Marine Pollution Bulletin 133 (2018) 320–327

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Use of a monoclonal antibody-based assay for the early detection of an invasive bivalve in plankton samples

Agar Montes[a,*], Silvia Lorenzo-Abalde[b,c], África González-Fernández[b,c], Elsa Vázquez[a], Celia Olabarria[a]

[a] Departamento de Ecoloxía e Bioloxía Animal, Facultade de Ciencias do Mar, and Estación de Ciencias Mariñas de Toralla (ECIMAT), Universidade de Vigo, 36200 Vigo, Spain
[b] Immunology, Biomedical Research Center (CINBIO), Centro Singular de Investigación de Galicia, Instituto de Investigación Sanitaria Galicia Sur (IISGS), Spain
[c] Universidade de Vigo, Campus Universitario s/n, 36310 Vigo, Spain

ARTICLE INFO

Keywords:
Xenostrobus securis
Larvae
Mussel
Biopollutant
Monoclonal antibodies
Immunofluorescence

ABSTRACT

The invasive mussel *Xenostrobus securis* was recorded for the first time in the Galician Rias Baixas (NW Spain) in 2007, within an area characterized by intense commercial culture *of Mytilus galloprovincialis*. The main aims of this study were to evaluate whether an immunological assay can be used to detect larvae of this species in field samples of plankton and to determine whether the distribution of larvae matched that of adults. The ability of two monoclonal antibodies to recognize the bivalve was tested by immunofluorescence. Only the M22.8 antibody recognized *X. securis* larvae. The staining pattern distinguished *X. securis* from *M. galloprovincialis* larvae in both laboratory cultures and field samples of plankton. The distribution of larvae did not match that of adults. This tool may prove very useful for monitoring the presence of this invasive species in the plankton, allowing rapid and specific recognition.

1. Introduction

The rate at which non-native species (NIS) are being introduced to aquatic ecosystems is increasing worldwide, particularly in estuarine areas (Ruiz et al., 1997). This situation is probably explained by stronger anthropogenic and propagule pressure, acting together with invasion processes in these areas (Allen and Williams, 2003). Although the impacts are context-dependent and species-specific, NIS may become invasive and displace native species, modify habitats, alter community structure and affect ecosystem functioning and processes (Katsanevakis et al., 2013) with important ecological and economic consequences (Castilla et al., 2004). Early detection of NIS is therefore crucial to prevent further introductions and impacts on marine ecosystems (Papacostas et al., 2017).

Molluscs are one of the main groups including invasive species, especially bivalves, with high ecological impact (Molnar et al., 2008). As ecosystem engineers, invasive bivalves can strongly affect ecosystem structure and functioning (Sax et al., 2007) through several mechanisms, e.g. suspension feeding, deposit feeding, grazing, biodeposition and bioturbation (Gutiérrez et al., 2003). For example, the filtering activity of certain bivalves greatly modulates the availability of resources and can even alter sediment properties through the biodeposition and stabilization of sediments (Crooks and Khim, 1999). In addition to the ecological impacts, non-native bivalves may also cause important economic losses, often directly affecting fisheries, shellfisheries and aquaculture (Bañón, 2012), as in the case of the zebra mussel *Dreissena polymorpha* Pallas, 1771 (Johnson and Padilla, 1996). Although some marine invasions occur as a result of natural dispersal mechanisms, NIS often occur as a result of human activities (Ruiz et al., 1997). In European waters in particular, more than 50% of the invasive species have been introduced by shipping, via ballast water and biofouling, followed by aquaculture activities (16%) and, to a much lesser extent, aquarium trade (3%) and inland canals (2%) (Katsanevakis et al., 2013). Methods of monitoring the presence of both adult and larval stages of non-native bivalves are therefore required.

Most of the diagnostic criteria for differentiating the larvae of different bivalve species rely on observation of external morphological shell traits, such as size, colour and texture (Lutz and Kennish, 1992). Traditionally, the most reliable morphological identifications have been based on analysis of the structure of the hinge teeth of the larval shell, which can be observed by scanning electron microscopy (Lutz and Hidu, 1979; Lutz and Jablonski, 1978; Lutz, 1985; Hare et al., 2000). However, during the D-veliger larval stage (straight-hinge stage), the morphology of larvae of different species is very similar (Lutz and

* Corresponding author.
E-mail address: amontes@alumnos.uvigo.es (A. Montes).

https://doi.org/10.1016/j.marpolbul.2018.05.004
Received 19 February 2018; Received in revised form 28 April 2018; Accepted 2 May 2018

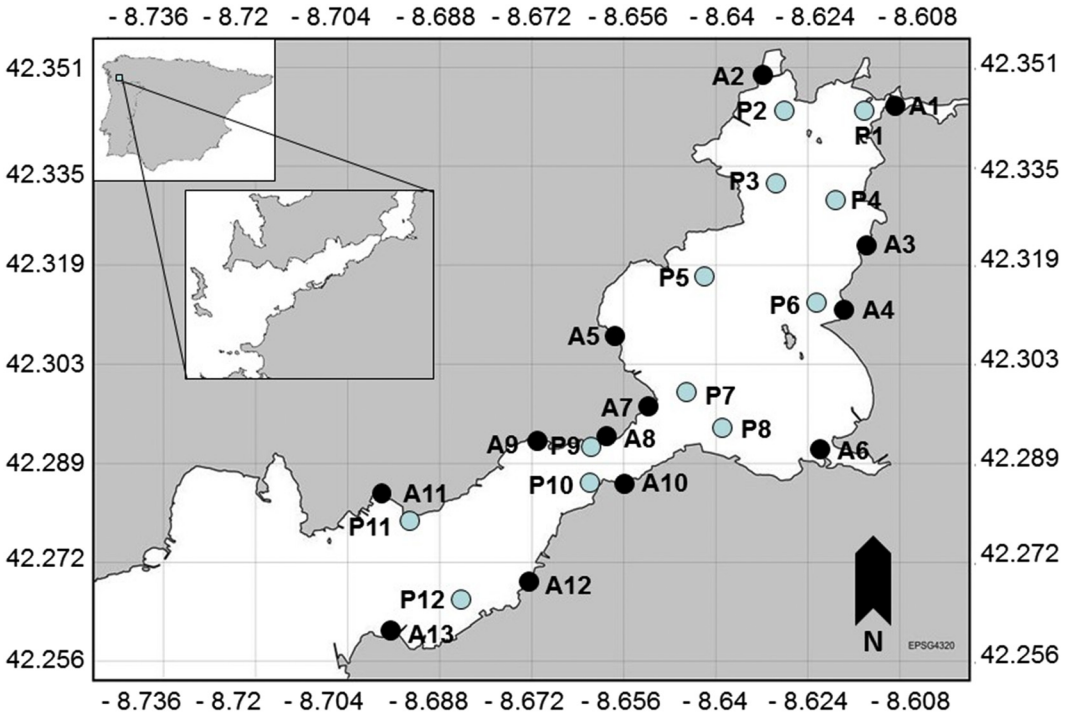

Fig. 1. Map of sampling locations in the Ría de Vigo (Galicia, NW Spain): Samples of adult populations (A) and of plankton (P) are indicated. P-A1: Pontesampaio, P-A2: Vilaboa, A3: San Antón, P3: Punta Cabalo, A-P4: Cesantes, A-P5: San Adrián, A-P6: Redondela, A-P7: Rande, A-P8: Domaio, A-P9: Punta do Mouro, A-P10: Cabanas, A-P11: Borna, A-P12: Chapela. A13: Etea.

Kennish, 1992), which makes identification difficult, especially when samples include a mixture of different bivalves at early stages of development (Mann, 1986; Pérez et al., 2009). In the last few decades, additional methods of identifying larvae have been developed, including molecular techniques based on analysis of variations in DNA sequences (Banks et al., 1993; Bell and Grassle, 1998; Hare et al., 2000) or gene amplification by PCR followed by restriction enzyme digestion and restriction fragment length polymorphism (RFLP) analysis (Toro, 1998). DNA-based techniques have been suggested to be effective for monitoring the presence of invasive species (Milbury et al., 2004; Santaclara et al., 2007; Provan et al., 2008; Kim et al., 2010; Pejovic et al., 2016). In addition, the cytochrome oxidase I gene has been used in DNA barcoding to develop species-specific primers for the rapid detection of bivalve larvae in water samples (Devloo-Delva et al., 2016).

Other methods of identification based on immunological techniques have additional advantages. They are very specific and allow faster and simpler analysis of a large number of samples. Overall, the most important advantage is that each individual larva can be easily recognized within a complex sample without need for prior isolation (Abalde et al., 2003). Two mouse monoclonal antibodies (mAb), M22.8 and M36.5, were generated and used to identify larvae of the commercial mussel *Mytilus galloprovincialis* Lamarck 1819 from the Galician Rias (NW Spain). These antibodies specifically recognized mussels and no cross-reactions occurred with larvae of other abundant species of bivalves coexisting in the area, such as *Cerastoderma edule* (Linnaeus 1758), *Ostrea edulis* (Linnaeus 1758), *Ruditapes decussatus* (Linnaeus 1758), *R. philippinarum* (Adams and Reeve 1850) or *Aequipecten opercularis* (Linnaeus 1758) (Abalde et al., 2003). Later studies indicated that these monoclonal antibodies may also be used in indirect immunofluorescence assays to identify *M. galloprovincialis* larvae in plankton samples obtained in the field (Lorenzo-Abalde et al., 2005). Moreover, both M22.8 and M36.5 recognize larvae of *M. galloprovincialis* from Galicia and also from the Mediterranean Sea and larvae of the closely-related species *M. edulis* from the Mediterranean Sea (Lorenzo-Abalde et al., 2005). The antibody-based immunofluorescence technique for recognizing mussel larvae was further optimized by Pérez et al. (2009).

The black pygmy mussel *Xenostrobus securis* (Lamarck 1819) is native to the brackish waters of Australia and New Zealand. It was reported for the first time as an invasive species in the coastal lagoons of Italy (Sabelli and Speranza, 1994), presumably arriving as fouling on the hulls of ships. Later expansion occurred across Mediterranean lagoons (Zenetos et al., 2004) and the Tyrrhenian Sea (Giutsi et al., 2008). The species was also cited as invasive in Japanese waters (Kimura et al., 1999; Kohama et al., 2001). In the Iberian Peninsula, *X. securis* was first reported in the Ría de Vigo (Garci et al., 2007) and later in the Ría de Pontevedra (Gestoso et al., 2012) and Bay of Biscay (Adarraga and Martínez, 2012). The species co-occurs with *M. galloprovincialis* in estuarine areas of the Galician Rias Baixas, forming mixed patchy aggregations of variable density on intertidal rocky shores and artificial substrates (Gestoso et al., 2012). Although facilitative rather than competitive interactions between the two species seem to occur, at least during the juvenile stage, the invader may be capable of outcompeting *M. galloprovincialis* at the earliest larval stages under optimal environmental conditions (Gestoso et al., 2014). Between 2007 and 2012, *X. securis* spread 6 km from the mouth of the river Verdugo towards the middle part of the Ría de Vigo (Gestoso et al., 2012).

The main objectives of the present study were to determine whether an immunological assay based on use of the monoclonal antibodies M22.8 and M36.5, which recognize *M. galloprovincialis* larvae, could also detect *X. securis* larvae and to verify the validity of this technique for identifying larvae of the invasive species in plankton samples obtained in the field. The assay was also used to test whether the distribution of *X. securis* adults matched that of larvae in the Ría de Vigo.