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ISSN: 0001-5113 AADRAY	ACTA ADRIAT., 60 (2): 137 - 146, 2019	ORIGINAL SCIENTIFIC PAPER

Distribution of non-native Pacific oyster *Magallana gigas* (Thunberg, 1793) along the eastern Adriatic coast

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Non-native Pacific oyster Magallana gigas *(Thunberg, 1793) was introduced to the Mediterranean Sea for aquaculture purposes in the 1960s. Although this species was not introduced for aquaculture to the Croatian part of the Adriatic Sea, in the 1970s, it was reported in the Lim Bay, in the North-eastern Adriatic. Until recently, there has been no research on the species in the Croatian part of the Adriatic. The aim of this research was to summarize existing and novel data on the distribution of* M. gigas *in coastal areas of the Eastern Adriatic and to provide a baseline for the future monitoring and assessment programmes of the species. Distribution of* M. gigas *was determined by three different methods: (i) a visual census of the presence of* M. gigas *specimens in the medio-littoral zone; (ii) DNA identification of* M. gigas *larvae in the water column; and (iii) the presence of* M. gigas *in the subtidal zone at depth between 25 and 40 m.* Magallana gigas *has a well-established population in the medio-littoral zone of natural and anthropogenic habitats along the coast of the North-eastern Adriatic Sea (west coast of Istria peninsula), but it is not present in the deeper layers . In the Central-eastern and South-eastern Adriatic Sea, the species was either absent or sporadically recorded with no evidence of fully established populations. Considering the great invasion success of* M. gigas *worldwide and effects that this species could have on the invaded ecosystem (e.g. competition for food and space with native species), detailed future monitoring is needed for the Eastern Adriatic Sea.*

Key words: *Crassostrea gigas,* DNA barcoding, invasion, *Magallana gigas*

INTRODUCTION

Non-native Pacific oyster *Magallana gigas* (previously *Crassostrea gigas*) was introduced to the Mediterranean Sea for aquaculture purposes in the 1960s as a response to the decrease of European flat oyster production caused by high mortalities due to parasitic diseases (GOSLING, 2008). Initially, *M. gigas* was limited to the aquaculture sites, but as environmental conditions in those areas were favourable for the species, it has since started to reproduce and spread outside aquaculture sites. *Magallana gigas* was however not introduced for

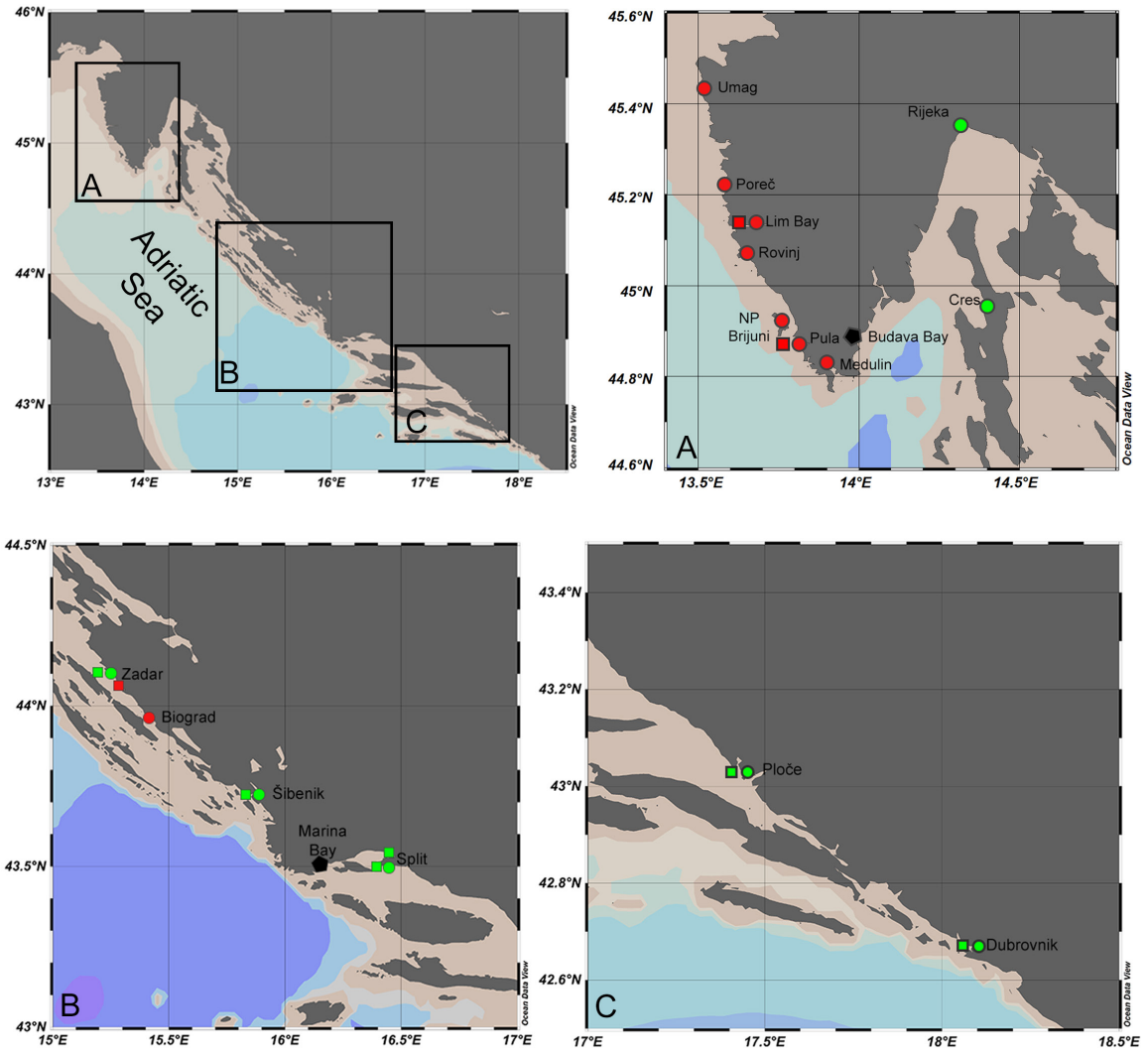

Fig. 1. Map of the locations where the study was conducted. Medio-littoral zone where visual census for the presence of Magallana gigas *was performed are marked with dots: green dots present locations were* M. gigas *was not found; red dots present location where* M. gigas *was found. Locations where M. gigas larva were analysed, are marked with squares - green squares present locations were* M. gigas *larvae were not detected; red squares present location where* M. gigas *larvae were detected. Black polygons present location where* M. gigas *was reported by ŠEGVIĆ-BUBIĆ et al. 2016*

aquaculture purposes to the Eastern Adriatic Sea (i.e. the Croatian coast). Interestingly, in the 1970s, the species was reported in the Lim Bay, the North-eastern Adriatic (FILIĆ & KRAJNOVIĆ-OZRETIĆ; 1978; HRS-BRENKO, 1982). Until recently, there was no research on the species in the Croatian part of the Adriatic (PEČAREVIĆ *et al.*, 2013). Then, PEČAREVIĆ et al. (2013) provided a review on introduced marine species, including *M. gigas*, and suggested that anthropogenic activities might be the vector of the species introduction to the Eastern Adriatic Sea. In the last few years, *M. gigas* has become a focus of several studies dealing only with its occurance, mostly using molecular methods for species determination (ŠEGVIĆ-BUBIĆ *et al.*, 2016; ŠPELIĆ *et al.,* 2018; SPAGNOLO *et al.,* 2019), but thorough assesment of the species distribution and establishemnt has not been conducted yet. The aim of this research was to summarize existing and novel data on the distribution of *M. gigas* in coastal areas of the Eastern Adriatic, and to

provide a baseline for the future monitoring and assessment program of this species. The specific objectives were to determine the presence of settled specimens and larvae of *M. gigas* in the medio-litoral and subtidal zones and water column, respectively.

MATERIAL AND METHODS

Data collection was conducted from October 2017 to October 2018 by: (i) a visual census of the presence of *M. gigas* in the medio-littoral zone; (ii) DNA identification of *M. gigas* larvae in the water column; and (iii) presence of *M. gigas* in the beam trawl catches. Visual census of the presence of *M.gigas* was done at nine locations in the North-eastern Adriatic (NE Adriatic) - Umag, Poreč, the Lim Bay, Rovinj, Pula, National Park "Brijuni", Medulin, Rijeka and Cres; four locations in the Central-eastern Adriatic (CE Adriatic) - Zadar, Biograd na Moru, Šibenik and Split; and two in the South-eastern Adriatic (SE Adriatic) - Ploče and Dubrovnik (Fig 1).

The first observations were conducted in the main shipping ports and aquaculture area as maritime transport and aquaculture were suggested as potential vectors for introduction of new species. If dense populations were found at the investigated locations, further surveys of nearby locations were done (e.g. along the west coast of Istria). Visual census in the medio-littoral zone was performed on harbour structures in commercial ports and on natural rocky shores (Fig 1, Table 1), and presence or absence of the species was recorded. In the shipping ports, a visual census was performed on at least 50% of the port coastline. The presence of *M. gigas* was categorised following a semi-quantitative scale: (i) absent; (ii) rare to occasional sightings of few specimens; and (iii) observed frequently and abundant in the given area. The presence of larvae was assessed in the main shipping ports along the Eastern Adriatic Sea (Fig 1, Table 2): the Ports of Pula, Zadar (two ports), Šibenik, Split (two ports), Ploče and Dubrovnik; and in one of the main aquaculture site in the NE Adriatic - the Lim Bay where two native species are cultivated: the black mussel *Mytilus galloprovincialis* and European flat oyster *Ostrea edulis*. Water samples for larval detection were collected during the warmer part of the year as this is considered as the period when *M. gigas* ripens and reproduce (EZGETA-BALIĆ, unpublished data). Three vertical hauls from 10m depth to the surface were done using a plankton net (i.e., vertical haul: 10 m; mesh size 53μm; mouth opening 35 cm) at all sampling locations in May and August 2018. Additionally, in September 2018, water samples were collected from the Port of Pula and Lim Bay as these two sites are the main shipping port and aquaculture site in the NE Adriatic where this species has already settled. Collected water samples were preserved in absolute ethanol and molecular DNA metabarcoding was conducted following ZHAN *et al.* (2013) to determinethe presence of *M. gigas* larvae in the water column. The hypervariable V4 region of nuclear small subunit ribosomal DNA (nSSU rDNA) was amplified using a specifically designed primer pair for zooplankton communities [Uni18S: AGGGCAAKYCTGGTGCCAGC; Uni18SR: GRCGGTATCTRATCGYCTT (ZHAN *et al.* 2013)]. Recovered sequences were submitted to Pangaea available at:https://doi.pangaea.de/10.1594/PANGAEA.900408.

Furthermore, the presence of *M. gigas* in the commercial beam trawl catch was conducted along the west coast of Istria. Catch and bycatch from 60 hauls (Fig 2.), from 2 to 15 nautical miles from the coast were analysed for the presence of the species. The depth of study area ranged from 25 to 40 m. The beam trawl frame length was 1.8 m and mesh size was 40 mm.

RESULTS AND DISCUSSION

Out of the fifteen surveyed locations, *M. gigas* was recorded at eight. The species was found in the medio-littoral zone, attached on artificial harbour structures, natural rocky shore and/or aquaculture facilities (Fig 1. & Table 1). At all locations on the west coast of Istria (NE Adriatic), the species was ubiquitous and commonly formed very dense colonies (Fig 3). In

Table 1. Presence of Magallana gigas *on at the investigated sites along the Eastern Adriatic*

	Location	Coordinates	Substrate type*	Presence	Occurrence	Previously reported in this area	Reference
NE Adriatic	Umag	45°26'03"N 13°31'11"E	Harbour structures	+	Abundant	No	
	Poreč	45°13'23"N 13°35'37"E	Harbour structures	+	Abundant	No	
	Lim Bay	45°07'53"N 13°44'04"E	Natural rocky shore and aquaculture facilities	+	Abundant	Yes	FILIĆ & KRAJNOVIĆ-OZRETIĆ, 1978; HRS-BRENKO, 1982; ŠEGVIĆ BUBIĆ et al., 2016; STAGLIČIĆ et al. 2018
	Rovinj	45°05'51"N 13°38'07"E	Harbour structures and natural rocky shore	+	Abundant	No	
	NP Brijuni	44°54'59"N 13°46'05"E	Harbour structures and natural rocky shore	+	Abundant	Yes	MZOPU 2001
	Pula	44°51'59"N 13°49'39"E	Harbour structures and natural rocky shore	+	Abundant	Yes	SPAGNOLO et al., 2017
	Medulin	44°49'13"N 13°55'38"E	Harbour structures and natural rocky shore	+	Abundant	Yes	ŠPELIĆ et al. 2018
	Rijeka	45°19'40"N 14°25'58"E	Harbour structures	-	Absent	No	
	Cres	44°57'29.8"N 14°24'25"E	Harbour structures and natural rocky shore	-	Absent	No	
CE Adriatic	Zadar	44°05'16"N 15°15'53"E	Harbour structures	-	Absent	No	
	Biograd N/M	43°56'20"N 15°26'22"E	Harbour structures	+	Few specimens	No	
	Šibenik	43°43'54"N 15°53'33"E	Harbour structures	-	Absent	No	
	Split	43°30'23"N 16°25'59"E	Harbour structures	-	Absent	No	
SE Adriatic	Ploče	43°03'04"N 17°26'01"E	Harbour structures	-	Absent	No	
	Dubrovnik	42°39'35"N 18°05'04"E	Harbour structures	-	Absent	No	

*Short description of substrata type: harbour structure – steep rock and/or concrete wall; natural rocky shore – rocky shore with different stope (from gentle slope to steep rocks); aquaculture facilities – wooden pillars

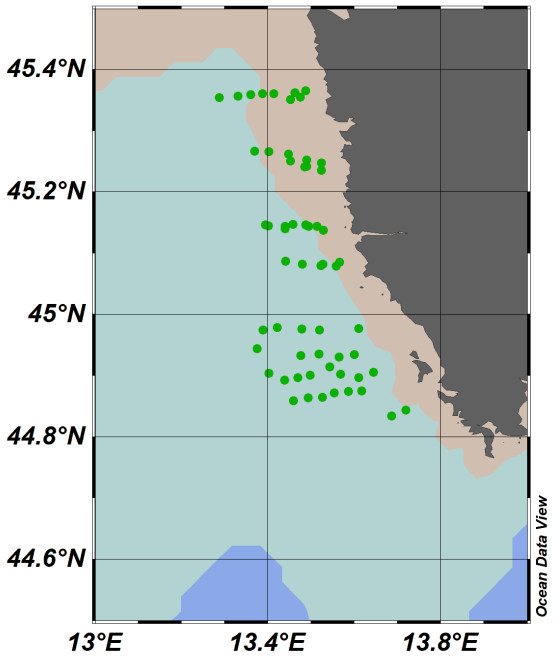

Fig. 2. Map of the beam trawl sampling sites along the west coast of Istria where the catch was analysed for the presence of Magallana gigas.

coastal region of Istria peninsula, the occurrence of *M. gigas* was previously reported in the Lim Bay, Medulin Bay, Port of Pula and Budava Bay (ŠEGVIĆ-BUBIĆ *et al*., 2016; ŠPELIĆ *et al.,* 2018; SPAGNOLO *et al.* 2019). *Magallana gigas* was also recorded in the National Park "Brijuni". Spatial management plan of the National Park "Brijuni" also sporadically mentioned the occurrence of *M. gigas* on harbour structures (MZOPU 2001), while during our study, *M. gigas* was frequently found on both harbour structures and natural rocky shores throughout the National Park. The Port of Rijeka and Cres Island were the only surveyed location in the NE Adriatic where the species was not recorded. In the CE Adriatic, *M. gigas* was found only at one location – Biograd na Moru, and this was the first record of the species in that area. The species was not present in high densities; only a few specimens were found on harbour structures. The presence of *M. gigas* could be related to aquaculture activities in this region, such as transfer of juvenile fish from supplier tanks together with untreated water to the cages. Though, this assumption needs to be

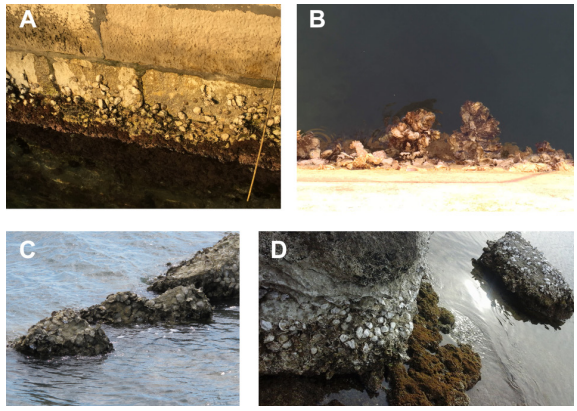

Fig. 3. Dense formations of Magallana gigas *along the west coast of Istria on both artificial and natural substrate: (A) Poreč, (B) Rovinj, (C) the Mirna River mouth and (D) Pula*

considered with caution and it should be further confirmed using molecular approaches. Importantly, this was not the first finding of *M. gigas* in the CE Adriatic as the species was previously reported by ŠEGVIĆ-BUBIĆ *et al.* (2016) in the Marina Bay, an important area for aquaculture. In the SE Adriatic Sea (i.e. the Ports of Ploče and Dubrovnik), the species was not detected during this study.

Larvae of *M. gigas* were found at three out of the nine surveyed locations (Fig 1. & Table 2). Here, we emphasize that the molecular marker used in our study was not able to distinguish between *M. gigas* and *M. angulate* (i.e. 18S); however, recent oyster survey along the NE Adriatic coastline using cytochrome c oxidase subunit I (COI) which is able to discriminate between the two species (HSIAO *et al.,* 2016), determined the presence of only *M. gigas*. Therefore, the species which our 18S marker detected is most likely *M. gigas*. In the NE Adriatic, larvae were detected in the Port of Pula and Lim Bay, which coincided with locations where wild populations of *M. gigas* were present. According to EZGETA-BALIĆ environmental parameters in the Lim Bay, such as temperature and chlorophyll *a* concentration, are favourable for the successful reproductive cycle of the species. Furthermore, considering that *M. gigas* larvae require water temperature of 22°C and above for its optimal development (RICO-VILLA *et al.,* 2009), the temperature after

Table 2. Presence of Magallana gigas *larvae on at the investigated sites along the Eastern Adriatic*

Location	Sampling date	Presence of *Magallana gigas*
Ports		
Port of Pula	May	-
	August	+
	September	-
Zadar - Port Gaženica	May	
	September	-
Zadar - City port	May	-
	September	+ (singleton unique sequence)
Port of Šibenik	May	-
	September	-
Split - City port	May	-
	September	-
Split - North port	May	-
	September	-
Port of Ploče	May	-
	September	-
Dubrovnik – Gruž Port	May	-
	September	-
Aquaculture site		
Lim Bay	May	+
	August	-
	September	+

the *M. gigas* spawning is also favourable for larval development (EZGETA-BALIĆ unpublished data). Therefore, the presence of *M. gigas* larvae at the survey sites together with the data on successful reproductive cycle in the same area and detection of small specimens (<20 mm; STAGLIČIĆ *personal communication*) suggested that *M. gigas* has fully established populations at the NE Adriatic. In the CE Adriatic, *M. gigas* larva was detected only in the Gaženica Port – Zadar. As we did not detect adults on the harbour structures in that area, and our DNA metabarcoding detected only a singleton unique sequence, there is possibility that *M. gigas* is present at very low abundance, or the larva detected was from discharged ships' ballast water and the species is not established there. Furthermore, this area is in a close proximity (around 20 km) to Biograd na Moru (Fig 1) where a few adult specimens were found on harbour structures. Consequently, we still believe that the species is not established in this region, but due to strong aquaculture and shipping activities, future detailed surveys of *M. gigas* are needed.

As *M. gigas* can inhabit depths up to 40 m (FAO, 2018; POPPE & GOTO, 2000), the aim of our study was also to determine if the species is present in deeper waters along the west coast of Istria where other bivalve species (e.g., the European oyster, scallops) are exploited by the beam trawl fisheries. Interestingly, there were no *M. gigas* specimens in any of the analysed beam trawl catches. The only oyster species present in the beam trawl catch was the European flat oyster *Ostrea edulis*. However, occasionally uncommonly large specimens of *O. edulis* were caught (Fig 4) and fisherman sometimes misidentified them as *M. gigas*.

In the natural rocky shore habitats of the North Adriatic, wild populations of *M. gigas* were recently recorded in the Gulf of Trieste, Italy (CROCETTA, 2011) and along the entire Slovenian coast, mainly in the lower medio-littoral area (LIPEJ *et al.,* 2012). Our study detected wild populations of *M. gigas* along the entire west coast of Istria, which is neighbouring area of the above mentioned regions and further extension of the Eastern Adriatic southwards. Confirming the previous study by SPAGNOLO *et al.* (2019) which assessed non-native macrozoobenthic species in twelve ports throughout the entire Adriatic basin during 2014, our study did also not record any *M. gigas* specimens in the CE and SE Adriatic, except at one location that was not included in their study (i.e., Biograd na Moru). SPAGNOLO *et al.* (2019) did not record the species neither in ports in Montenegro nor Albania. However, the authors reported the presence of the species in all Italian and Slovenian ports surveyed, as well as in the Port of Pula in Croatia. Furthermore, VIDJAK *et al.* (2019) assessing zooplankton diversity in eastern Adriatic ports, including the Ports of Pula, Šibenik and Split, did not also report *M. gigas* larvae. It should be emphasized, though, that their results were based on micro-

Fig. 4. Large specimens of Ostrea edulis *caught by the beam trawl that fisherman misidentified as* Magallana gigas.

scopic analysis, which are not able to accurately identify bivalve larvae to species level. Interestingly, they reported a high abundance of bivalve larvae in the Port of Pula; some of these larvae could be *M. gigas* as our study confirmed the presence of *M. gigas* larvae in the water column of the same port. Taking into account intensive maritime traffic among Croatian ports, it is surprising that the species is established only in the NE Adriatic. Consequently, our current findings point towards natural dispersal of the species, as main water current circulation of the Adriatic Sea, that goes northward along the eastern coast (i.e., Croatia) and returns southward along the western coast (i.e. Italy; ORLIĆ *et al.,* 1992), most probably counteracts natural dispersal of larvae to southern parts of the Croatian Adriatic Sea. However, long-term monitoring together with DNA analysis of adult specimens are needed to properly determine the vectors of spread and origin of the established populations.

CONCULUSIONS

Magallana gigas has well-established populations in the medio-littoral zone of natural and anthropogenic habitats along the coast of the North-eastern Adriatic Sea (i.e. western coast of Istria). In the Central-eastern and South-eastern Adriatic Sea, the species is either absent or sporadically recorded with no evidence of established populations. Presence of larvae on the west coast of Istria (i.e., the NE Adriatic) where *M. gigas* wild populations were recorded, together with the evidence of successful reproductive cycle (EZGETA-BALIĆ, unpublished data) and detection of small specimens (<20 mm; STAGLIČIĆ, unpublished data) suggest self-sustaining populations and possibility of natural spread in this area. Larvae present in the central Adriatic could be related to aquaculture and shipping activities. However, additional research is needed to determine the transport vectors and prevent further unintentional spread of *M. gigas*.

This study focused on the main ports and aquaculture areas where commercial shipping, increasing nautical tourism and aquaculture production may consequently increase the spread of the species to new habitats. However, it is still not clear if vectors of spread are connected to shipping industry, aquaculture activities, simple natural dispersal, or any combination of them. Considering great invasion success that *M. gigas* has already demonstrated worldwide (TROOST 2010 and references therein), and its positive and negative effects on ecosystems (LAUGEN *et al.* 2015; HERBERT *et al.* 2016; MORTENSEN *et al.* 2017), future monitoring studies are essential to keep track of the status of the *M. gigas* populations in the Adriatic Sea, and investigation of possible vectors of its introduction in new areas.

ACKNOWLEDGEMENTS

This work has been supported by the Unity through Knowledge Fund (UKF) project „Competition between native *Ostrea edulis* and invasive *Crassostrea gigas* oysters in the Adriatic Sea – effects on the ecosystem, fisheries and aquaculture“ (COCOA) under the UKF Grant Agreement no. 2/17. Permission for the beam trawl survey was obtained from the Ministry of Agriculture-Directorate of Fisheries. EB was additionally supported by the Alexander von Humboldt Sofja Kovalevskaja Award.

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Received: 18 April 2019
Accepted:11 September 2019

Rasprostranjenost nezavičajne vrste kamenice *Magallana gigas* (Thunberg, 1793) duž istočne obale Jadrana

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SAŽETAK

Alohtona pacifička kamenica *Magallana gigas* (Thunberg, 1793) unesena je u akvakulturu Mediterana 1960tih godina. Iako ova vrsta nije unesena u uzgoj u hrvatskom dijelu Jadrana, 1970tih godina zabilježena je u Limskom zaljevu, sjeverni Jadran. Sve do nedavno kamenica *M. gigas* nije bila predmet istraživanja na ovom području. Cilj ovog istraživanja bilo je prikazati postojeća i nova saznanja o distribuciji kamenice *M. gigas* u obalnom dijelu istočnog Jadrana te dati temeljne podatke za budući monitoring i procjenu stanja ove vrste.

Rasprostranjenost kamenice *M. gigas* određivana je primjenom tri metode: (i) vizualni cenzus prisutnosti odraslih primjeraka kamenice *M. gigas* u mediolitoralnom području; (ii) DNK identifikacija ličinki *M. gigas* u vodenom stupcu; (iii) prisutnost odraslih jedinki *M. gigas* u ulovu rampona. Rezultati su pokazali kako kamenica *M. gigas* ima dobro razvijene populacije na prirodnim i antropogenim podlogama mediolitorala duž zapadne obale Istre (sjeverni Jadran), ali nije bila prisutna u dubljim slojevima (25 – 40 m).

U srednjem Jadranu, pacifička kamenica bila je ili odsutna ili tek sporadično zabilježena bez dokaza da su uspostavljene stabilne populacije. Uzimajući u obzir invazivnost koju je ova vrsta već pokazala diljem svijeta, detaljni monitoring i sustavna istraživanja potrebni su za ovu vrstu duž istočne obale Jadrana.

Ključne riječi: *Crassostrea gigas*, DNA barcoding, invazija, *Magallana gigas*