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RESEARCH ARTICLE

Wild Pacific oyster *Magallana gigas* (Thunberg, 1793) populations in Romanian Black Sea waters – friend or foe?

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Abstract
A relatively small population of *Magallana gigas* was discovered near the Agigea harbor (Constanța, Romania) in 2017. The DNA barcoding method was used to confirm the morphological identification of the species. We consider this colony to be the first instance of a possibly stable Pacific oyster population in the Black Sea, outside of farming activities. The possible impact on native ecosystems is briefly discussed.

Keywords
benthic ecosystems, climate change, *Crassostrea gigas*, DNA barcoding, ecological niche, feral populations, invasive species.

Introduction

The Pacific oyster, *Magallana* (*Crassostrea*) *gigas* (Thunberg, 1793), is a highly appreciated shellfish species. Native to the Far East Pacific, it is being used worldwide in aquaculture and has been extensively and successfully introduced in temperate and tropical areas (Ruesink et al. 2005). The first introduction in Europe took place in France in 1966, followed by a series of similar events along the northern European

Atlantic coasts where it established stable and even flourishing wild populations (Shatkin et al. 1997, Lallias et al. 2015). In Romania, *M. gigas* was first recorded in 1995 (Micu 2004). Between 2001 and 2003, intensive off-shore procedures were carried out for the introduction and establishment of the species in the Black Sea for farming purposes (Zaharia and Crivăț 2017). Live isolated specimens have been found in the Romanian Black Sea waters since 2003 (Micu 2004), some of them fixed on the rocky substrate near Constanța (Skolka and Gomoiu 2004). Also, after winter storms, fresh shells of Pacific oyster can frequently be seen on the beaches near Constanța, as was the case in the 2003–2004 winter when valves of 1–8 cm in length were found (Skolka and Gomoiu 2004).

Ostreid species have widely variable shells depending on the substrate where the spat settles (Poppe and Goto 2000). These highly variable morphological characters make it quite difficult to differentiate between ostreid species (Dridi et al. 2008). This is where molecular methods, such as DNA barcoding, are implemented in order to help identify such difficult species. The ostreid specimens discovered in the Romanian Black Sea were identified based on morphological characters and the DNA barcoding method was used to confirm the morphological identification.

This paper presents the first record of a wild colony of *M. gigas* discovered during diving sessions carried out in the Agigea harbor (south of Constanța) for quantitative and qualitative studies of the zoobenthos, in 2017–2018. Possible interactions with native fauna are also discussed.

Materials and methods

Diving sessions were carried out in August and October 2017 in Agigea harbor (south of Constanța, Romania), for a quantitative and qualitative study of the zoobenthos. The oyster colony was discovered in a rocky bottom area in the Agigea harbor (44.098204°N, 28.695071°E) (Figs 1, 2). All oyster individuals were counted and measured in length using an underwater measuring tape in order to estimate the age related to the growth rate. The entire surface occupied by the oyster colony and the depth range were measured using a graded rope with lead weights. The oyster density was assessed by applying the relevé method using a 1m side metallic quadrate. Two individuals were collected and preserved at -20 °C for further DNA analyses.

Morphological identification. Characters such as shell color, shape and length, aspect and color of the adductor muscle scar were assessed and compared to oyster descriptions published in literature (Lucas 1982, Poppe and Goto 2000, Zenethos et al. 2003).

DNA barcoding. Genomic DNA was extracted from foot muscle tissue of two oyster individuals using the commercial ISOLATE II Genomic DNA Kit (Bioline meridian BIOSCIENCE, London, UK), following the producer's instructions. A partial COI (Cytochrome C oxidase subunit I) fragment was selected as barcode and amplified using the HCO2198/LCO1490 primer pair (Folmer et al. 1994). The PCR reaction was carried out in 50 µl final reaction volume containing about 100 ng DNA template, 1 U of GoTaq®G2 Flexi DNA Polymerase (Promega Corporation, Madison, WI, USA), 1X Green GoTaq®Flexi Buffer (pH 8.5), 2 mM $MgCl_2$, 0.1 mM of each dNTP, 0.1 µM of each primer and water up to final volume. The PCR conditions were comprised of an initial denaturation at 95˚C for 2min, 5 cycles of 94˚C for 30s, 45˚C for 1min 30s, 72˚C for 1min, followed by 35 cycles of 94˚C for 30s, 52˚C for 1min 30s, 72˚C for 1min, and a final elongation step of 5min at 72˚C. The amplified fragments were visualized on a 1.5% agarose gel stained with ethidium bromide and purified using the FavorPrep™ Gel/PCR Purification Kit (FAVORGEN® Biotech Corp., Changzhi, Taiwan). The amplicons were sequenced using the commercial services of Macrogen Europe (Amsterdam, Netherlands). The sequences were manually checked for potential sequencing errors in CodonCode Aligner v3.7.1 (CodonCode Corporation, Dedham, MA, USA), then used to query the GenBank online database Nucleotide collection (nr/nt) using the Megablast algorithm (optimized for highly similar sequences) (Zhang et al. 2000, Morgulis et al. 2008) in the BLAST online application (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

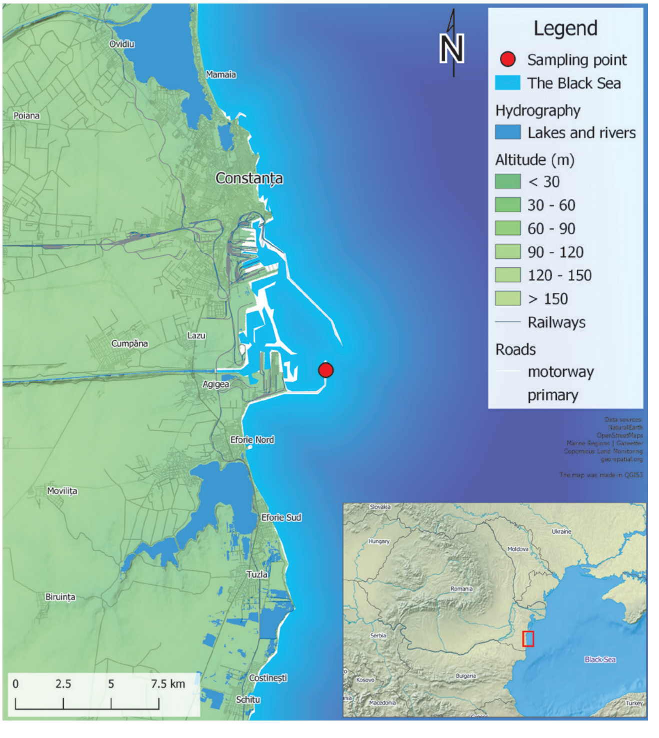

Figure 1. The location of the new *Magallana gigas* population at the Romanian Black Sea coast is indicated by the red dot.

Figure 2. *Magallana gigas* habitat from the Romanian Black Sea littoral

Results

The depth range where the oyster colony was located varied between 4 and 10 m, with a total covered surface of ~350 m^2. The density was relatively low, with an average of 1.16 oysters/m^2.

Diagnosis. Most of the analyzed oysters presented a roundish shell with extensive fluting, similar to *M. gigas* individuals described from hard substrata (Lucas 1982, Zenethos et al. 2003), and only a few young individuals presented an elongated shell. Shell inequivalve with the right valve flattened and the left valve larger and convex, white with purple patches, similar to the description of Zenethos et al. (2003). The adductor muscle scar heavily colored (Poppe and Goto 2000). Shell length varied between 5.2 and 18.6 cm, with an average size of 10.35 cm (Fig. 3).

DNA barcoding. Two 638bp COI sequences were obtained and used to query the GenBank online nucleotide database. 25 matches to sequences of *Crassostrea*/*Magallana gigas* specimens from the native area of the species (Pacific Ocean, China and Japan) were obtained, with similarity levels between 99.4% and 100%. These results confirmed the species identification based on morphological criteria.

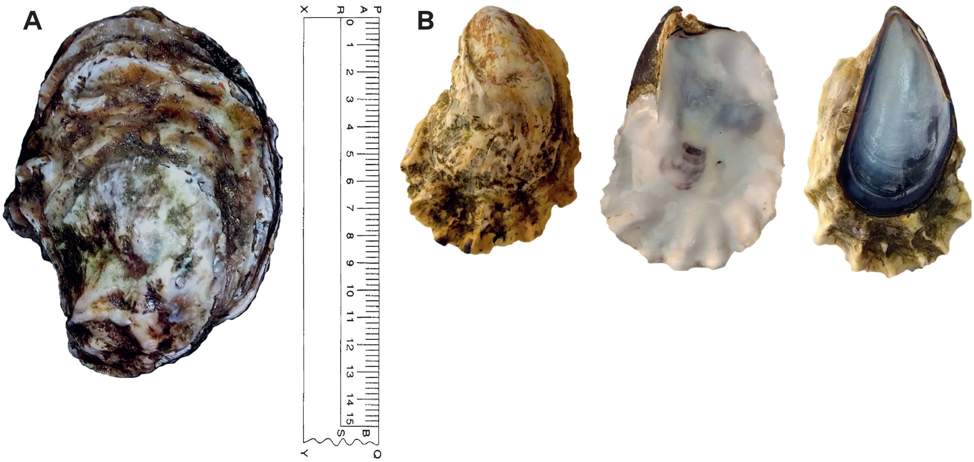

Figure 3. *Magallana gigas* specimens from the Romanian Black Sea waters (scale in cm). (A) The largest collected specimen; (B) Medium sized specimens.

Discussion

M. gigas was first introduced in the northern Black Sea coastal waters from the Sea of Japan to replace the native *Ostrea edulis* that was steadily harvested until the arrival of *Rapana venosa* in the \`30s–\`40s, the voracious gastropod that decimated the native oyster populations (Grossu 1986). A sole individual (only the shell) of *M. gigas* was previously discovered in the Black Sea outside of mariculture facilities, in the Odessa Bay in 2007. The shell was assessed as being over 12 years old and most likely the result of mariculture attempts in the coastal waters of the Caucasus since the 1980s (Kovtun and Zolotarev 2008). Despite being listed as one of the worst 100 invaders due to its high reproductive potential and plasticity (DAISIE 2009), it was generally considered that the Pacific oyster was unable to survive in the Black Sea without artificial hydrobiological conditions. This is why, despite intensive farming activities on the northern shores, it could not survive outside farming facilities, due to the special conditions of the Black Sea, mainly the salinity, the rhopic factor and the average water temperatures,which have a strong effect on the spawning, growth, and survival of this species (Fabioux et al. 2005, Anglès d'Auriac et al. 2017, Ibarra et al. 2017). Also, the Romanian Black Sea conditions are far from optimal for the Pacific oyster in regards to exposure, waves, depth, average minimum temperature, and salinity (Zaharia and Crivăț 2017). Therefore, the discovery of the wild population near the Agigea harbor was unexpected. Several acclimating attempts of the Pacific oyster to farming in the Romanian Black Sea coastal waters were carried out by the National Institute for Marine Research and Development "Grigore Antipa" in collaboration with a private mariculture farm. These experiments were conducted between 2001–2003, in laboratory and off-shore conditions in the Agigea area (Zaharia and Crivăț 2017). Thus, the most likely source of the *M. gigas* colony near Agigea is the stock used by the mariculture farm established here, but in order to confirm or infirm the origins of the colony, further molecular studies will be needed.

The relatively small number of Pacific oyster individuals found in the Agigea population and their small average size, correlated with the average growth rate of 4–5 cm in the first year and up to approximately 7.5 cm in the second year (Diederich 2006), suggest that this population is young, around 5 years old. Only larger oysters were attached to the rocky substrate, while most of the small and medium sized individuals were attached to Mediterranean mussels (*Mytilus galloprovincialis*). This might suggest that either the oyster and mussel colonies developed roughly at the same time, or the mussels settled at a later time and developed at a faster rate, covering a wider rocky area than the oysters. Both situations can be explained by the fact that the conditions in the Romanian Black Sea waters are not optimal for the settlement and development of *M. gigas* (Zaharia and Crivăț 2017), while *M. galloprovincialis* is a native species that thrives in these conditions, even forming a specific littoral biocenosis associated with several benthic communities characteristic to the Romanian Black Sea coast, habitats such as infralittoral rock with *M. galloprovincialis* and biogenic reefs (Băcescu et al. 1971, Teacă et al. 2010, Galatchi et al. 2014).

M. gigas is considered, along with all ostreid species, a successful ecosystem engineer. These species can modify ecosystems by changing or providing new substrates, creating new habitats, altering local hydrodynamics or dynamics of nutrients and sediments (Ruesink et al. 2005, Padilla 2010). The interaction of the Pacific oyster with native species in local ecosystems was assessed so far in several studies with quite opposite results, depending on the habitat, species assemblages, and climate conditions (Ruesink et al. 2005, Padilla 2010, Troost 2010). There is evidence of negative impact of rapidly developing Pacific oyster colonies on blue mussel populations *Mytilus edulis* in the Wadden Sea, Denmark (Reise 1998, Diederich 2006, Reise et al. 2017). However, there are signs of increased biodiversity associated with non-native Pacific oyster reefs in the Oosterchelde estuary, Netherlands (van Broekhoven 2005). Also, relatively short-term studies have shown no discrepancy between associated communities of native *O. edulis* and non-native *M. gigas* populations in different locations (Zwerschke et al. 2016), while studies on sympatric populations of *O. edulis* and *M. gigas* over a longer period of time revealed significant differences between associated epibiota (Guy et al. 2018). In the case of *M. gigas* populations established in the Strait of Georgia, Canada, though, the Pacific oyster has occupied an ecosystem niche that was available at the time of introduction, and offered a very good substrate for native barnacle species (Ruesink et al. 2005). There are also some cases that lead to our tentative hypothesis of *M. gigas* filling the empty niche of *O. edulis* where the habitat requirements of the two species overlap (e.g. Troost 2010).

As was expected, no Black Sea native oysters were found during the zoobenthos survey. This encourages us to believe that the nonindigenous *M. gigas* might occupy the empty spot left in rocky benthic ecosystems at the Romanian Black Sea littoral by the disappearance of native *O. edulis*. Thus, the newly established population of *M. gigas* in the Black Sea could fill a vacant ecosystem niche and act as a positive ecological development, as the Black Sea native oyster banks have disappeared from the Romanian coast due to the invasive veined rapa whelk *Rapana venosa* (Gomoiu 1972).

Conclusions

The presence of the wild *M. gigas* colony in the Romanian Black Sea waters confirms the high adaptability to salinity variations and rhopic factor differences of the species and suggests that it is already acclimated to the special conditions of these waters. The relatively small number of individuals and their small average size suggest that this colony is young, about 5 years old. The source of this colony is most likely the mariculture farm located not very far, thus this population could be considered feral. The young individuals that were found (~5 cm in length) and the long period of time time between acclimating attempts of the Pacific oyster for farming in the Romanian Black Sea coastal waters (~15 years) suggest that the reproduction of *M. gigas* in natural conditions is quite possible, but further studies and monitoring are needed to determine whether *M. gigas* managed to successfully reproduce and form stable colonies in the Black Sea conditions, without human intervention. A genetic approach may indicate the origin of the settled *M. gigas* and ecological studies will assess the future development of this young population in the Romanian Black Sea waters in the context of climate change and ocean acidification.

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