinity falls below 22 PSU, and temperature varies from 11,4 °C to 28,1 °C (BOLOTIN, unpublished data).

During the first analysis on November 19, 2006, 6 to 43 individuals of younger *P. nobilis* with a size from 17 to 93 mm and a mean value of 38.8 ± 16.6 mm were found per meter of rope. The second analysis on November 28, 2006 yielded from 6 to 36 juvenile fan mussels slightly larger (18-95 mm length) than those from the first collector analysis. The mean shell length was 45.7 ± 16.4 mm. A total of 322 juveniles of *P. nobilis* ranging in size from 16 to 95 mm with a mean value of 36.7 ± 15.2 mm were collected on the "Christmas tree" collector, including those parts of the collector where only individual juveniles were observed. According to RICHARDSON *et al.* (2004) Pinnids such as *P. nobilis* and *Pinna bicolor* Gmelin, 1791 exhibit the fastest growth rate of any shell-fish during first two-three years (Fig. 3).



Fig. 3. Measuring of juvenile Pinna nobilis individuals.

From all collected specimens of *P. nobilis* the 120 smallest were separated to monitor the growth rate in suspension (KožuL *et al.*, 2012), and the remaining part was placed in the natural environment. During two-year trials, mortality was observed only during the first few months (8 deaths out of 120 individuals, 6,6%). KATSANEVAKIS (2007) also notes low mortality in natural conditions in Lake Vougliameni, Greece (7 deaths in 160 individuals). ACARLI *et al.* (2011b) monitored the mortality of young *P. nobilis* in suspended PVC cages in Izmir Bay. A 7% mortality rate was recorded in a group of smaller individuals (shell lenght 32 mm). In a group with larger individuals (shell length 73 mm) no mortality was recorded. Wu & SHIN (1998) note 18% mortality in cage rearing of *P. bicolor* and 24% mortality in raft rearing, during 12 months. Survival rate of *P. bicolor* in suspended culture was 95% after 23 weeks and 78% after 80 weeks (BEER & SOUTHGATE, 2006). For the same culture conditions, *P. carnea* Gmelin, 1791 exhibits a high survival rate of over 96% (NARVÁEZ *et al.*, 2000).

According to RICHARDSON *et al.* (2004) fan mussels generally settle on the substrate in late autumn and winter, and in the Bay of Mali Ston the settlement of *P. nobilis* is more intensive in late autumn than in summer (late summer). The same authors also cite data for sea lakes on the island of Mljet (South Adriatic), where juvenile *P. nobilis* with lengths between 8.6 and 9.6 cm were observed in Little Lake during the summer and individuals between 10.7 and 11.2 cm in the Great Lake in late summer and early autumn.

In contrast to the southern Adriatic, in the northwestern Mediterranean, in Spain, KERSTING & GARSIA-MARCH (2017) observed two recruitment peaks of *P. nobilis*, in the periods July-September and September-November. During these studies, in addition to monitoring of natural recruitment, larval settlement in artificial collectors was also monitored. On vertically placed collectors consisting of plastic mesh bags (eight liter volume and 0.8 cm mesh-size) filled with tangled nylon fishing net, a maximum of between 160 and 164 individuals were found, and a minimum of 2 to 25 recruits. Juvenile size ranged from 0.6 to 9.1 cm.

Earlier research from the western Mediterranean shows a marginally longer period of recruitment of *P. nobilis*, from July to December. Six hundred and fourteen juveniles with a mean shell length of 2.76 cm were collected on vertically placed collector type I ropes, after 68 days of immersion. On polyethylene mesh bags (collector type II) after six months of immersion (July to December), 691 juveniles with mean shell length of 5.38 cm were collected (CABANELLAS *et al.*, 2009). ACARLI *et al.* (2011a) note that during one-year period in eastern Mediterranean *P. nobilis* settles only on collectors placed during July for 26 days. They collected 122 individuals with a mean shell length of 7.2 mm.

Spat settlement on artificial structures was monitored for other species within the *Pinna* genus: *P. bicolor, P. rugosa* and *P. carnea*. BUTTLER (1987) notes that in four-year trials in south Australia a negligible number of juvenile *P. bicolor* settled on plastic mesh onion bags. According to BEER & SOUTHGATE (2006) juvenile settlement of *P. bicolor* was significantly greater in north Australia, where they collected 950 juveniles on plastic mesh bags during 12 months. The same type of mesh bag collectors was used in California to collect 1031 young individuals of *P. rugosa* during six months (CENDEJAS *et al.,* 1985). This type of collector has also been shown to be efficient in Venezuela, where 80 individuals per collector were recorded during 16 months of an experiment (NARVÁEZ *et al.,* 2000).

The experimentally installed "Christmas tree" rope collector for collecting juvenile mussels in the Mali Ston Bay did not come into commercial use because the shellfish cooperative was soon disbanded due to difficulties in operation and ceased to operate. Thus, further monitoring of settlement of fan mussels on this type of collector was not possible.

In Croatia, through cooperation of Fund for Environmental Protection and Energy Efficiency and the Institute for Environmental Protection and Nature of the Ministry of Economy and Sustainable Development, the project "Preservation of *Pinna nobilis* in the Adriatic Sea" is underway (2020-2022) (http://www.haop.hr/hr/projekti). As part of the project, collectors for the collection of juvenile *P. nobilis* were set up in the Mali Ston Bay, but without results, no juvenile *P. nobilis* having been found on them so far. Therefore, it might be useful to consider that in addition to the already used type of collector, a "Christmas tree" rope collector be placed, given the relatively simple installation and, in the opinion of the authors of this paper, very good acceptance by younger *P. nobilis* during its experimental use in the Mali Ston bay in the South Adriatic.

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SAŽETAK

Mlađ periske *Pinna nobilis* Linneaus, 1758 na kolektoru "Christmas tree" u području Malostonskog zaljeva (južni Jadran)

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U studenom 2006. g. na godinu dana prije eksperimentalno postavljenom kolektoru za mlađ dagnje (*Mytilus galloprovincialis* Lamarck, 1819) "Christmas tree" u dužini od 500 metara, opažen je i znatan broj mlađi periske *Pina nobilis* Linnaeus, 1758. U dvije analize isključivo na dubljim dijelovima konopa nađene su 322 žive juvenilne jedinke *P. nobilis* dužine ljušturice od 17 do 93 mm sa srednjom vrijednošću od 38.8±16.6 mm. Opažena je gustoća naseljavanja od šest do 43 komada po metru dužnom kolektora. Ukupno 120 najmanjih juvenilnih jedinki *P. nobilis* odvojeno je u svrhu praćenja stope rasta u suspenziji (KožuL *et al.*, 2012), a preostali dio mlađi je položen u prirodni okoliš.