Continental Shelf Research 267 (2023) 105101

Contents lists available at ScienceDirect

Continental Shelf Research

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

Distribution of the alien bivalve *Xenostrobus securis* (Lamarck, 1819) in the coast of Bizkaia (northern Iberian Peninsula)

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A R T I C L E I N F O

Keywords:
Limnoperna securis
Mytilus galloprovincialis
Invasive species
Estuary
Iberian peninsula
Biscay bay

A B S T R A C T

The present work provides new information about the distribution of the invasive bivalve *Xenostrobus securis* (Lamarck, 1819) in the coast of Bizkaia (Bay of Biscay), including the first report of its presence at the estuaries of Barbadun, Butroe, Oka, Lea and Artibai. The knowledge of its distribution at the estuary of Nerbioi, where the species had been previously reported, is also expanded. Field observations were contrasted by molecular identification (MiniCOI gene; Glu-5') of several individuals collected in every estuary. Present results prove that *X. securis* has the potential to carry out a successful colonization of a Basque estuary, with the ability to reach a very wide distribution. The ecological implications of this invasion and the affections to the ecosystems of the Bay of Biscay deserve further investigations.

1. Introduction

Biological invasions are among the biggest problems ecosystem conservation currently faces (Hobbs and Huenneke 1992), as alien species have the potential to severely alter ecosystem characteristics and functioning if they become dominant in the introduced area or displace key organisms from their habitat (Ruiz et al., 1997). Coastal and brackish water environments linked to port activities are particularly prone to suffer these kinds of hazards, as ship traffic is one of the most important ways of invasive species spreading (Keller et al., 2011; Ruiz et al., 1997). One such species is *Xenostrobus securis* (Lamarck, 1819), also known as black-pygmy mussel, a bivalve of the family Mytilidae original from Australia and New Zealand (Wilson 1968) which has been spreading far from its original range during the last 3 decades (Adarraga and Martínez, 2012). In Asia, the presence of *X. securis* has been reported in China, Japan and Korea (Iwasaki 2006; Iwasaki et al., 2004; Kimura et al., 1999; Shirafuji and Sato 2003), whereas in Europe, the first records are from 1992, at the Italian Adriatic coast (Lazzari and Rinaldi 1994; Sabelli 1993). Today, *X. securis* can be found widespread at Italian, French and Spanish Mediterranean coasts (Barbieri et al., 2011; Giusti et al., 2008; Gofas and Zenetos 2003), and in the shores of the Spanish Atlantic Ocean and Cantabrian sea (Adarraga and Martínez, 2012; Bañón et al., 2008; Devloo-Delva et al., 2016; Garci et al., 2007; Gestoso et al., 2012; Pejovic et al., 2016). In the Basque Country, its presence was reported in 2012 only at the estuary of Nerbioi, Bizkaia (Adarraga and Martínez, 2012), but no further data of its distribution or consequences of the invasion have been published. *Xenostrobus securis* has been recently introduced in the Exotic Invasive Fauna list performed by the Basque Government (Eusko Jaurlaritza - Gobierno Vasco, 2022), thus, it is important to fill the knowledge gap about the distribution of this species, in order to perform a better management of environmental resources.

Invasions involving bivalve molluscs deserve special attention because they are key elements of the marine and estuarine food webs (Newell 2004) and they act as ecosystem architects (Gutiérrez et al., 2003), mainly due to shell production and the formation of dense aggregates. These enhance habitat complexity and alter resource availability, providing the local flora and fauna different services, such as

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https://doi.org/10.1016/j.csr.2023.105101
Received 13 February 2023; Received in revised form 26 July 2023; Accepted 14 August 2023
Available online 16 August 2023

refuge and attachment points, among others, as reviewed by Gutiérrez et al. (2003). For these reasons, the colonization of a new habitat by an invasive bivalve species or the displacement of a local one could cause great alterations in the structure and functioning of the recipient ecosystem (Sousa et al., 2009).

A key aspect usually shared by successful invaders is their adaptability to fluctuating environmental conditions (Astudillo et al., 2017). In the case of *X. securis*, this ability is manifested in many forms. Its wide eurihalinity is one of the main factors explaining its distribution and invasive success, as adults can survive in salinities between 1 and 31 PSU (Wilson 1968) and the larvae between 8 and 17.5 PSU (Wilson 1969). Similarly, it is highly tolerant to temperature changes in a range from 14 °C to 30 °C (Astudillo et al., 2017), and it is also able to withstand short periods of extreme temperatures of up to 42 °C (Olabarria et al., 2016). Additionally, it has the ability to attach to a wide range of natural substrata of different granulometric characteristics (Pascual et al., 2010), soft or hard, and it also colonizes artificial substrata (Garci et al., 2007). This is due to the properties of its byssus threads, which are remarkably numerous, thin and long, making them suitable for different kinds of attachment points (Babarro and Lassudrie 2011). Small animals with short lifespans and high reproductive capacity are also usually found on the lists of successful invaders, traits *X. securis* possesses (Babarro and Lassudrie, 2011), as well as a very long spawning period that can last up to 10 months (Montes et al., 2020).

Apart from the aforementioned characteristics intrinsic to the invader species, traits of local species and the interaction between them are equally important in determining the potential of an invader (Gestoso et al., 2014). The mussel *Mytilus galloprovincialis* Lamarck, 1819, is an ecologically and economically relevant autochthonous bivalve species in the Bay of Biscay that could compete with *X. securis* for food and space (Garci et al., 2007; Veiga et al., 2011). Both mussels are filter feeders, they have a sessile lifestyle attached to hard substrata (even though *X. securis* can also live in muddy sediments) and they tend to live forming dense aggregations (Garci et al., 2007; Gestoso et al., 2012; Pascual et al., 2010). The interaction of these two species is very complex. The local mussel shows some competitive advantages over the alien one like stronger shell and byssus production (Babarro and Lassudrie, 2011) and a wider gaping capacity (Comeau and Babarro 2014). However, *X. securis* is able to outperform it in certain places, especially in the middle areas of the estuaries (Garci et al., 2007; Gestoso et al., 2014). The adaptation capacity of *X. securis* could also be related to a lower predatory pressure (Veiga et al., 2011) and parasite load (Pascual et al., 2010) it may suffer. The alteration in the population density of an ecosystem architect like *M. galloprovincialis* could have huge implications. It has already been reported that the macrofaunal communities inhabiting *X. securis* beds are different from those found at *M. galloprovincialis* assemblages, as significantly less number of individuals tend to live associated to the invasive species (Gestoso et al., 2012).

In this context, the presence of *X. securis* in the Basque coast is expected to be wider than previously recorded. Therefore, the objectives of the present study are to record the presence or absence of *X. securis* in the estuaries of Bizkaia, and to quantify the spread level of the species inside the estuaries.

2. Materials and methods

2.1. Area of research

The estuaries of the province of Bizkaia, western Basque Country, Spain (UTM zone 30T) were analysed; from East to West are Barbadun (UTM: 30T VN 99), Nerbioi (UTM 30T VP 99 + 30T VN 99 + 30T WN 09 + 30T WN 08), Butroe (UTM 30T WP 00), Oka (UTM 30T WP 20 + 30T WN 29), Lea (UTM 30T WP 40) and Artibai (UTM 30T WN 49) (Fig. 1).

Out of all the estuaries considered for the research, only one (Nerbioi) crosses a highly populated area (the city of Bilbao and its surroundings), and it suffers the most intense anthropic pressure. Other relevant activities in the study area are the Port of Bilbao, which is the biggest commercial port of the northern Iberian Peninsula (Nerbioi estuary, -N-) and the Port of Ondarroa (the most important fishing port of the Cantabrian Sea, Artibai estuary, -A-) (Borja et al., 2004). Overall, the ecological status of these estuaries is good, except in Nerbioi (moderate) and Oka (moderate - bad) (Borja et al., 2021). This last estuary (Oka, -O-) is the backbone of the Urdaibai Biosphere Reserve, a place of ecological interest designated by UNESCO in 1984. A summary of the most relevant characteristics of each estuary is presented at Table 1.

2.2. Sampling 1: estuaries of Nerbioi and Butroe

First, a visual survey was performed to identify the areas colonized by *X. securis* at the estuaries of Butroe and Nerbioi (Fig. 1), the two estuaries where the invader had been previously noticed (Adarraga and Martínez, 2012; personal communication). Both estuaries were thoroughly sampled in May 2019, from the mouth of the river and upstream to the end of the tidal influence. 10 sampling points were chosen in Butroe (from closest to the sea; mouth -B1- to furthest; end of the tidal influence -B10-) and 16 in Nerbioi (from closest to the sea -N1- to furthest -N16-). Coordinates and characteristics of the sampling points can be found in Table S1. At this stage, visual discrimination of *X. securis* and *M. galloprovincialis* was performed, and whole soft tissues of four *X. securis* of each estuary were dissected for further genetic identification procedures.

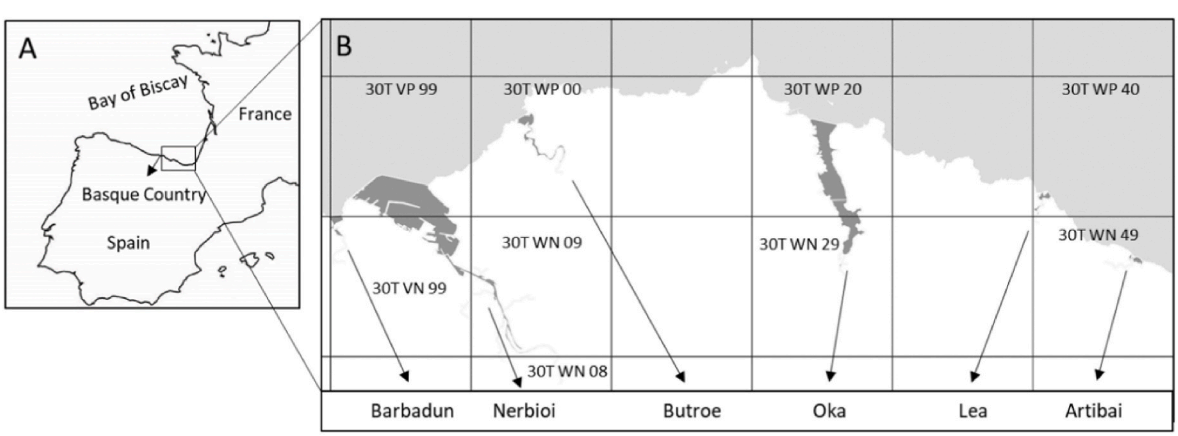

Fig. 1. The map of the studied area. (A) The location of the Basque Country in the Iberian Peninsula. (B) Amplification of the western coast of the Basque Country. Highlighted in grey the six estuaries monitored in this work, from the west to the east, Barbadun, Nerbioi, Butroe, Oka, Lea and Artibai. Squares indicate 10 × 10 km UTM squares. The squares where the study has been carried out are labelled with the correspondent UTM.

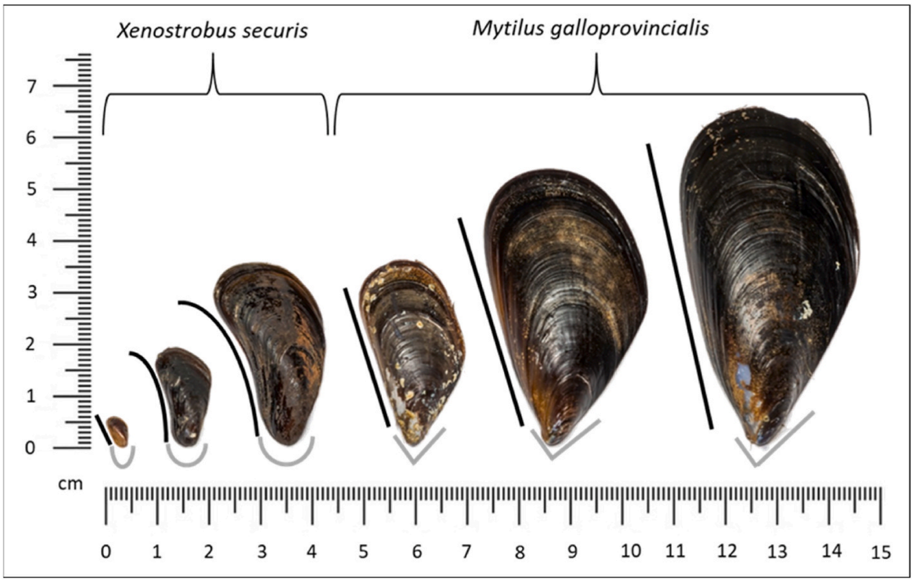

Fig. 2. Shape comparison between *Xenostrobus securis* and *Mytilus galloprovincialis*. The black lines emphasize the outline of the ventral side of the mussels (curved in the biggest *X. securis* and straight in *M. galloprovincialis* and the smallest *X. securis*). The grey lines emphasize the shape of the umbo (round in *X. securis* and sharp in *M. galloprovincialis*).

Table 1
Characteristics of the estuaries of Bizkaia. Data for length, population, industrial activity and port presence are taken from Borja et al. (2004), and the ecological status from Borja et al. (2021).

Estuary	UTM (10x10)	Length (km)	Population	Industrial activity	Ecological status	Commercial	Ports Fishing	Leisure
Barbadun	30T VN 99	4,4	<25.000	High	Good	No	No	No
Nerbioi	30T VP 99	22	>800.000	High	Moderate	Yes	Yes	Yes
	30T VN 99							
	30T WN 09							
	30T WN 08							
Butroe	30T WP 00	8	<25.000	Low	Good	No	No	Yes
Oka	30T WP 20	12,5	<25.000	Low	Moderate-bad	No	No	Yes
	30T WN 29							
Lea	30T WP 40	2	<25.000	Low	Good	No	Yes	Yes
Artibai	30T WN 49	3,5	<25.000	High	Good	No	Yes	Yes

In July 2023, three sampling points were chosen at each estuary in order to estimate the population densities of both species in a wide range of abiotic conditions. These sampling points were representative of the outer (closest to the sea; B2 and N1), middle (B6 and N3) and inner (furthest from the sea; B9 and N8) zones. In each of the points, three transects were drawn perpendicular to the shoreline. In every transect, three 50 × 50 cm squares were placed, and all mussels found in them were collected. Those mussels were visually identified to the species level, counted and weighed (wet weight).

2.3. Sampling 2: estuaries of Barbadun, Oka, Lea and Artibai

From September to October 2020, three points were sampled in Barbadun, Oka, Lea and Artibai estuaries, representative of the outer, middle and inner zones. These sampling points will be referred from now on as follows, from closest to the sea to furthest: Barbadun (M1 - M3), Oka (O1 - O3), Lea (L1 - L3) and Artibai (A1 - A3). Coordinates and characteristics of the sampling points can be found in Table S1. Here, *X. securis* and *M. galloprovincialis* presence was visually checked, and samples were collected for genetic identification. Considering the amount of mussels observed (see Results section), no quantitative analysis of population density or biomass were carried out.

2.4. Molecular identification of Xenostrobus securis

In order to molecularly confirm the presence of the species and to validate the visual identification performed, the individuals collected in each estuary were processed as follows. Briefly, up to 100 μg of each tissue was chemically homogenized and DNA extracted using DNeasy Blood and Tissue Kits (Qiagen, Hilden, Germany). DNA concentration was measured by Nanodrop (Agilent, Santa Clara, USA).

Xenostrobus securis-specific MiniCOI primers were used as described by Devloo-Delva et al. (2016). Conventional PCR was carried out by adding 0,5-1 μg/μl of DNA to a 25 μL PCR reaction mixture using Taq polymerase (NZY Tech, Lisbon, Portugal) and 1 μl Bovine Serum Albumin (BSA) in a BioRad thermocycler, with an annealing temperature of 53 °C with 35 cycles. The same protocol was used to test the Glu-5' gene, using specific primers designed by Inoue et al. (1995) to discern between *M. galloprovincialis, Mytilus edulis* Linnaeus, 1758, and *Mytilus trossulus* Gould, 1850, both as a control and to test if the samples that gave negative results with the MiniCOI gene belong to the mentioned species. In both tests (MiniCOI and Glu-5') two individuals of *M. galloprovincialis* from the estuary of Nerbioi and *M. edulis* from the North Sea (provided by the Biscay Bay Environmental Biospecimen Bank; BBEBB) were included as controls.

Amplicons were visualized in 2% agarose gel electrophoresis stained by EthBromide. Positive amplicons were purified and sequenced in the Sequencing and Genotyping service of the Genomic and Proteomic Unit of the University of the Basque Country (SGIker-UPV/EHU). Results were homologically blasted (blastN) against GenBank database (NCBI, 2022).

2.5. Data treatment

The maps were made with the software QGIS (QGIS Development Team, 2019), using the maps available at GeoEuskadi (Eusko Jaurlaritza/Gobierno Vasco.geoEuskadi). Distribution maps are shown with two UTM grids: 10 × 10 km (big squares) and 1 × 1 km (small squares). Only 10 × 10 km UTM coordinates where *X. securis* was found are labelled.

Average mussel abundance and biomass of every transect were calculated and extrapolated to m^2. After checking the normality of the data using the Shapiro-Wilk test, abundance and biomass of both species were compared in every sampling point using the Student T test. All statistical procedures were carried out using the IBM SPSS Statistics 28.0 software (IBM Corp. 2021).

3. Results

Specimens of *X. securis* were found in all the estuaries sampled for this research, which were identified *de visu* and confirmed molecularly. At the same time, *M. galloprovincialis* was also found at every estuary. No other mytilid species was identified.

3.1. Identification of *X. securis*

Xenostrobus securis individuals were visually identified following the curvature present on the ventral area of the shell, as well as the rounded shape of the umbo, as none of these characteristics is found in *M. galloprovincialis* (Fig. 2). Overall, the umbo shape was found to be a more reliable characteristic for the discernment between both species, especially when considering very small individuals (up to approximately 10 mm in length).

All the specimens visually identified as *X. securis* that where molecularly tested with the MiniCOI gene showed an amplicon at the expected length of 310 bp (Fig. 3), proving to be positive for *X. securis*. SANGER sequencing results of the amplicons were similar to known *X. securis* sequences available at the GenBank NCBI database. The followings are the Genbank IDs of the sequences with the highest homology (as of April 2022): HQ396442.1 (E value = $3e^{-151}$), HQ396456.1 (E value = $3e^{-156}$) and HQ396462.1 (E value = $4e^{-158}$). The obtained MiniCOI *X. securis* sequences were uploaded to Genbank NCBI database with accession numbers ranging from ON310811 to ON310819. None of the samples visually identified as *X. securis* amplified the Glu-5' gene. However, some samples from the estuary of Nerbioi initially identified as *M. galloprovincialis* and used here as controls proved to be hybrids between *M. galloprovincialis* and *M. edulis* (Bierne et al., 2002; Brooks et al., 2015), as shown by the two bands that appeared in some *M. galloprovincialis* samples when amplifying the Glu-5' gene (Inoue et al., 1995) (Fig. 3).

Fig. 3. Agarose gel electrophoresis image showing the amplification of *Xenostrobus securis* DNA for MiniCOI gene (-upper gel image-using *X. securis* specific primers described at Devloo-Delva et al. (2016)), and Glu-5' gene (-lower gel image-using *Mytilus* specific primers described at Inoue et al. (1995)). Letters M, N, B, O, L an A mean the places where *X. securis* was *de visu* identified and collected. M: Barbadun; N: Nerbioi; B: Butroe; O: Oka; L: Lea; A: Artibai; Mg: *Mytilus galloprovincialis* (control); Mg*: Mytilid hybrid (control; initially identified as *M. galloprovincialis*); Me: *Mytilus edulis* (control); NTC: Non template control. The DNA ladder used added in each edge of the gel is a 50 bp ladder, in particular NZYDNA ladder VI (NZY Tech, Lisbon, Portugal).

3.2. Distribution of *X. securis* in the coast of Bizkaia

In the samplings of Butroe and Nerbioi, *X. securis* was found widely distributed at both estuaries. In the estuary of Butroe, *X. securis* was present at all sampling points (Fig. 4 A). In Nerbioi, *X. securis* was found at all sampling points except in the two furthest points from the sea (N15 - N16) (Fig. 4 B).

In the outer part of Butroe estuary, the abundance of *M.galloprovincialis* was higher than *X. securis*, however, this difference was not significant (Table 2). On the contrary, *M. galloprovincialis* had a significantly higher biomass than *X. securis* in this point. The opposite was found in the estuary of Nerbioi, as *M. galloprovincialis* was significantly more abundant, but no significant differences were found in biomass, despite the autochthonous species having a higher mean value. In the middle areas, *X. securis* was significantly more abundant at both estuaries, although in terms of biomass, differences were only found at Butroe. At the upper zones, only *X. securis* was found.

In the rest of the estuaries analysed, the presence of the invader in every one of them was confirmed. In Barbadun, Lea and Artibai *X. securis* was found at the sampling points representative of the outer and middle zones (M1, M2, L1, L2, A1, A2) of the estuaries (Fig. 5 A, C, D). At these points, the alien species coexisted with *M. galloprovincialis*. Neither of the two mussel species was present in the inner points of Barbadun and Artibai (M3, A3), whereas in Lea, *M. galloprovincialis* was abundant (L3). In the Oka estuary, *X. securis* was only present at the outer point (O1) alongside *M. galloprovincialis*, and in the middle (O2) and inner (O3) areas, only the latter was found (Fig. 5 B). In this estuaries *X. securis* individuals found were always few and small, so it was decided not to consider these estuaries for the quantitative samplings.

4. Discussion

After the successful molecular confirmation of the specimens suspected to be *X. securis* using the MiniCOI gene (Devloo-Delva et al., 2016), and *M. galloprovincialis* individuals by Glu-5' gene (Inoue et al., 1995), it can be confirmed that the visual identification of the individuals is accurate when big mussels are selected (>10 mm in length). However, the smallest mussels present less marked morphological differences and thus errors in the identification could happen, making the molecular identification advisable in some cases.

This highlights the need of a scrupulous visual identification of mussels for the routine pollution monitoring programmes carried out in the Basque coast. The distribution of *X. securis* can help to determine the critical estuaries where the misidentification between mussel species could occur. An expert in the field will rarely misidentify large specimens of *X. securis* as *M. galloprovincialis.* Moreover, in the biomonitorings of environmental pollution programmes of the Basque coast, the standardized mussel size is 3.5–4.5 cm (Garmendia et al., 2011), thus it can be considered that the identification has been accurate. Nevertheless,

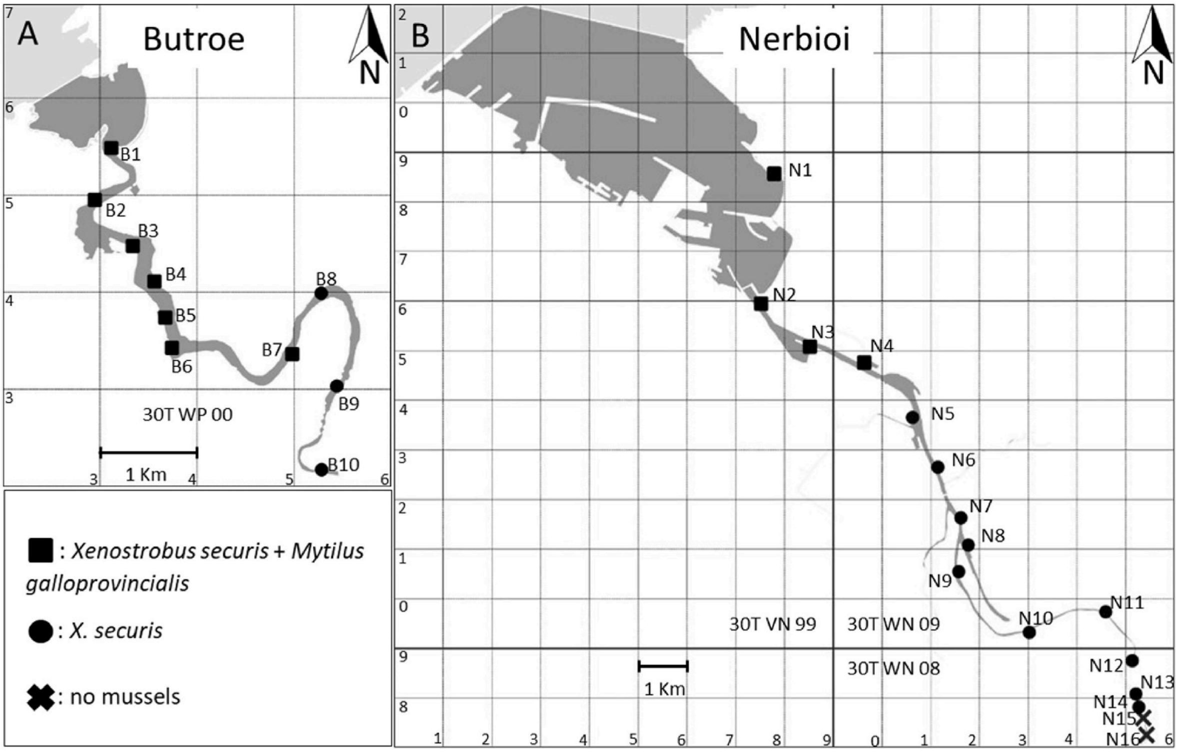

Fig. 4. Distribution maps of *Xenostrobus securis* and *Mytilus galloprovincialis* in the (A) Butroe (B1 - B10) and (B) Nerbioi (N1 - N16) estuaries (Bizkaia, Basque Country). Big squares indicates 10 × 10 km areas, whereas small squares 1 × 1 km ones. UTM coordinates are indicated for each 10 × 10 km area.

Table 2
Abundances (individuals/m^2) and biomass (g/m^2) of *Xenostrobus securis* and *Mytilus galloprovincialis* at the Butroe and Nerbioi estuaries represented as means ± standard deviation. Asterisks (*) represent statistically significant differences ($p < 0.05$) among species at a given sampling point.

Estuaries		Abundance (individuals/m2)		Biomass (g/m2)	
		X. securis	M. galloprovincialis	X. securis	M. galloprovincialis
Butroe	**Outer**	8 ± 4	16.4 ± 8.7	3 ± 1.6	67.9 ± 51.6*
	Middle	37.8 ± 10.9*	1.8 ± 0.8	36.7 ± 15.8*	8 ± 8.6
	Inner	74.7 ± 51.3*	0	95.2 ± 81*	0
Nerbioi	**Outer**	1.3 ± 1.4	40 ± 18.9*	0.2 ± 0.2	471.8 ± 552.2
	Middle	135.6 ± 65.2*	13.3 ± 4.8	204.4 ± 102.2	100.8 ± 39.9
	Inner	200.9 ± 146.2*	0	132.5 ± 105.8*	0

the possibility of misidentification has to be seriously considered if the mussels collected are smaller than 10 mm, which could be interesting for different research areas such as ecotoxicological, physiological or population studies. For instance, for the present study molecular identification was necessary, as the invasive species does not reach big sizes in every part of an estuary.

The present research reports the presence of the alien bivalve *X. securis* for the first time at the estuaries of Barbadun, Butroe, Oka, Lea and Artibai, and new information of its distribution in the estuary of Nerbioi has been provided. These results demonstrate that the *X. securis* distribution in the Basque coast is wider than previously reported, where the distribution was described at a few points in the estuary of Nerbioi (Adarraga and Martínez, 2012; Gil-Uriarte et al., 2016). Definitively, *X. securis* inhabits all the estuaries of Bizkaia, and it is probably present in more eastern estuaries as well, which could be confirmed in future studies following the methodology presented herein.

Even though all six estuaries considered in this research were positive for the existence of *X. securis* populations, the invasion was not equal in all of them. Nerbioi and Butroe are the estuaries where the invasion has reached its highest prevalence, as the alien was present in almost all the sampling points (only excluding the innermost areas of Nerbioi), and the populations found there reached relatively high densities and biomass, in some cases. However, the picture in other estuaries was different; in Barbadun, Oka, Lea and Artibai very few individuals of *X. securis* were found, and their sizes and abundances were almost negligible in every case. In order to consider a species as invasive, it has to successfully overcome a series of stages, which are transportation, release, establishment and spread (Kolar and Lodge 2001). Considering the observations made in the present research, it is clear that *X. securis* has reached the final invasion stage at Nerbioi and Butroe estuaries. For the other estuaries, the amount of mussels observed does not allow to confirm this situation, but the presence of *X. securis* is molecularly proven. Therefore, the presence of *X. securis* in Barbadun, Oka, Lea and Artibai estuaries could either be the result of an unsuccessful introduction, or an invasion at an early state that could potentially become as large as in Butroe and Nerbioi. Further research is needed to confirm the invasion state of *X. securis* in the mentioned estuaries.

Due to the high abundance of both *X. securis* and *M. galloprovincialis* found in Nerbioi and Butroe, a clear differential distribution could be drawn along the estuaries. In the areas closest to the sea of both Butroe and Nerbioi, more individuals of *M. galloprovincialis* than of *X. securis* were found, even though this difference was only significant at Nerbioi. The invasive species also had lower biomass values at these points. In fact, the presence of *X. securis* was very hard to notice at first sight, and only some small individuals were collected, most of the times attached to the byssus threads of the much bigger *M. galloprovincialis*. All of this suggests a dominance of the indigenous species close to the sea, which

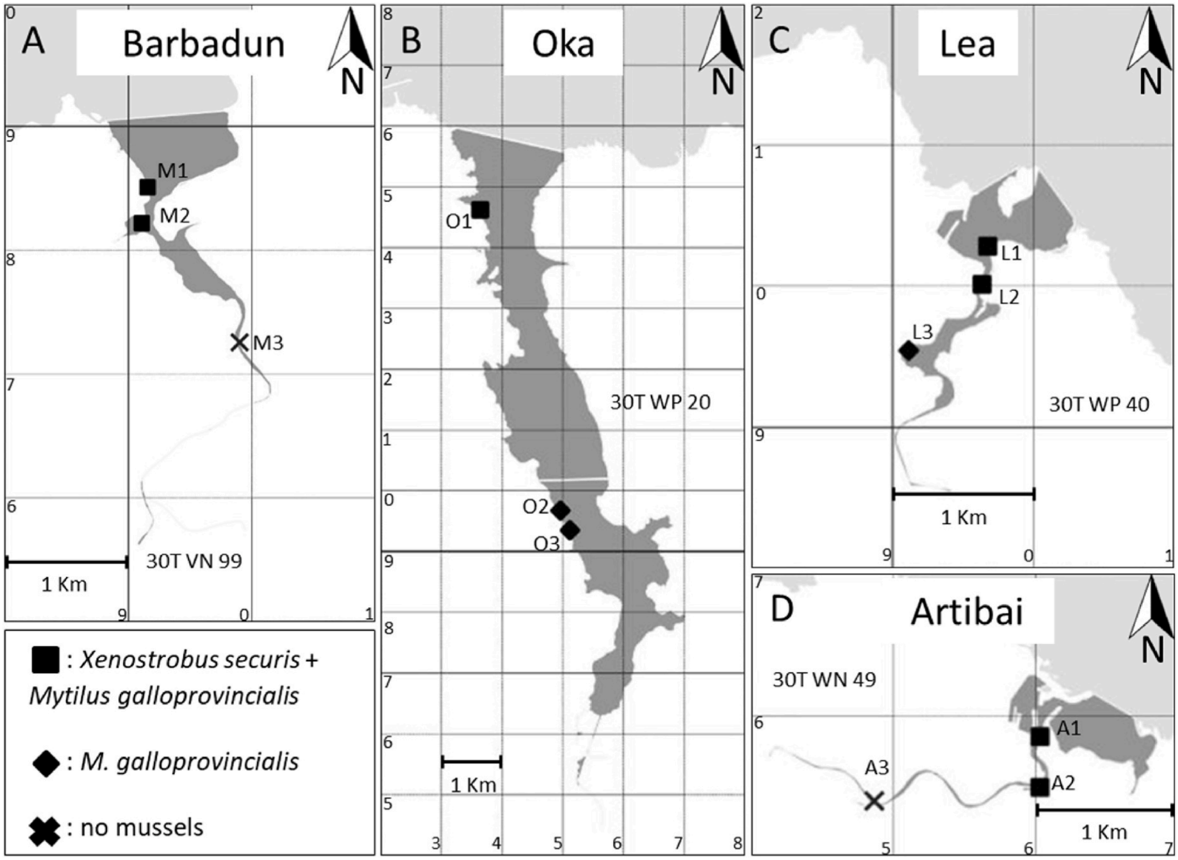

Fig. 5. Distribution maps of *Xenostrobus securis* and *Mytilus galloprovincialis* in the (A) Barbadun (M1 - M3), (B) Oka (O1 - O3), (C) Lea (L1 - L3) and (D) Artibai (A1 - A3) estuaries (Bizkaia, Basque Country). Big squares indicate 10 × 10 km areas, whereas small squares 1 × 1 km ones. UTM coordinates are indicated for each 10 × 10 km area.

could be expected due to the optimal salinity conditions for the invader, which are found at brackish waters (Wilson 1968). It is worth mentioning that the mussel populations at the lowest part of Butroe were remarkably small, as evidenced by the low density and biomass recorded. This is in agreement with observations carried out by our research group in the last years, as a marked decline in mussel populations has been noticed in several estuaries of the Basque Coast. This could be related to some recurring bivalve mass mortality events documented in other places such as the French coast (Lupo et al., 2021) or to the overall decline in European mussel production, linked to lower spat recruitment, among other causes (Avdelas et al., 2021). In the middle zones of the estuaries, mussel communities continued to be mixed, but *X. securis* was more abundant and had higher biomass at both of them. Despite being cohabiting species with similar ecological niches, there is no direct competition between *X. securis* and *M. galloprovincialis* (Gestoso et al., 2014). The factors driving the population patterns of both species in these coexistence areas seem to be a complex interaction of different forces. The invader has shown to be able to colonize a wider range of substrata type than the indigenous *M. galloprovincialis,* due to the vast amount of thin byssus threads that it secretes, and the ability to alter key byssus characteristics in response to fluctuating abiotic conditions such as water flow speed (Babarro and Lassudrie, 2011). Indeed, the water flow speed is highly variable in the estuaries of the Basque Country, generally short and subjected to high pluviosity (Borja et al., 2004). This, coupled with its high eurihalinity (Wilson, 1968) and tolerance to thermal stress (Olabarria et al., 2016), gives *X. securis* the capacity to colonize a wide variety of habitats inside an estuary, and makes it highly competitive in areas of great abiotic instability. On top of that, it has been reported that the predator crab *Carcinus maenas* (Linnaeus, 1758), a common species in Butroe and Nerbioi, feeds preferentially on the indigenous mussel in laboratory conditions (Veiga et al., 2011) possibly facilitating the spreading of *X. securis*. A similar phenomenon has been observed in the field, where *X. securis* seems to suffer a lesser predatory pressure than *M. galloprovincialis* in mixed aggregations, pointing towards a facilitative rather than competitive interaction between the two (Gestoso et al., 2014). Another key factor of the success of the alien mussel could be its continuous 10 month-long spawning season recorded at Galician estuaries (Montes et al., 2021), in contrast to *M. galloprovincialis*, which spawns from April to July in the Basque coast, with the possibility of having a second spawning peak in October (Ortiz-Zarragoitia et al., 2010). Mussel spawning is very dependent on abiotic factors such as temperature, salinity and food availability (Cáceres-Martínez and Figueras, 1997), and vary among species and localities. In this frame, the reproductive ability of *X. securis* in Basque waters is still unknown, and further seasonal researches are needed to determine the gamete development stages and reproduction success. In the inner parts of the estuaries, *X. securis* was the only mytilid species to be found, and it completely disappeared at the innermost area of Nerbioi. All these observations are, in general, concordant with previous descriptions carried out in Galician estuaries, where an increasing abundance of the invader was found at the inner areas of the estuaries, while the abundance of the indigenous species decreased, a pattern mostly explained by the salinity of the environment (Garci et al., 2007; Gestoso et al., 2012).

In conclusion, the molecular analysis could be relevant for studies involving small individuals of *X. securis* (<10 mm length) to avoid the misidentification with other mussel species present in the Basque Coast. *Xenostrobus securis* is present at every estuary of Bizkaia, but the state of the invasion is not equal in all of them. The species is highly prevalent at Nerbioi and Butroe but not at Barbadun, Oka, Lea and Artibai. It has to be confirmed in further research if these estuaries are at an early stage of invasion, which is especially interesting in Oka, as it is part of the UNESCO Biosphere Reserve of Urdaibai. Once established, *X. securis* could potentially inhabit any place within an estuary in the Basque coast. *Mytilus galloprovincialis* seems to dominate the areas closer to the sea, but *X. securis* is more successful when going inwards the estuary. In the innermost areas, only the invasive mussel is able to live. This is the first attempt to document and understand the invasion of *X. securis* in Basque waters, and more research has to be done in order to complete the knowledge about this species in terms of the state of the invasion and interaction with the local fauna and flora. The wider understanding of the species and the effects of its presence will lead to a better decision-making and management, especially important in the light of its recent inclusion in the Exotic Invasive Fauna list of the Basque Country. Moreover, considering the wide range of thermic and salinity tolerance and reproductive capacity, it is provable that it will keep spreading north, and more estuaries of the eastern Basque and French Atlantic coasts must also be studied.

Funding

The present research was funded by the Basque Government (IT1743-22). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank for technical and human support provided by the Sequencing and Genotyping service of the SGIker (UPV/EHU/ERDF, EU), and to the Biscay Bay Environmental Biospecimen Bank (BBEBB).

Special thanks to Denis Benito for the photographs of mussel shells featured in Fig. 2.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.csr.2023.105101.

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