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3 1 **Evaluating the genetic effects of the invasive *Ocenebra inornata* on the native oyster drill**

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5 2 *Ocenebra erinacea*

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33 13 Keyword: *Ocenebra erinaceus*; *Ocenebrellus inornatus*; haplotype diversity; evolutionary
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35 14 response; evolutionary ecology; invasive species; glacial refugium; cryptic species

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16 **Abstract**

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18 Studies focusing on the effect of invasive species on the genetic diversity of native marine
19 invertebrates remain scant. Here we report diversity among French populations of the
20 intertidal gastropod *Ocenebra erinacea* (Linnaeus, 1758) sampled in the presence and absence
21 of the invasive *Ocenebra inornata* (Recluz, 1851). Between 1999 and 2004 a total of 352
22 individuals of *O. erinacea* were collected from 15 sites, five of which in the presence of the
23 invasive, and genotyped at the mitochondrial locus *coxI*. No statistical difference was
24 observed between polymorphism levels recorded within native populations exposed to the
25 invasive, compared to populations sampled in the absence of *O. inornata*. No sign of native
26 population decline was detected in response to the invader. While significant shifts in native
27 *O. erinacea* population sizes were previously reported in the literature, genetic effects may
28 take longer to accumulate, or may be undetectable without a larger panel of genetic markers.
29 In contrast, large genetic distances and significant population differentiation were recorded
30 between Atlantic and Mediterranean *O. erinacea* samples, suggesting that these populations
31 have distinct evolutionary histories. Comparison of genetic divergence within the closely-
32 related genus *Nucella* suggests that the Atlantic populations and the Mediterranean *O.*
33 *erinacea* populations from Thau Lagoon may belong to different species or subspecies.

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3 35 **Introduction**
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10 Natural movements of species ranges on the surface of the world (expansions,
11 regressions, displacements) play a considerable role in the evolution of species. Most of the
12 time, these phenomena are progressive and marked by the tempo of geological processes (e.g.
13 Hewitt 1996). However, during the last few decades a growing number of species has
14 undergone changes in their natural range due to both changes at a global scale (e.g. Parmesan
15 & Yohe 2003) and artificial transfers due to human activities (e.g. Carlton 1989; Seebens *et al.*
16 2013).
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24 The number of biological invasions has strongly increased during the last decades (e.g.
25 Mack *et al.* 2000; Mooney & Cleland, 2001; Ruiz *et al.* 2000). In the marine environment,
26 these phenomena are mainly due to aquaculture and especially to shellfish farming, which
27 represents a major cause of introduction, intentional or not, of exogenous species (Elton 1958;
28 Carlton 1992).
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36 Apart from potentially important economic consequences, the arrival of these
37 introduced species can also cause serious ecological impacts on local fauna. Introduced
38 species are likely to decrease the abundance of indigenous species, excluding them from part
39 of their distributional area or even causing their extinction by modifying invaded habitats,
40 hybridizing with native species, exchanging pathogens, preying on them, or competing with
41 them (e.g. Lockwood *et al.* 2007 and references therein). Moreover, when they exert strong
42 selective pressures, introduced species can also reduce the genetic diversity of native
43 populations (Kim *et al.* 2003; Wittmann *et al.* 2013).
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54 Such impacts, although poorly known (e.g. Strauss *et al.* 2006), may have heavy
55 ecological consequences since adaptive potential depends on the genetic diversity of a
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3 59 population. Reductions in genetic diversity are generally considered detrimental (e.g.
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5 60 Frankham 1995; Lande 1995; Strauss *et al.* 2006) and might contribute to extinction
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7 61 (Wittmann *et al.* 2013). Thus, various authors showed a link between the fitness of a species
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9 62 and its genetic diversity, particularly in mollusks (Mitton & Grant 1984; Garton & Haag 1991;
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11 63 Zouros 1993; Launey & Hedgecock 2001; Hedgecock *et al.* 2007). In addition, the reduced
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13 64 genetic variability of an indigenous population could promote the expansion process of other
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15 65 species that are phylogenetically close. However, very few studies address changes in genetic
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17 66 diversity of an indigenous species under the competitive pressure generated by a biological
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19 67 invader.
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23 68 The oyster drills *Ocenebra erinacea* (Linnaeus, 1758) and *Ocenebra inornata* (Recluz,
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25 69 1851) constitute a noteworthy model to study the genetic effects of indigenous-invasive
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27 70 interactions on indigenous populations. A native of the northwestern Pacific (Choe & Park
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29 71 1997; Amano & Vermeij 1998), *O. inornata* (previously known as *Ocenebrellus inornatus*;
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31 72 see Houart and Sirenko 2003; Bouchet and Houart 2014) has recently invaded European
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33 73 coasts, probably following massive oyster imports (Pigeot 2000; De Montaudouin & Sauriau
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35 74 2000; review of Lützen *et al.* 2012). Genetic data suggest that French populations may come
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37 75 from Asia and the United States (Martel *et al.* 2004a). The introduction of *O. inornata* may
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39 76 have important economical consequences, as it is a predator of cultivated mollusks (e.g.
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41 77 oysters, blue mussels, Gouletquer *et al.* 2002). It coexists at several French sites with an
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43 78 indigenous muricid, *O. erinacea* (Linnaeus, 1758), which ranges from the straits of Gibraltar
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45 79 to the Netherlands, and inhabits all British and Mediterranean coasts (Graham 1988).
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47 80 Although *O. erinacea* and *O. inornata* differ in some life history traits (Martel *et al.* 2004c),
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49 81 these muricid gastropods fill similar ecological niches, and may compete for habitat (both
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51 82 species live on hard substrates and drill the shell of bivalves to feed on them; e.g. Lützen *et al.*
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53 83 2012). Pigeot *et al.* (2000) recorded a decrease in population density of *O. erinacea*, in
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3 84 parallel to an increase in numbers of *O. inornata* in Marennes-Oléron (Charente-Maritime,
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5 85 France), between 1997 and 1999 (two years after the invasive was first detected). While the
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7 86 introduction and expansion patterns of *O. inornata* have been investigated in previous studies
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9 87 (Martel *et al.* 2004a; Martel *et al.* 2004b), its ecological impacts on the native *O. erinacea* are
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11 88 poorly known.

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14 89 In previous studies (Martel 2003), seven allozyme loci were analyzed on populations
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16 90 of *Ocenebra erinacea* and *Ocenebra inornata* collected in 7 sites of the French Atlantic coast
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18 91 where the two species live in sympatry. These markers revealed genetic diversity indices
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20 92 systematically weaker within the native *O. erinacea* than within the invasive *O. inornata*. This
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22 93 result was counterintuitive, as (i) the founder effect linked to an introduction event should lead
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24 94 to a low genetic diversity within the populations of the introduced species, (ii) this
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26 95 phenomenon should be all the more marked as the invasion is recent (review of Sakai *et al.*
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28 96 2001). Consequently, a lower genetic diversity within the populations of the exogenous
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30 97 species compared to the populations of the indigenous species was expected. It is thus of
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32 98 importance to test whether the genetic diversity of the indigenous species *O. erinacea* is
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34 99 correlated with the presence of the introduced species *O. inornata* in zones of sympatry.
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36 100 Indeed, *O. inornata* could induce a selective pressure on *O. erinacea* leading to decrease of
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38 101 polymorphism in this local species.

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41 102 Here, we test this hypothesis by sampling *Ocenebra erinacea* from the Atlantic and
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43 103 Mediterranean French coasts, in the presence and absence of *Ocenebra inornata*, and by
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45 104 measuring genetic diversity of the native species using the mitochondrial marker *cox1*. While
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47 105 investigating the genetic effects that the presence of *O. inornata* may have on sympatric
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49 106 populations of *O. erinacea*, we came across a very strong genetic break between Atlantic and
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51 107 Mediterranean populations. This break is detailed and potential biogeographic causes are
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53 108 discussed.

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5 110 **Materials and methods**6
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9 112 Sampling

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12 A total of 352 adult specimens of *Ocenebra erinacea* were collected between 1999 and
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14 2004 at 15 sites on the French coast, along line transects (<200 m in length). At each site,
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16 specimens from different rocks were collected to reduce sampling bias in favor of a particular
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18 lineage. The sites were located both within oyster farming zones and unexploited areas (Table
19
20 1). In order to show a possible impact of the presence of *Ocenebra inornata* on genetic
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22 diversity of *O. erinacea*, five locations where the two species live in sympatry and 10 sites
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24 free of *O. inornata* have been sampled. The presence of *O. inornata* was assessed by direct
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26 observation. After collection, specimens were stored in 95% ethanol before DNA extraction.
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33 122 DNA extraction, amplification and sequencing

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36 Total DNA was extracted from <15 mg of foot muscle using Dneasy™ Tissue Kit
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38 according to the manufacturer's protocol (Qiagen, Germany). Part of the mitochondrial *cox1*
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40 gene was PCR-amplified with the HCO2198/LCO1490 primers (Folmer *et al.* 1994), which
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42 have proved useful for neogastropod studies (e.g. Zou *et al.* 2011, 2012). Polymorphism at
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44 *cox1* is high in *Ocenebra inornata*, a phylogenetically close species (Martel *et al.* 2004a).
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48 PCR's were done in 50 µL, with 1X PCR buffer, 1.85 mM MgCl₂, 125 µM dNTPs,
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50 0.25 µM of each primer, 1.6 U of Red Hot® DNA Polymerase (ABgene) and about 10 ng of
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52 DNA template. The following cycling profile was performed using a MJResearch PTC 100
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54 Thermal Cycler: initial 5-min denaturation step at 94°C followed by 40 cycles of 30 sec at
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3 132 94°C, 30 sec at 50°C and 1 min at 72°C, and by a final 5-min extension period at 72°C. PCR
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5 133 products were purified using MultiScreen-PCR MANU03010 plates (Millipore).
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8 134 Sequencing was done by GenoScreen (Lille - France) using an ABI PRISM® 3730 XL
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10 135 Automated DNA Sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA). Sequences
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12 136 were aligned using ClustalX (Thompson *et al.* 1994).
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18 138 Data analyses.

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20 139 Genetic analyses aimed at (i) quantifying and comparing genetic diversity among
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22 140 populations, (ii) analyzing the spatial distribution of polymorphism and genetic exchanges
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24 141 among populations, and (iii) studying the evolutionary relationships among populations.
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26 142 Haplotype number H , number of polymorphic sites S , haplotype diversity H_e and average per
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28 143 site nucleotide diversity π (Nei 1987) were calculated for each population using the software
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30 144 DnaSP 5.10.1 (Librado & Rozas 2009).
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34 145 We tested the null hypothesis of the standard neutral model in Arlequin v.3.5 (Excoffier
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36 146 & Lischer 2010), by calculating the D and F_s statistics, as defined by Tajima (1989) and Fu
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38 147 (1997). When these statistics are significantly different from zero, populations may have
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40 148 undergone purifying selection, a selective sweep and/or expansion (<0), or balancing selection
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42 149 and/or a population decline (>0). Statistical significance was tested by generating 10,000
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44 150 random samples under the hypothesis of selective neutrality and population equilibrium.
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46 151 These tests were performed for each sampling site separately, and also for pooled sites in
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48 152 presence or absence of the invasive.
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52 153 The differentiation index Φ_{ST} (Excoffier *et al.* 1992), an estimator of F_{ST} (Wright 1951)
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54 154 calculated from frequency values and distances between haplotypes, was computed with
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56 155 Arlequin v.3.5. The Kimura 2 Parameter (K2P) model of nucleotide substitution was used to
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3 156 estimate genetic distances, and 10,000 permutations were used to test statistical significance
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5 157 under the null hypothesis of no difference between populations (Excoffier *et al.* 1992).
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7 158 Finally, a haplotype network was built using the median-joining algorithm
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9 159 implemented in Network 4.6.1.1 (fluxus-engineering.com, Bandelt 1999). This method is one
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11 160 of the most accurate for inferring intra-specific networks in the absence of recombination
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13 161 (Woolley *et al.* 2008).
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16 162 To help interpret the large genetic divergence observed between Atlantic and
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18 163 Mediterranean specimens of *Ocenebra erinacea*, we looked for mitochondrial *cox1* data from
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20 164 the BOLD database (Ratnasingham & Hebert 2007). However, besides three other BOLD
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22 165 *cox1* sequences from Spanish specimens of *O. erinacea*, we produced the only available
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24 166 mitochondrial sequences for the genus *Ocenebra*. We therefore used *cox1* sequences from six
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26 167 species of the closely-related genus *Nucella* Röding, 1798 (e.g. Pascal 2004) to measure intra-
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28 168 specific and inter-specific genetic distances. *Nucella* and *Ocenebra* are both characterized by
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30 169 a non-planktonic larval development and lay egg capsules on hard substrates (Martel *et al.*
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32 170 2004c; review by Krug 2011). We used the K2P model of nucleotide substitution (Kimura
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34 171 1980), widely used in DNA barcoding (Hebert *et al.* 2003; Barrett & Hebert 2005), to
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36 172 measure genetic distances among *cox1* haplotypes.
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43 174 **Results**

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47 176 A 550 bp fragment of *cox1* was sequenced for 352 individuals, and 29 haplotypes were
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49 177 identified (GenBank accession numbers AY995771-99; Popset 63109090). Sequences include
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51 178 37 polymorphic sites, 20 of which are parsimony informative, and one of which has three
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53 179 character states. No indels were observed (Table 2).
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3 181 Population-level genetic diversity and demographic stability
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5 182 Genetic diversity is comparable among Atlantic populations of *Ocenebra erinacea*, but
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7 183 values for the different diversity indices are low compared to other recently-studied marine
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10 184 mollusks. The number of polymorphic sites between two different sequences varies between 0
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12 185 and 6 among Atlantic populations. Two of these populations (Morbihan and St Quay) are each
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14 186 characterized by a single haplotype, and the 12 other Atlantic sites have no more than five
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16 187 haplotypes (sample sizes provided in Table 1). Consequently, haplotype diversity H_e is low,
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18 188 except for Oléron ($H_e = 0.564$; Table 2). In Fouras, where *Ocenebra inornata* and *O. erinacea*
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20 189 are sympatric, the haplotype and nucleotide diversities are respectively four- and six-fold
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22 190 higher in the invasive drill ($H_e = 0.348$ and $\pi \times 10^{-3} = 0.83$; data from Martel 2003) than in the
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24 191 local one ($H_e = 0.074$ and $\pi \times 10^{-3} = 0.14$).
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28 192 A single Mediterranean population (Thau) was sampled. The haplotype diversity ($H_e =$
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30 193 0.342) and the nucleotide diversity ($\pi \times 10^{-3} = 2.52$) are respectively two- and five-fold higher
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32 194 than on the whole of Atlantic populations (Table 2). Moreover, 22 polymorphic sites were
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34 195 found among 37 individuals sampled in Thau, a value considerably higher than the 24
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36 196 polymorphic sites observed among 315 Atlantic individuals.
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40 197 Last, haplotype and nucleotide diversities of *Ocenebra erinacea* populations co-
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42 198 occurring with *Ocenebra inornata* were lower ($H_e = 0.247 \pm 0.0535$ and $\pi \times 10^{-3} = 0.57 \pm 0.65$)
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44 199 than for populations located in zones where *O. inornata* was not detected ($H_e = 0.399 \pm$
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46 200 0.3976 , $\pi \times 10^{-3} = 10.04 \pm 5.52$) (Table 2). However, this pattern is entirely due to the higher
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48 201 diversity encountered at Thau; when removing this site from the group of populations that
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50 202 were not found in contact with *O. inornata*, diversity values dropped significantly ($H_e = 0.179$
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52 203 ± 0.0368 , $\pi \times 10^{-3} = 0.38 \pm 0.51$). Comparing molecular diversity at the site level revealed the
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54 204 same pattern (Welch Two Sample t-test, including Thau, for H_e : $t = -0.83$, $df = 6.22$, $p = 0.44$;
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3 205 for π : $t = -0.16$, $df = 12.81$, $p = 0.88$. In both cases, results were also non-significant when
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5 206 removing the population of Thau).

7 207 Except for one case, the D and F_s statistics were never positive (Table 2). Furthermore,
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9 208 the only slightly positive D value (deviation from zero non significant) was observed when
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11 209 sites where the invasive was absent were pooled, including Thau, and this result was therefore
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13 210 likely influenced by the underlying population structure (see “Genetic differentiation among
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15 211 populations” Results section). There is therefore no supporting evidence that *Ocenebra*
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17 212 *erinacea* populations exposed to *O. inornata* suffered from a population decline. Some sites
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19 213 exhibited significant negative values of D and F_s , which can be interpreted as signs of
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21 214 purifying selection, selective sweep and/or population expansion. Particularly, the pooled
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23 215 Atlantic sites showed significantly negative values for both tests, regardless of whether the
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25 216 invasive was present or not. These molecular signatures must, however, be interpreted with
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27 217 care, as they might reflect older demographic events.
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35 219 Genealogical relationships and spatial distribution of haplotypes

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38 220 Two haplogroups, separated by 18 mutational steps, were observed using the median-
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40 221 joining network (Fig. 1). The first haplogroup (23 haplotypes) was mainly composed of
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42 222 Atlantic specimens, and included one specimen from Thau, characterized by haplotype H26
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44 223 (separated from other Mediterranean haplotypes by 20 to 22 mutational steps). Haplotypes
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46 224 from this group diverged by ≤ 2 mutations. Haplotype H1 was common (represented in 89%
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48 225 of Atlantic individuals) and central to the Atlantic haplogroup, while the other 22 haplotypes
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50 226 were rare (7 individuals for H3, 3 for H13, 2 for H6, H9, H15, H19 and a single individual for
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52 227 the others) and peripheral to H1. The second haplogroup was strictly composed of
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55 228 Mediterranean individuals. Of six haplotypes from this group, one was common (H21,
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3 229 represented in 82% of Thau individuals), and four were rare (3 individuals for H22 and a
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5 230 single individual for H23-25). Haplotypes from this group diverged by one mutation. The
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7 231 Atlantic samples share no haplotype with the Mediterranean sample (Fig. 2). Except for H1
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9 232 (present at all sites except Thau), H3 (shared between Loix and Aytré) and H13 (shared
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11 233 between Loix, Aytré and Trébeurden), all haplotypes are private (observed only within one
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13 234 population).

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236 Genetic differentiation among populations

237 Genetic differentiation among pairs of populations was measured using Φ_{ST} . The
238 population from Thau was significantly differentiated from all other populations. Pairwise Φ_{ST}
239 values ranged between 0.94 and 0.96, corresponding to a substantial genetic differentiation
240 between the Mediterranean and Atlantic populations (Table 3). Inside the Atlantic group, no
241 significant differentiation was observed after sequential Bonferroni correction (lowest
242 corrected alpha level for Atlantic populations: 0.00055).

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244 Levels of intra-specific divergence in *Ocenebra* compared to *Nucella*

245 Given the differences in haplotype composition and divergence between
246 Mediterranean and Atlantic sites, we investigated whether the genetic distances correspond to
247 intra- or inter-specific divergence by comparing *Ocenebra* to its close relative *Nucella*. The
248 pairwise K2P distance between *Ocenebra erinacea* haplotypes ranged between 0.18 and 4.54%
249 (maximum observed between haplotypes 20 from Loire and 25 from Thau Lagoon). Within
250 *Nucella*, K2P was calculated for 532 sequences and 117 haplotypes distributed among six
251 species, along a 434 bp stretch of *cox1*. Intra-specific distances ranged from 0 to 3.32%, while
252 inter-specific distance ranged from 4.81 to 12.2%, for specimens distributed over 1000 to

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3 253 2000 km (East and West coasts of North America, respectively; BOLD database). For
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5 254 comparison, Zou et al (2011), analyzing 108 neogastropod *cox1* sequences (same gene region
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7 255 as analysed here; not including *Ocenebra* or *Nucella*) found maximum intra-specific K2P
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9 256 distances of 2.2% and minimum inter-specific distances of 2.1%.

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13 14 15 258 **Discussion**

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18 19 260 Relation between *Ocenebra erinacea* and the invasive species *Ocenebra inornata*

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22 261 While a decrease in genetic diversity in response to invaders was reported in the past
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24 262 (Kim *et al.* 2003), we did not detect such a pattern among *Ocenebra erinacea* exposed to the
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26 263 invasive species *Ocenebra inornata*. The relatively low polymorphism levels recorded may
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28 264 have hampered our ability to detect genetic effects of the invasive on the native species, and
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30 265 the use of additional molecular markers such as nuclear microsatellites or SNPs might further
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32 266 help detect possible demographic events associated with the presence *O. inornata*. However,
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34 267 this remains to be tested, as even genome-wide scans can fail at detecting recent demographic
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36 268 events and selective pressures. Riquet *et al.* (2013), for instance, used AFLPs to compare
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38 269 native and invasive populations of the marine mollusk *Crepidula fornicata*. They reported
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40 270 little genetic differentiation among these populations, and detected no F_{ST} outliers out of 344
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42 271 tested loci. An alternative hypothesis explaining the apparent absence of genetic effects of the
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44 272 invasive on the native is that the competitive and selective pressures inflicted on *O. erinacea*
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46 273 are too low to have genetic effects (e.g. Wittmann *et al.* 2013). Finally, deviations from
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48 274 neutrality observed for pooled Atlantic sites suggest selection could have shaped the current
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50 275 genetic diversity and could have blurred signatures of demographic processes.

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55 276 *Ocenebra inornata* was first documented on the French Atlantic coast in 1995 (De
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57 277 Montaudouin & Sauriau 2000, Pigeot *et al.* 2000), and the specimens of *Ocenebra erinacea*

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3 278 used in this study were collected between 1999 and 2004. The introduction of *O. inornata*
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5 279 may have been too recent at the time of sampling for genetic consequences on the native
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7 280 species to be detectable. About ten years later, the distributional landscape of *O. inornata* on
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9 281 Atlantic coasts have significantly changed, and the invasive is now found as far north as the
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11 282 entrance of the Baltic Sea (Lützen *et al.* 2012). A new survey of the genetic diversity of *O.*
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13 283 *erinacea* may today unfold the genetic consequences of the invasion by *O. inornata*, and this
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15 284 study therefore provides a snapshot in time that may help better understand the temporal
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17 285 dynamics of loss of genetic diversity. In addition to sampling in the field, we searched for
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19 286 *Ocenebra* specimens in the collections of the Museum national d'Histoire naturelle in Paris
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21 287 (France), in order to look for genetic diversity in *O. erinacea* specimens collected prior to, or
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23 288 soon after the invasion by *O. inornata* (MNHN voucher numbers IM-2008-7101, IM-2008-
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25 289 7102, IM-2008-7103). Unfortunately, we were not able to amplify the *cox1* marker from these
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27 290 specimens.
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35 292 Remarkably low genetic diversity of *Ocenebra erinacea* populations

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37 293 Mitochondrial genetic diversity, as measured using part of *cox1*, was low relative to
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39 294 what was observed in other marine mollusks. Overall, *Ocenebra erinacea* haplotype and
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41 295 nucleotide diversities were (disregarding the sample from Thau, see below) $H_e = 0.18-0.25$
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43 296 and $\pi \times 10^{-3} = 0.38-0.57$ (Table 2). Comparatively, $H_e = 0.684$ and $\pi \times 10^{-3} = 2.25$ for *Ocenebra*
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45 297 *inornata* in its natural range (data from Martel *et al.* 2004a), $H_e = 0.734$ and $\pi \times 10^{-3} = 14.78$ in
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47 298 the gastropod *Cyclope neritea* (Simon-Bouhet *et al.* 2006), and $H_e = 0.720$ and $\pi \times 10^{-3} = 89.84$
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49 299 in the bivalve *Macoma balthica* (Becquet *et al.* 2012).
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54 300 The low genetic diversity observed at *cox1* was consistent with the low diversity
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56 301 observed using allozymes: Martel (2003) reported that the number of alleles N_{all} and the
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3 302 observed heterozygosity H_o characterizing the Atlantic populations of *Ocenebra erinacea* are
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5 303 respectively two to four times, and twenty to thirty times lower ($N_{all} = 1.1 \pm 0.1$; $H_o = 0.01 \pm$
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7 304 0.01 ; mean \pm SD) than in other marine gastropods sampled in their native range, such as
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9 305 *Bedevea hanleyi* ($N_{all} = 2.2 \pm 0.1$, $H_o = 0.30 \pm 0.02$; Hoskin 2000), *Drupella sp.* ($N_{all} = 2.3 \pm$
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11 306 1.0 , $H_o = 0.25$; Johnson & Cumming 1995), and *Littorina striata* ($N_{all} = 4.2 \pm 1.0$, $H_o = 0.18 \pm$
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13 307 0.17 ; De Wolf *et al.* 2000). Congruent patterns across mitochondrial and allozyme markers
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15 308 thus suggest a low genetic diversity in these populations rather than an absence of variability
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17 309 at *cox1*. Still, our sampling remains restricted compared to the native range of the species, and
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19 310 additional monitoring may reveal new patterns of genetic diversity.
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312 Genetic diversity and connectivity among populations

313 The genetic diversity of the Mediterranean population was among the highest (Table
314 2). The oyster farmers of the Thau lagoon, one of the main shell farming areas of the French
315 Mediterranean coasts, carry out commercial exchanges with distant production sites, and
316 *Ocenebra erinacea* is likely to be transferred during these exchanges. In fact, the
317 morphological survey done by Berrou *et al.* (2004) evidenced exchanges from Oléron Island
318 to Thau Lagoon (this is congruent with the observation of the Atlantic haplotype H26 being
319 observed at Thau; Figs. 1-2). In our study, the high genetic diversity observed in Thau Lagoon
320 could be the result of the introduction of Mediterranean specimens imported from other
321 production sites such as Oléron. As no Mediterranean site was sampled other than Thau
322 Lagoon, the artificial mixing induced by shellfish exchanges cannot be further evaluated here.

323 Alternatively, the difference in genetic diversity observed between the Atlantic and the
324 Mediterranean population of Thau lagoon could be explained by historical and
325 biogeographical factors, and/or selection. *Ocenebra erinacea* is not well adapted to cold water

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3 326 and Belgium currently constitutes the northern limit of its natural range (Graham 1988).
4
5 327 Consequently, the species may have found, as other marine species (e.g. Nikula & Väinölä
6
7 328 2003, Ladhar-Chaabouni *et al.* 2010), a refugium on the Iberian coast or in the Mediterranean
8
9 329 basin during past glaciations. *O. erinacea* may have disappeared from the French Atlantic
10
11 330 coasts during the Würm Glacial period (115,000 to 10,000 years BP) but survived on the
12
13 331 coasts of the Iberian peninsula, which is known as one of the major Pleistocene refugia
14
15 332 (Gómez & Lunz 2007). At the end of this climatic crisis, a reduced number of individuals
16
17 333 from southern refuges may have reached northern coasts. Maggs *et al.* (2008), reviewing
18
19 334 molecular signatures of glacial refugia on marine species, made predictions of low genetic and
20
21 335 haplotype diversity in northern regions previously covered by ice sheets, and comparatively
22
23 336 high diversity in refugial southern regions (and see Hewitt 1996). These predictions are
24
25 337 generally met for *O. erinacea*, but additional sampling from the Iberian peninsula and the
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27 338 Mediterranean Sea would be necessary to further characterize the historical biogeography of
28
29 339 this species. Given *O. erinacea*'s maladaptation to cold-water, another possibility is that
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31 340 selection (either purifying selection, or selective sweeps) linked to differences in water
32
33 341 temperature between Thau and the Atlantic sites produced the observed patterns of genetic
34
35 342 diversity. The negative Tajima's *D* and Fu's *F_s* observed for the Atlantic population (in
36
37 343 absence and presence of the invasive) would support this scenario.
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43 344 One potential consequence of biogeographic divergence between Atlantic and
44
45 345 Mediterranean populations is the emergence of new species (Hewitt 1996, 2004). Recently,
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47 346 Salicini *et al.* (2013) have shown that in the bat *Myotis nattereri*, a complex of four cryptic
48
49 347 species exist in the Western Palearctic region (central and southern Europe, northwestern
50
51 348 Maghreb), each species coinciding with a glacial refugium. In *Ocenebra erinacea*, inter-clade
52
53 349 divergence overlaps with the inter-specific distances observed in the closely-related genus
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55 350 *Nucella* (even though the geographical distances separating *Nucella* specimens were greater
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3 351 than the distances separating *O. erinacea* specimens; see Bergsten *et al.* 2012). In addition, *O.*
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5 352 *erinacea* specimens from the Atlantic and Mediterranean can readily be distinguished using
6
7 353 morphology, and the morphological distance between Atlantic and Mediterranean *O. erinacea*
8
9 354 is comparable to what is observed between *O. erinacea* and *Ocenebra brevirobusta* Houart
10
11 355 2000 (Berrou *et al.* 2004). It is therefore possible that the Atlantic and Mediterranean clades
12
13 356 sampled for this study belong to groups in incipient stages of speciation, or even undescribed
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15 357 species.
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19 358

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556 Table 1. *Ocenebra erinacea* collection sites (listed from North to South, following the
 557 coastline), site name abbreviations (Ab., as used in Figure 2), number of *O. erinacea*
 558 specimens collected and sequenced (n), geographical coordinates, year of collection, and
 559 characteristics of the locations: presence (+) or absence (-) of oyster farms and of *O. inornata*.

560

Location	Ab.	n	Latitude	Longitude	Year of collection	Shellfish area	Presence of <i>O. inornata</i>
Blainville	Bl	31	49°03' N	1°36' W	2003	+	-
Chaussey	Ch	25	48°52' N	1°48' W	2003	+	-
Saint Malo	SM	22	48°39' N	2°01' W	2004	-	-
Saint Quay	SQ	23	48°39' N	2°50' W	2004	-	-
Trébeurden	Tr	24	48°48' N	3°35' W	2004	-	-
Crozon	Cr	23	48°17' N	4°27' W	2004	-	-
Le Croisic	LC	22	47°18' N	2°31' W	2004	-	-
Morbihan	Mo	12	47°33' N	2°51' W	2004	-	-
Bourgneuf	Bo	24	47°01' N	2°01' W	2003	+	+
Loix	Lo	28	46°13' N	1°24' W	2003	+	+
Aytré	Ay	20	46°06' N	1°07' W	2003	+	+
Fouras	Fo	27	46°00' N	1°07' W	2004	+	+
Oléron	OI	13	45°53' N	1°10' W	2004	+	+
Arcachon	Ar	21	44°40' N	1°12' W	1999	+	-
Thau	Th	37	43°24' N	3°35' W	1999	+	-

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563 Table 2. Molecular diversity of populations and results of the neutrality tests. The number of
 564 segregating sites (S), the number of haplotypes (H), the haplotype diversity ($H_e \pm$ one standard
 565 deviation), and the nucleotide diversity ($\pi \pm$ one standard deviation) are given for each
 566 sampling site, site groups in presence and in absence of the invasive (with and without the
 567 Mediterranean population of Thau), and for the entire data set. Sites where *Ocenebra inornata*
 568 was present are indicated by an asterisk. For neutrality tests of Tajima and Fu, statistical
 569 significance after sequential Bonferroni correction is indicated with an asterisk.

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Sampling sites	S	H	$H_e \pm SD$	$\pi \pm SD (x10^{-3})$	Tajima's D	Fu's F_s
Blainville	1	2	0.065 \pm 0.059	0.12 \pm 0.28	-1.14	-1.24*
Chaussey	3	4	0.230 \pm 0.110	0.44 \pm 0.58	-1.73	-3.08*
Saint Malo	1	2	0.091 \pm 0.081	0.17 \pm 0.34	-1.16	-0.96
Saint Quay	0	1	0	0	0	0
Trébeurden	5	5	0.377 \pm 0.122	0.90 \pm 0.89	-1.83	-2.80*
Crozon	2	3	0.316 \pm 0.118	0.60 \pm 0.70	-0.86	-0.87
Le Croisic	4	4	0.333 \pm 0.124	0.81 \pm 0.84	-1.67	-1.74
Morbihan	0	1	0	0	0	0
Bourgneuf *	2	2	0.083 \pm 0.075	0.30 \pm 0.47	-1.51	-0.19
Loix *	5	5	0.270 \pm 0.109	0.65 \pm 0.72	-2.01*	-3.57*
Aytré *	4	4	0.363 \pm 0.131	0.89 \pm 0.89	-1.64	-1.61
Fouras *	1	2	0.074 \pm 0.067	0.14 \pm 0.30	-1.15	-1.12
Oléron *	2	3	0.564 \pm 0.112	1.12 \pm 1.06	-0.13	-0.17
Arcachon	1	2	0.095 \pm 0.084	0.17 \pm 0.35	-1.16	-0.92
Thau	22	6	0.342 \pm 0.098	2.52 \pm 1.76	-2.50*	-0.42
Sites in presence of invasive	11	9	0.247 \pm 0.0535	0.57 \pm 0.65	-2.10*	-9.42*
Sites in absence of invasive	31	22	0.399 \pm 0.3976	10.04 \pm 5.52	0.22	-0.06
Sites in absence of invasive (without Thau)	16	16	0.179 \pm 0.0368	0.38 \pm 0.51	-2.38*	< -10*
All populations	37	29	0.356 \pm 0.0325	7.63 \pm 4.20	-0.81	-4.97

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574 Table 3. Pairwise Φ_{ST} values calculated using the Kimura 2 Parameter model of nucleotide substitution. Only pairwise comparisons involving the
575 population of Thau are statistically significant after sequential Bonferroni correction.

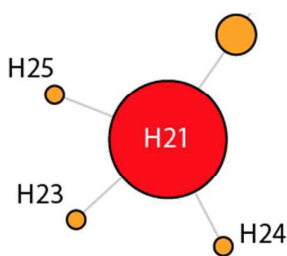
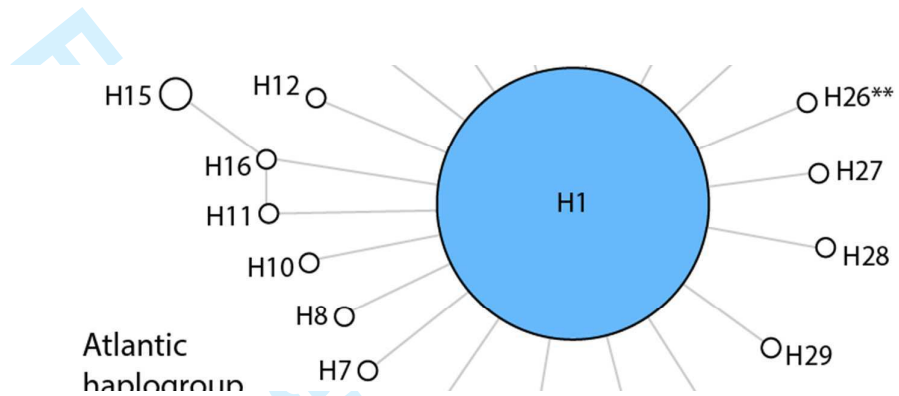
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	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Blainville	0														
2. Chausey	0.0048	0													
3. Saint Malo	0.00242	0.00244	0												
4. Saint Quay	0.01001	0.00356	0.00207	0											
5. Trebeurden	0.02194	0.01045	0.00921	0.01207	0										
6. Crozon	0.05037	0.02772	0.03452	0.04529	0.02682	0									
7. Le Croisic	0.03001	0.01442	0.01577	0.02118	0.01654	0.01135	0								
8. Morbihan	0.03708	0.03371	0.03067	0	0.02156	0.00476	0.01543	0							
9. Bourgneuf	0.00458	0.00021	0.00102	0.00181	0.01083	0.0316	0.01598	0.03274	0						
10. Loix	0.00242	-0.0008	0.00559	0.00747	0.00258	0.02217	0.012	-0.0358	0.01337	0					
11. Aytré	0.03811	0.01838	0.02156	0.0293	0.00956	0.03281	0.02045	0.01049	0.00555	0.01096	0				
12. Oleron	0.28743	0.18725	0.23275	0.28709	0.1342	0.16138	0.13706	0.18898	0.15611	0.12163	0.04004	0			
13. Fouras	0.0004	0.00162	0.00096	0.00611	0.01656	0.04351	0.02394	0.03502	0.00196	0.00096	0.03104	0.26425	0		
14. Arcachon	0.0032	0.00323	0.00014	0.00443	0.00763	0.03266	0.01404	0.02941	0.00153	0.00658	0.01955	0.22606	0.00145	0	
15. Thau	0.96208	0.95559	0.95617	0.95856	0.94988	0.95236	0.95001	0.94928	0.95614	0.9547	0.9484	0.94167	0.95959	0.95541	0

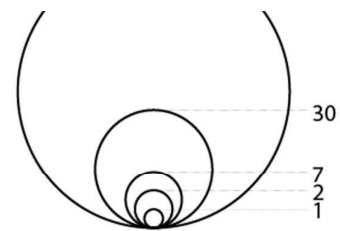
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579 Fig. 1. Median-joining haplotype network. Each circle represents a haplotype, which
 580 frequency is proportional to circle diameter (legend: bottom right). Distances between
 581 haplotypes are proportional to the number of mutation events (see text). The Atlantic
 582 haplogroup contains one Mediterranean population, represented by haplotype H26 (marked
 583 with **).

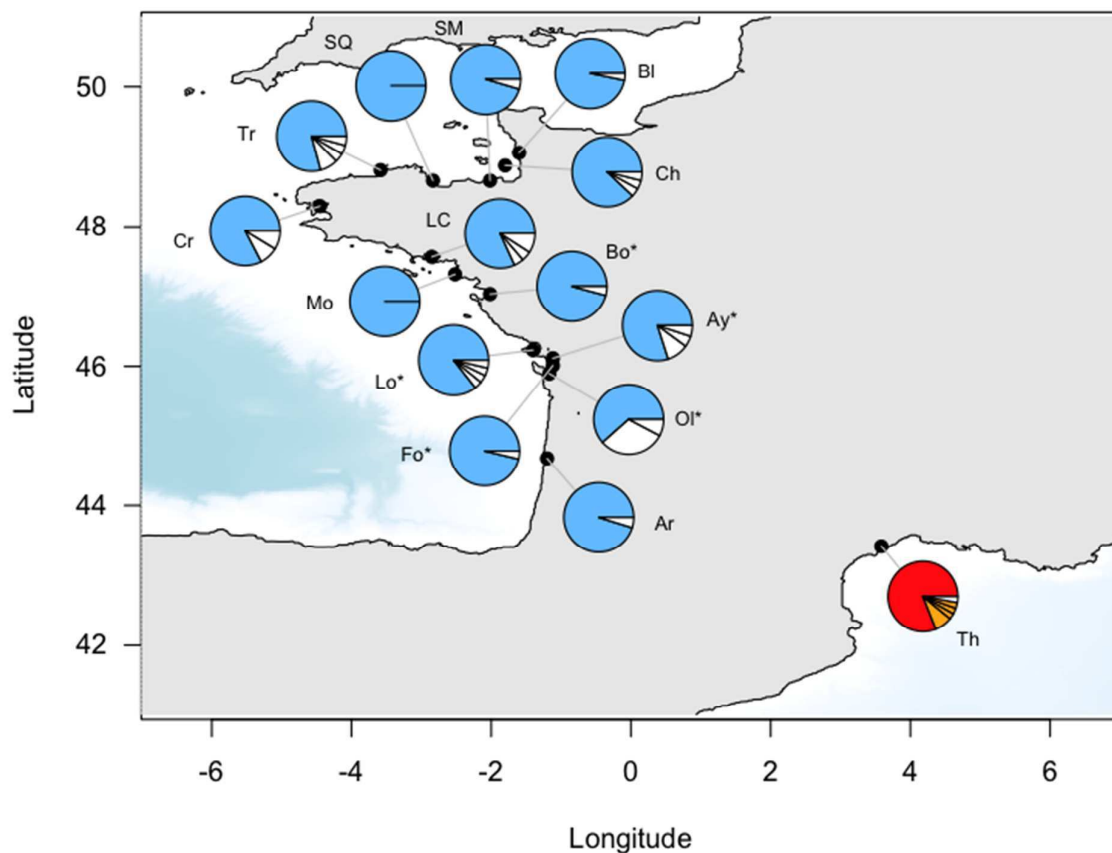


napiogroup



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3 585 Fig. 2. Distribution of haplotype frequencies along the French coast. Abbreviations for site
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5 586 names are detailed in Table 1. Haplotype colors correspond to the colors used in Figure 1. The
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7 587 stations where both *Ocenebra erinacea* and *Ocenebra inornata* were observed are marked
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10 588 with an asterisk. Map constructed with R package marmap (Pante & Simon-Bouhet 2013).



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7 1 | Evaluating the genetic effects of the invasive *Ocenebrelluscenebra inornatus* on the
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9 2 | native oyster drill *Ocenebra erinacea*
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13 4 Eric Pante¹, Pierre-Yves Pascal², Vanessa Becquet¹, Amélia Viricel¹, Benoit Simon-Bouhet¹,
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15 and Pascale Garcia¹
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33 13 | Keyword: *Ocenebra erinaceus*; *Ocenebrellus inornatus*; haplotype diversity; evolutionary
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35 14 response; evolutionary ecology; invasive species; glacial refugium; cryptic species
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16 **Abstract**

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18 Studies focusing on the effect of invasive species on the genetic diversity of native marine
19 invertebrates remain scant. Here we report diversity among French populations of the
20 intertidal gastropod *Ocenebra erinacea* (Linnaeus, 1758) sampled in the presence and absence
21 of the invasive ~~*Ocenebrellus inornatus*~~*Ocenebra inornata* (Recluz, 1851). Between 1999 and
22 2004 a total of 352 individuals of *O. erinacea* were collected from 15 sites, five of which in
23 the presence of the invasive, and genotyped at the mitochondrial locus *cox1*. No statistical
24 difference was observed between polymorphism levels recorded within native populations
25 exposed to the invasive, compared to populations sampled in the absence of ~~*O. inornatus*~~*O.*
26 *inornata*. No sign of native population ~~bottleneck-decline~~ was detected in response to the
27 invader. While significant shifts in native *O. erinacea* population sizes were previously
28 reported in the literature, genetic effects may take longer to accumulate, ~~or may be~~
29 ~~undetectable without a larger panel of genetic markers~~ ~~or may be detectable using a larger~~
30 ~~panel of genetic markers~~. In contrast, large genetic distances and significant population
31 differentiation were recorded between Atlantic and Mediterranean *O. erinacea* samples,
32 suggesting that these populations have distinct evolutionary histories. Comparison of genetic
33 divergence within the closely-related genus *Nucella* suggests that the Atlantic populations and
34 the Mediterranean *O. erinacea* populations from Thau Lagoon may belong to different species
35 or subspecies.

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7 37 **Introduction**
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11 39 Natural movements of species ranges on the surface of the world (expansions,
12 40 regressions, displacements) play a considerable role in the evolution of species. Most of the
13 41 time, these phenomena are progressive and marked by the tempo of geological processes (e.g.
14 42 Hewitt 1996). However, ~~for a~~ during the last few decades a growing number of species has
15 43 undergone changes in their natural range due to both ~~global~~ changes at ~~the planetary~~ global scale
16 44 (e.g. Parmesan & Yohe 2003) and artificial transfers due to human activities (e.g. Carlton
17 45 1989; Seebens *et al.* 2013).

18 46 The number of biological invasions has strongly increased during the last decades (e.g.
19 47 Mack *et al.* 2000; Mooney & Cleland, 2001; Ruiz *et al.* 2000). In the marine environment,
20 48 these phenomena are mainly due to aquaculture and especially to shellfish farming, which
21 49 represents a major cause of introduction, intentional or not, of exogenous species (Elton 1958;
22 50 Carlton 1992).

23 51 Apart from potentially important economic consequences, the arrival of these
24 52 introduced species can also cause serious ecological impacts on local fauna. Introduced
25 53 species are likely to decrease the abundance of indigenous species, excluding them from part
26 54 of their distributional area or even causing their extinction by modifying invaded habitats,
27 55 hybridizing with native species, exchanging pathogens, preying on them, or competing with
28 56 them (e.g. Lockwood *et al.* 2007 and references therein). Moreover, when they exert strong
29 57 selective pressures, introduced species can also reduce the genetic diversity of native
30 58 populations (Kim *et al.* 2003; Wittmann *et al.* 2013).

31 59 Such impacts, although poorly known (e.g. Strauss *et al.* 2006), may have heavy
32 60 ecological consequences since adaptive potential depends on the genetic diversity of a

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7 61 population. Reductions in genetic diversity are generally considered detrimental (e.g.
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9 62 Frankham 1995; Lande 1995; Strauss *et al.* 2006) and ~~may lead~~might contribute to extinction
10 63 (Wittmann *et al.* 2013). Thus, various authors showed a link between the fitness of a species
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12 64 and its genetic diversity, particularly in mollusks (Mitton & Grant 1984; Garton & Haag 1991;
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14 65 Zouros 1993; Launey & Hedgecock 2001; Hedgecock *et al.* 2007). ~~Conversely~~In addition, the
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16 66 reduced genetic variability of an indigenous population could promote the expansion process
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18 67 of other species that are phylogenetically close. However, very few studies address changes in
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20 68 ~~the evolution of~~ genetic diversity of an indigenous species under the competitive selection
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22 69 pressure generated by a biological invader.

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24 70 The oyster drills *Ocenebra erinacea* (Linnaeus, 1758) and ~~*Ocenebrellus*~~
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26 71 ~~*inornatus*~~*Ocenebra inornata* (Recluz, 1851) constitute a noteworthy model to study the
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28 72 genetic effects of indigenous-invasive interactions on indigenous populations. A native of the
29
30 73 northwestern Pacific (Choe & Park 1997; Amano & Vermeij 1998), ~~*Ocenebrellus inornatus*~~*O.*
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32 74 *inornata* (previously known as *Ocenebrellus inornatus*; see Houart and Sirenko 2003; Bouchet
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34 75 and Houart 2014) (Recluz, 1851) has recently invaded European coasts, probably following
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36 76 massive oyster imports (Pigeot 2000; De Montaudouin & Sauriau 2000; review of Lützen *et*
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38 77 *al.* 2012). Genetic data suggest that French populations may come from Asia and the United
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40 78 States (Martel *et al.* 2004a). The introduction of ~~*O. inornatus*~~*O. inornata* ~~may~~ has important
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42 79 economical consequences, as it is a predator of cultivated mollusks (e.g. oysters, blue mussels,
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44 80 Gouilletquer *et al.* 2002). It coexists ~~on~~at several French sites with an indigenous muricid,
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46 81 ~~*O. enebra*~~ *erinacea* (Linnaeus, 1758), which ranges from the straits of Gibraltar to the
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48 82 Netherlands, and inhabits all British and Mediterranean coasts (Graham 1988). Although *O.*
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50 83 *erinacea* and ~~*O. inornatus*~~*O. inornata* differ in some life history traits (Martel *et al.* 2004c),
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52 84 ~~they~~these muricid gastropods fill similar ecological niches, and may compete for habitat
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54 85 (both species live on hard substrates and drill the shell of bivalves to feed on them; e.g.

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7 86 | Lutzen et al 2012). Pigeot et al. (2000) recorded a decrease in population density of *O.*
8 87 | *erinacea*, in parallel to an increase in numbers of *O. inornata* in Marennes-Oléron
9 88 | (Charente-Maritime, France), between 1997 and 1999 (two years after the invasive was first
10 89 | detected). While the introduction and expansion patterns of ~~*O. inornatus*~~*O. inornata* have
11 90 | been investigated in previous studies (Martel et al. 2004a; Martel et al. 2004b), its ecological
12 91 | impacts ~~and the influence of on~~ the ~~local native *O. erinacea* species on its capacity of~~
13 92 | ~~integration~~ are poorly known.

14 93 | In previous studies (Martel 2003), seven allozyme loci were analyzed on populations
15 94 | of ~~*Ocenebra*~~*O. erinacea* and ~~*O. inornatus*~~*Ocenebra inornata* collected in 7 sites of the French
16 95 | Atlantic coast where the two species live in sympatry. These markers revealed genetic
17 96 | diversity indices systematically weaker within the native *O. erinacea* than within the invasive
18 97 | ~~*O. inornatus*~~*O. inornata*. This result was counterintuitive, as (i) the founder effect linked to an
19 98 | introduction event should lead to a low genetic diversity within the populations of the
20 99 | introduced species, (ii) this phenomenon should be all the more marked as the invasion is
21 100 | recent (review of Sakai et al. 2001). Consequently, a lower genetic diversity within the
22 101 | populations of the exogenous species compared to the populations of the indigenous species
23 102 | was expected. It is thus of importance to test whether the genetic diversity of the indigenous
24 103 | species *O. erinacea* is correlated with the presence of the introduced species ~~*O. inornatus*~~*O.*
25 104 | ~~*inornata*~~ in zones of sympatry. Indeed, ~~*O. inornatus*~~*O. inornata* could induce a selective
26 105 | pressure on *O. erinacea* leading to decrease of polymorphism in this local species.

27 106 | Here, we test this hypothesis by sampling ~~*Ocenebra*~~*O. erinacea* from the Atlantic and
28 107 | Mediterranean French coasts, in the presence and absence of ~~*O. inornatus*~~*Ocenebra inornata*,
29 108 | and by measuring genetic diversity of the native species using the mitochondrial marker *coxI*.
30 109 | While investigating the genetic effects that the presence of ~~*O. inornatus*~~*O. inornata* may have
31 110 | on sympatric populations of *O. erinacea*, we came across a very strong genetic break between

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7 111 Atlantic and Mediterranean populations. This break is detailed and potential biogeographic
8 112 causes are discussed.
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13 114 **Materials and methods**

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16 116 Sampling

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19 117 A total of 352 adult specimens of *Ocenebra erinacea* were collected between 1999 and
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21 118 2004 at 15 sites on the French coast, along line transects (<200 m in length). At each site,
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23 119 specimens from different rocks were collected to reduce sampling bias in favor of a particular
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25 120 lineage. The sites were located both within oyster farming zones and unexploited areas (Table
26
27 121 | 1). In order to show a possible impact of the presence of *O. inornatus**Ocenebra inornata* on
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29 122 genetic diversity of *O. erinacea*, five locations where the two species live in sympatry and 10
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31 123 sites free of *O. inornatus**O. inornata* have been sampled. The presence of *O. inornata* was
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33 124 assessed by direct observation. After collection, specimens were stored in 95% ethanol before
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35 125 DNA extraction.
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39 127 DNA extraction, amplification and sequencing

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41 128 Total DNA was extracted from <15 mg of foot muscle using Dneasy™ Tissue Kit
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43 129 according to the manufacturer's protocol (Qiagen, Germany). Part of the mitochondrial *coxI*
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45 130 gene was PCR-amplified with the HCO2198/LCO1490 primers (Folmer *et al.* 1994), which
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47 131 are have particularly proved useful for neogastropod studies (e.g. Harasewych *et al.* 1997 Zou
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49 132 et al 2011, 2012). Polymorphism at *coxI* is high in *O. inornatus**Ocenebra inornata*, a
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51 133 phylogenetically close species (Martel *et al.* 2004a).
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7 134 PCRs were done in 50 μ L, with 1X PCR buffer, 1.85 mM $MgCl_2$, 125 μ M dNTPs,
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9 135 0.25 μ M of each primer, 1.6 U of Red Hot® DNA Polymerase (ABgene) and about 10 ng of
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11 136 DNA template. The following cycling profile was performed using a MJResearch PTC 100
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13 137 Thermal Cycler: initial 5-min denaturation step at 94°C followed by 40 cycles of 30 sec at
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15 138 94°C, 30 sec at 50°C and 1 min at 72°C, and by a final 5-min extension period at 72°C. PCR
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17 139 products were purified using MultiScreen-PCR MANU03010 plates (Millipore).

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19 140 Sequencing was done by GenoScreen (Lille - France) using an ABI PRISM® 3730 XL
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21 141 Automated DNA Sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA). Sequences
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23 142 were aligned using ClustalX (Thompson *et al.* 1994).

24 25 143 26 27 144 Data analyses.

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29 145 Genetic analyses aimed at (i) quantifying and comparing genetic diversity among
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31 146 populations, (ii) analyzing the spatial distribution of polymorphism and genetic exchanges
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33 147 among populations, and (iii) studying the evolutionary relationships among populations.
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35 148 Haplotype number H , number of polymorphic sites S , haplotype diversity H_e/H_e and average
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37 149 per site nucleotide diversity π (Nei 1987) were calculated for each population using the
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39 150 software DnaSP 5.10.1 (Librado & Rozas 2009).

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41 151 We tested the null hypothesis of the standard neutral model in Arlequin v.3.5 (Excoffier
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43 152 & Lischer 2010), by calculating the D and F_s statistics, as defined by Tajima (1989) and Fu
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45 153 (1997). In each location, we checked the demographic equilibrium of the populations in
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47 154 Arlequin v.3.5, by calculating the D and F_s statistics, as defined by Tajima (1989) and Fu
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49 155 (1996). When these statistics are significantly different from zero, populations may have
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51 156 undergone purifying selection, a selective sweep and/or expansion (<0), or balancing selection
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53 157 and/or a population decline or overdominant selection and/or a bottleneck (>0). Statistical

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7 158 significance was tested by generating 10,000 random samples under the hypothesis of
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9 159 selective neutrality and population equilibrium. These tests were performed for each sampling
10 160 site separately, and also for pooled sites in presence or absence of the invasive.

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13 161 The differentiation index Φ_{ST} (Excoffier *et al.* 1992), an estimator of F_{ST} (Wright 1951)
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15 162 calculated from frequency values and distances between haplotypes, was computed with
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17 163 Arlequin v.3.5 (Excoffier & Lischer 2010). The Kimura 2 Parameter (K2P) model of
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19 164 nucleotide substitution was used to estimate genetic distances, and 10,000 permutations were
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21 165 used to test statistical significance under the null hypothesis of no difference between
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23 166 populations (Excoffier *et al.* 1992).

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25 167 Finally, a haplotype network was built using the median-joining algorithm
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27 168 implemented in Network 4.6.1.1 (fluxus-engineering.com, Bandelt 1999). This method is one
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29 169 of the most accurate for inferring intra-specific networks in the absence of recombination
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31 170 (Woolley *et al.* 2008).

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33 171 To help interpret the large genetic divergence observed between Atlantic and
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35 172 Mediterranean specimens of *O.cenebra erinacea*, we looked for mitochondrial *cox1* data from
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37 173 the BOLD database (Ratnasingham & Hebert 2007). However, besides three other BOLD
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39 174 *cox1* sequences from Spanish specimens of *O. erinacea*, we produced the only available
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41 175 mitochondrial sequences for the genus *Ocenebra*. We therefore used *cox1* sequences from
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43 176 seven six species of the closely-related genus *Nucella* Röding, 1798 (e.g. Pascal 2004) to
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45 177 measure intra-specific and inter-specific genetic distances. *Nucella* and *Ocenebra* are both
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47 178 characterized by a non-planktonic larval development and lay egg capsules on hard substrates
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49 179 (Martel *et al.* 2004c; review by Krug 2011). We used the K2P model of nucleotide
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51 180 substitution (Kimura 1980), a widely used metric in DNA barcoding (Hebert *et al.* 2003;
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53 181 Barrett & Hebert 2005), to measure genetic distances among *cox1* haplotypes.

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7 183 **Results**

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10 185 A 550 bp fragment of *cox1* was sequenced for 352 individuals, and 29 haplotypes were
11 186 identified (GenBank accession numbers AY995771-99; Popset 63109090). Sequences include
12 187 37 polymorphic sites, 20 of which are parsimony informative, and one of which has three
13 188 character states. No indels were observed (Table 2).

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21 190 Population-level genetic diversity and demographic stability

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23 191 Genetic diversity is comparable among Atlantic populations of *Ocenebra- erinacea*,
24 192 but values for the different diversity indices are low compared to other recently-studied
25 193 marine mollusks. The number of polymorphic sites between two different sequences varies
26 194 between 0 and 6 among Atlantic populations. Two of these populations (Morbihan and St
27 195 Quay) are each characterized by a single haplotype, and the 12 other Atlantic sites have no
28 196 more than five haplotypes (sample sizes provided in Table 1). Consequently, haplotype
29 197 diversity H_e is low, except for Oléron ($H_e = 0.564$; Table 2). In Fouras, where ~~Θ~~
30 198 ~~*inornatus*~~ *Ocenebra inornata* and *O. erinacea* are sympatric, the haplotype and nucleotide
31 199 diversities are respectively four- and six-fold higher in the invasive drill ($H_e = 0.348$ and π
32 200 $\times 10^{-3} = 0.8378$; data from Martel 2003) than in the local one ($H_e = 0.074$ and $\pi \times 10^{-3} = 0.14$).

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42 201 A single Mediterranean population (Thau) was sampled. The haplotype diversity ($H_e =$
43 202 0.342) and the nucleotide diversity ($\pi \times 10^{-3} = 2.52$) are respectively two- and five-fold higher
44 203 than on the whole of Atlantic populations (Table 2). Moreover, 22 polymorphic sites were
45 204 found among 37 individuals sampled in Thau, a value considerably higher than the 24
46 205 polymorphic sites observed among 315 Atlantic individuals.

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206 Last, haplotype and nucleotide diversities of *O-cenebra erinacea* populations co-
 207 occurring with *O-inornatusOcenebra inornata* were lower ($H_e = 0.247 \pm 0.0535$ and $\pi \times 10^{-3} =$
 208 0.57 ± 0.65) than for populations located in zones where *O-inornatusO. inornata* was not
 209 detected ($H_e = 0.399 \pm 0.3976$, $\pi \times 10^{-3} = 10.04 \pm 5.52$) (Table 2). However, this pattern is
 210 entirely due to the higher diversity encountered at Thau; when removing this site from the
 211 group of populations that were not found in contact with *O-inornatusO. inornata*, diversity
 212 values dropped significantly ($H_e = 0.179 \pm 0.0368$, $\pi \times 10^{-3} = 0.38 \pm 0.51$). Comparing
 213 molecular diversity at the site level revealed the same pattern (Welch Two Sample t-test,
 214 including Thau; for H_e : $t = -0.83$, $df = 6.22$, $p = 0.44$; for π : $t = -0.16$, $df = 12.81$, $p = 0.88$. In
 215 both cases, results were also non-significant when removing the population of Thau).

216 Except for one case, the D and F_s statistics were never positive (Table 2). Furthermore,
 217 the only slightly positive D value (deviation from zero non significant) was observed when
 218 sites where the invasive was absent were pooled, including Thau, and this result was therefore
 219 likely influenced by the underlying population structure (see “Genetic differentiation among
 220 populations” Results section). Except for one case (all sites without *O. inornatus* pooled,
 221 deviation from zero non significant), the D and F_s statistics were never positive. There is
 222 therefore no supporting evidence that *O-cenebra erinacea* populations exposed to *O-*
 223 *inornatusO. inornata* suffered from a population declinebottleneck. Some sites populations
 224 exhibited significant negative values of D and F_s , which can be interpreted as signs of
 225 purifying selection, selective sweep and/or population expansion. Particularly, the pooled
 226 Atlantic sites showed significantly negative values for both tests, regardless of whether the
 227 invasive was present or not. These molecular signatures must, however, be interpreted with
 228 care, as they might reflect older demographic events.

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6 230 | Phylogenetic-Generational relationships and spatial distribution of haplotypes

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9 231 | Two haplogroups, separated by 18 mutational steps, were observed using the median-
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11 232 | joining network (Fig. 1). The first haplogroup (23 haplotypes) was mainly composed of
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13 233 | Atlantic specimens, and included one specimen from Thau, characterized by haplotype H26
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15 234 | (separated from other Mediterranean haplotypes by 20 to 22 mutational steps). Haplotypes
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17 235 | from this group diverged by ≤ 2 mutations. Haplotype H1 was common (represented in 89%
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19 236 | of Atlantic individuals) and central to the Atlantic haplogroup, while the other 22 haplotypes
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21 237 | were rare (7 individuals for H3, 3 for H13, 2 for H6, H9, H15, H19 and a single individual for
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23 238 | the others) and peripheral to H1. The second haplogroup was strictly composed of
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25 239 | Mediterranean individuals. Of six haplotypes from this group, one was common (H21,
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27 240 | represented in 82% of Thau individuals), and four were rare (3 individuals for H22 and a
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29 241 | single individual for H23-25). Haplotypes from this group diverged by one mutation. The
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31 242 | Atlantic samples share no haplotype with the Mediterranean sample (Fig. 2). Except for H1
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33 243 | (present at all sites except Thau), H3 (shared between Loix and Aytré) and H13 (shared
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35 244 | between Loix, Aytré and Trébeurden), all haplotypes are private (observed only within one
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37 245 | population).

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40 247 | Genetic differentiation among populations

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43 248 | Genetic differentiation among pairs of populations was measured using Φ_{ST} . The
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45 249 | population from Thau was significantly differentiated from all other populations. Pairwise Φ_{ST}
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47 250 | values ranged between 0.94 and 0.96, corresponding to an ~~important~~-substantial genetic
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49 251 | differentiation between the Mediterranean and Atlantic populations (Table 3). Inside the
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51 252 | Atlantic group, no significant differentiation was observed after sequential Bonferroni
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53 253 | correction (lowest corrected alpha level for Atlantic populations: 0.00055).

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7 2548 255 Levels of intra-specific divergence in *Ocenebra* compared to *Nucella*9
10 256 Given the differences in haplotype composition and divergence between

11 257 Mediterranean and Atlantic sites, we investigated whether the genetic distances correspond to

12 258 intra- or inter-specific divergence by comparing *Ocenebra* to its close relative *Nucella*. The13 259 pairwise K2P distance between *Ocenebra* ~~*O.*~~ *erinacea* haplotypes ranged between 0.18 and

14 260 4.54% (maximum observed between haplotypes 20 from Loire and 25 from Thau Lagoon).

15 261 Within *Nucella*, K2P was calculated for 532 sequences and 117 haplotypes distributed among16 262 six species, along a 434 bp stretch of *cox1*. Intra-specific distances ranged from 0 to 3.32%,17 263 while inter-specific distance ranged from 4.81 to 12.2%, for specimens distributed over 100018 264 to 2000 km (East and West coasts of North America, respectively; BOLD database). For19 265 comparison, Zou et al (2011), analyzing 108 neogastropod *cox1* sequences (same gene region20 266 as analysed here; not including *Ocenebra* or *Nucella*) found maximum intra-specific K2P21 267 distances of 2.2% and minimum inter-specific distances of 2.1%.

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23 269 **Discussion**

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25 271 Relation between *Ocenebra* *erinacea* and the invasive species ~~*O. inornatus*~~ *Ocenebra*26 272 *inornata*

27 273 While a decrease in genetic diversity in response to invaders was reported in the past

28 274 (Kim *et al.* 2003), we did not detect such a pattern among *Ocenebra* ~~*O.*~~ *erinacea* exposed to29 275 the invasive species ~~*O. inornatus*~~ *Ocenebra inornata*. The relatively low polymorphism levels

30 276 recorded may have hampered our ability to detect genetic effects of the invasive on the native

31 277 species, and the use of ~~more sensitive~~ additional molecular markers such as nuclear

32 278 microsatellites or SNPs might further help detect possible demographic events associated with

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7 279 | the presence ~~*O. inornatus*~~*O. inornata*. However, this remains to be tested, as even genome-
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9 280 | wide scans can fail at detecting recent demographic events and selective pressures. Riquet *et*
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11 281 | *al.* (2013), for instance, used AFLPs to compare native and invasive populations of the marine
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13 282 | mollusk *Crepidula fornicata*. They reported little genetic differentiation among these
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15 283 | populations, and detected no F_{ST} outliers out of 344 tested loci. An alternative hypothesis
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17 284 | explaining the apparent absence of genetic effects of the invasive on the native is that the
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19 285 | competitive and selective pressures inflicted on *O. erinacea* are too ~~mild~~low to have genetic
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21 286 | effects (e.g. Wittmann *et al.* 2013). Finally, deviations from neutrality observed for pooled
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23 287 | Atlantic sites suggest selection could have shaped the current genetic diversity and could have
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25 288 | blurred signatures of demographic processes.

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27 289 | *Ocenebra* ~~*O. inornatus*~~ invaded was first documented on the French Atlantic coast in
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29 290 | ~~the 1997-2000~~s (De Montaudouin & Sauriau 2000, Pigeot *et al.* 2000), and the specimens of
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31 291 | ~~*Ocenebra*~~ *erinacea* used in this study were collected between 1999 and 2004. The
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33 292 | introduction of *O. inornata* may have been too recent at the time of sampling for genetic
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35 293 | consequences on the native species to be detectable. About ten years later, the distributional
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37 294 | landscape of ~~*O. inornatus*~~*O. inornata* on Atlantic coasts have significantly changed, and the
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39 295 | invasive is now found as far north as the entrance of the Baltic Sea (Lützen *et al.* 2012). A
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41 296 | new survey of the genetic diversity of *O. erinacea* may today unfold the genetic consequences
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43 297 | of the invasion by ~~*O. inornatus*~~*O. inornata*, and this study therefore provides a snapshot in
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45 298 | time that may help better understand the temporal dynamics of loss of genetic diversity. In
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47 299 | addition to sampling in the field, we searched for *Ocenebra* specimens in the collections of
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49 300 | the Museum national d'Histoire naturelle in Paris (France), in order to look for genetic
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51 301 | diversity in *O. erinacea* specimens collected prior to, or soon after the invasion by *O. inornata*
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53 302 | ~~*Ocenebrellus*~~ (MNHN voucher numbers IM-2008-7101, IM-2008-7102, IM-2008-7103).
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55 303 | Unfortunately, we were not able to amplify the *coxI* marker from these specimens.

Remarkably low genetic diversity of *Ocenebra*-*erinacea* populations

Mitochondrial genetic diversity, as measured using part of *cox1*, was low relative to what was observed in other marine mollusks. Overall, *Ocenebra*-*erinacea* haplotype and nucleotide diversities were (disregarding the sample from Thau, see below) $H_e = 0.18-0.25$ and $\pi \times 10^{-3} = 0.38-0.57$ (Table 2). Comparatively, $H_e = 0.684$ and $\pi \times 10^{-3} = 2.25$ for *O. inornatus* *Ocenebra inornata* in its natural range (data from Martel *et al.* 2004a), $H_e = 0.734$ and $\pi \times 10^{-3} = 14.78$ in the gastropod *Cyclope neritea* (Simon-Bouhet *et al.* 2006), and $H_e = 0.720$ and $\pi \times 10^{-3} = 89.84$ in the bivalve *Macoma balthica* (Becquet *et al.* 2012).

The low genetic diversity observed at *cox1* was consistent with the low diversity observed using allozymes: Martel (2003) reported that the number of alleles N_{all} and the observed heterozygosity H_o characterizing the Atlantic populations of *Ocenebra erinacea* are respectively two to four times, and twenty to thirty times lower ($N_{all} = 1.1 \pm 0.1$; $H_o = 0.01 \pm 0.01$; mean \pm SD) than in other marine gastropods sampled in their native range, such as *Bedevea hanleyi* ($N_{all} = 2.2 \pm 0.1$, $H_o = 0.30 \pm 0.02$; Hoskin 2000), *Drupella sp.* ($N_{all} = 2.3 \pm 1.0$, $H_o = 0.25$; Johnson & Cumming 1995), and *Littorina striata* ($N_{all} = 4.2 \pm 1.0$, $H_o = 0.18 \pm 0.17$; De Wolf *et al.* 2000). Congruent patterns across mitochondrial and allozyme markers thus suggest a low genetic diversity in these populations rather than an absence of variability at *cox1*. Still, our sampling remains restricted compared to the native range of the species, and additional monitoring may reveal new patterns of genetic diversity.

Genetic diversity and connectivity among populations

The genetic diversity of the Mediterranean population was among the highest ~~str than that of each Atlantic population (except Oléron,~~ Table 2). The oyster farmers of the Thau

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7 328 lagoon, one of the main shell farming areas of the French Mediterranean coasts, carry out
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9 329 commercial exchanges with distant production sites, and *O-cenebra erinacea* is likely to be
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11 330 transferred during these exchanges. In fact, the morphological survey done by Berrou *et al.*
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13 331 (2004) evidenced exchanges ~~between-from~~ Oléron Island ~~and-to~~ Thau Lagoon (this is
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15 332 congruent with the observation of the Atlantic haplotype H26 being observed at Thau; Figs. 1-
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17 333 2). In our study, the high genetic diversity observed in Thau Lagoon could be the result of the
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19 334 introduction of Mediterranean specimens imported from other production sites such as Oléron.
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21 335 As no Mediterranean site was sampled other than Thau Lagoon, the artificial mixing induced
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23 336 by shellfish exchanges cannot be further evaluated here.

24 337 Alternatively, the difference in genetic diversity observed between the Atlantic and the
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26 338 Mediterranean population of Thau lagoon could be explained by historical and
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28 339 biogeographical factors, and/or selection. *Ocenebra- erinacea* is not well -adapted to cold
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30 340 water and Belgium currently constitutes the northern limit of its natural range (Graham 1988).
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32 341 Consequently, the species may have found, as other marine species (e.g. Nikula & Väinölä
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34 342 2003, Ladhar-Chaabouni *et al.* 2010), a refugium on the Iberian coast or in the Mediterranean
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36 343 basin during past glaciations. *O. erinacea* may have disappeared from the French Atlantic
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38 344 coasts during the Würm Glacial period (115,000 to 10,000 years BP) but survived on the
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40 345 coasts of the Iberian peninsula, which is known as one of the major Pleistocene refugia
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42 346 (Gómez & Lunz 2007). At the end of this climatic crisis, a reduced number of individuals
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44 347 from southern refuges may have reached northern coasts. Maggs *et al.* (2008), reviewing
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46 348 molecular signatures of glacial refugia on marine species, made predictions of low genetic and
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48 349 haplotype diversity in northern regions previously covered by ice sheets, and comparatively
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50 350 high diversity in refugial southern regions (and see Hewitt 1996). These predictions are
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52 351 generally met for *O. erinacea*: a mitochondrial genealogy matching geography, but additional
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54 352 sampling from the Iberian peninsula and the Mediterranean Sea would be necessary to further

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7 353 | characterize the historical biogeography of this species. Given *O. erinacea*'s maladaptation to
8 354 | cold-water, another possibility is that selection (either purifying selection, or selective sweeps)
9 | linked to differences in water temperature between Thau and the Atlantic sites produced the
10 355 | observed patterns of genetic diversity. The negative Tajima's *D* and Fu's *F_s* observed for the
11 356 | Atlantic population (in absence and presence of the invasive) would support this scenario, and
12 357 | Hewitt's "northern purity, southern richness" genetic diversity pattern (Magg's et al. Model
13 | Ha).

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20 360 | One potential consequence of biogeographic divergence between Atlantic and
21 361 | Mediterranean populations is the emergence of new species (Hewitt 1996, 2004). Recently,
22 362 | Salicini *et al.* (2013) have shown that in the bat *Myotis naterreri*, a complex of four cryptic
23 363 | species exist in the Western Palearctic region (central and southern Europe, northwestern
24 364 | Maghreb), each species coinciding with a glacial refugium. In *Ocenebra- erinacea*, inter-clade
25 365 | divergence overlaps with the inter-specific distances observed in the closely-related genus
26 366 | *Nucella* (even though the geographical distances separating *Nucella* specimens were greater
27 367 | than the distances separating *O. erinacea* specimens; see Bergsten *et al.* 2012). In addition, *O.*
28 368 | *erinacea* specimens from the Atlantic and Mediterranean can readily be distinguished using
29 369 | morphology, and the morphological distance between Atlantic and Mediterranean *O. erinacea*
30 370 | is comparable to what is observed between *O. erinacea* and *Ocenebra- brevirobusta* Houart
31 371 | 2000 (Berrou *et al.* 2004). It is therefore possible that the Atlantic and Mediterranean clades
32 372 | sampled for this study belong to groups in incipient stages of speciation, or even undescribed
33 373 | species.

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576 Table 1. Sampling sites-Ocenebra erinacea Collection-collection sites (listed from North to
 577 South, following the coastline), site name abbreviations (Ab., as used in Figure 2), number of
 578 *O. erinacea* specimens collected and sequenced (n), geographical coordinates, year of
 579 collection, and characteristics of the locations: presence (+) or absence (-) of oyster farms and
 580 of *O. inornatus*/*O. inornata*.

Location	Ab.	n	Latitude	Longitude	Shellfish area	<i>O. inornatus</i>
Blainville	Bl	31	49°03' N	1°36' W	+	-
Chaussey	Ch	25	48°52' N	1°48' W	+	-
Saint Malo	SM	22	48°39' N	2°01' W	-	-
Saint Quay	SQ	23	48°39' N	2°50' W	-	-
Trébeurden	Tr	24	48°48' N	3°35' W	-	-
Crozon	Cr	23	48°17' N	4°27' W	-	-
Le Croisic	LC	22	47°18' N	2°31' W	-	-
Morbihan	Mo	12	47°33' N	2°51' W	-	-
Bourgneuf	Bo	24	47°01' N	2°01' W	+	+
Loix	Lo	28	46°13' N	1°24' W	+	+
Aytré	Ay	20	46°06' N	1°07' W	+	+
Fouras	Fo	27	46°00' N	1°07' W	+	+
Oléron	Ol	13	45°53' N	1°10' W	+	+
Areache	Ar	21	44°40' N	1°12' W	+	-
Thau	Th	37	43°24' N	3°35' W	+	-

Location	Ab.	n	Latitude	Longitude	Year of collection	Shellfish area	Presence of <i>O. inornata</i>
<u>Blainville</u>	<u>Bl</u>	<u>31</u>	<u>49°03' N</u>	<u>1°36' W</u>	<u>2003</u>	<u>+</u>	<u>-</u>
<u>Chaussey</u>	<u>Ch</u>	<u>25</u>	<u>48°52' N</u>	<u>1°48' W</u>	<u>2003</u>	<u>+</u>	<u>-</u>
<u>Saint Malo</u>	<u>SM</u>	<u>22</u>	<u>48°39' N</u>	<u>2°01' W</u>	<u>2004</u>	<u>-</u>	<u>-</u>
<u>Saint Quay</u>	<u>SQ</u>	<u>23</u>	<u>48°39' N</u>	<u>2°50' W</u>	<u>2004</u>	<u>-</u>	<u>-</u>
<u>Trébeurden</u>	<u>Tr</u>	<u>24</u>	<u>48°48' N</u>	<u>3°35' W</u>	<u>2004</u>	<u>-</u>	<u>-</u>
<u>Crozon</u>	<u>Cr</u>	<u>23</u>	<u>48°17' N</u>	<u>4°27' W</u>	<u>2004</u>	<u>-</u>	<u>-</u>
<u>Le Croisic</u>	<u>LC</u>	<u>22</u>	<u>47°18' N</u>	<u>2°31' W</u>	<u>2004</u>	<u>-</u>	<u>-</u>
<u>Morbihan</u>	<u>Mo</u>	<u>12</u>	<u>47°33' N</u>	<u>2°51' W</u>	<u>2004</u>	<u>-</u>	<u>-</u>

<u>Bourgneuf</u>	<u>Bo</u>	<u>24</u>	<u>47°01' N</u>	<u>2°01' W</u>	<u>2003</u>	<u>±</u>	<u>±</u>
<u>Loix</u>	<u>Lo</u>	<u>28</u>	<u>46°13' N</u>	<u>1°24' W</u>	<u>2003</u>	<u>±</u>	<u>±</u>
<u>Aytré</u>	<u>Ay</u>	<u>20</u>	<u>46°06' N</u>	<u>1°07' W</u>	<u>2003</u>	<u>±</u>	<u>±</u>
<u>Fouras</u>	<u>Fo</u>	<u>27</u>	<u>46°00' N</u>	<u>1°07' W</u>	<u>2004</u>	<u>±</u>	<u>±</u>
<u>Oléron</u>	<u>Ol</u>	<u>13</u>	<u>45°53' N</u>	<u>1°10' W</u>	<u>2004</u>	<u>±</u>	<u>±</u>
<u>Arcachon</u>	<u>Ar</u>	<u>21</u>	<u>44°40' N</u>	<u>1°12' W</u>	<u>1999</u>	<u>±</u>	<u>-</u>
<u>Thau</u>	<u>Th</u>	<u>37</u>	<u>43°24' N</u>	<u>3°35' W</u>	<u>1999</u>	<u>±</u>	<u>-</u>

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Table 2. Molecular diversity of populations and results of the neutrality tests. The number of segregating sites (S), the number of haplotypes (H), the haplotype diversity ($H_e \pm$ one standard deviation), and the nucleotide diversity ($\pi \pm$ one standard deviation) are given for each sampling site, site groups in presence and in absence of the invasive (with and without the Mediterranean population of Thau), and for the entire data set. Sites where *O. inornatus*/*Ocenebra inornata* was present are indicated by an asterisk. For neutrality tests of Tajima and Fu, statistical significance after sequential Bonferroni correction is indicated with an asterisk.

Sampling sites	S	H	$H_e \pm SD$	$\pi \pm SD (x10^{-3})$	Tajima's D	Fu's F_s
Blainville	1	2	0.065 \pm 0.059	0.12 \pm 0.28	-1.14	-1.24*
Chaussey	3	4	0.230 \pm 0.110	0.44 \pm 0.58	-1.73	-3.08*
Saint Malo	1	2	0.091 \pm 0.081	0.17 \pm 0.34	-1.16	-0.96
Saint Quay	0	1	0	0	0.00	0.00
Trébeurden	5	5	0.377 \pm 0.122	0.90 \pm 0.89	-1.83	-2.80*
Crozon	2	3	0.316 \pm 0.118	0.60 \pm 0.70	-0.86	-0.87
Le-Croisie	4	4	0.333 \pm 0.124	0.81 \pm 0.84	-1.67	-1.74
Morbihan	0	1	0	0	0.00	0.00
Bourgneuf*	2	2	0.083 \pm 0.075	0.30 \pm 0.47	-1.51	-0.19
Loix*	5	5	0.270 \pm 0.109	0.65 \pm 0.72	-2.01*	-3.57*
Aytré*	4	4	0.363 \pm 0.131	0.89 \pm 0.89	-1.64	-1.61
Fouras*	1	2	0.074 \pm 0.067	0.14 \pm 0.30	-1.15	-1.12
Oléron*	2	3	0.564 \pm 0.112	1.12 \pm 1.06	-0.13	-0.17
Arcahon	1	2	0.095 \pm 0.084	0.17 \pm 0.35	-1.16	-0.92
Thau	22	6	0.342 \pm 0.098	2.52 \pm 1.76	-2.50*	-0.42
Sites in presence of invasive	11	9	0.247 \pm 0.0535	0.57 \pm 0.65	-2.10*	-9.42*
Sites in absence of invasive	31	22	0.399 \pm 0.3976	10.04 \pm 5.52	0.22	-0.06
Sites in absence of invasive (without Thau)	16	16	0.179 \pm 0.0368	0.38 \pm 0.51	-2.38*	<-10*
All populations	37	29	0.356 \pm 0.0325	7.63 \pm 4.20	-0.81	-4.97
Sampling sites	S	H	$H_e \pm SD$	$\pi \pm SD (x10^{-3})$	Tajima's D	Fu's F_s
Blainville	1	2	0.065 \pm 0.059	0.12 \pm 0.28	-1.14	-1.24*
Chaussey	3	4	0.230 \pm 0.110	0.44 \pm 0.58	-1.73	-3.08*
Saint Malo	1	2	0.091 \pm 0.081	0.17 \pm 0.34	-1.16	-0.96

<u>Saint Quay</u>	<u>0</u>	<u>1</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
<u>Trébeurden</u>	<u>5</u>	<u>5</u>	<u>0.377 ± 0.122</u>	<u>0.90 ± 0.89</u>	<u>-1.83</u>	<u>-2.80*</u>
<u>Crozon</u>	<u>2</u>	<u>3</u>	<u>0.316 ± 0.118</u>	<u>0.60 ± 0.70</u>	<u>-0.86</u>	<u>-0.87</u>
<u>Le Croisic</u>	<u>4</u>	<u>4</u>	<u>0.333 ± 0.124</u>	<u>0.81 ± 0.84</u>	<u>-1.67</u>	<u>-1.74</u>
<u>Morbihan</u>	<u>0</u>	<u>1</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
<u>Bourgneuf *</u>	<u>2</u>	<u>2</u>	<u>0.083 ± 0.075</u>	<u>0.30 ± 0.47</u>	<u>-1.51</u>	<u>-0.19</u>
<u>Loix *</u>	<u>5</u>	<u>5</u>	<u>0.270 ± 0.109</u>	<u>0.65 ± 0.72</u>	<u>-2.01*</u>	<u>-3.57*</u>
<u>Aytré *</u>	<u>4</u>	<u>4</u>	<u>0.363 ± 0.131</u>	<u>0.89 ± 0.89</u>	<u>-1.64</u>	<u>-1.61</u>
<u>Fouras *</u>	<u>1</u>	<u>2</u>	<u>0.074 ± 0.067</u>	<u>0.14 ± 0.30</u>	<u>-1.15</u>	<u>-1.12</u>
<u>Oléron *</u>	<u>2</u>	<u>3</u>	<u>0.564 ± 0.112</u>	<u>1.12 ± 1.06</u>	<u>-0.13</u>	<u>-0.17</u>
<u>Arcachon</u>	<u>1</u>	<u>2</u>	<u>0.095 ± 0.084</u>	<u>0.17 ± 0.35</u>	<u>-1.16</u>	<u>-0.92</u>
<u>Thau</u>	<u>22</u>	<u>6</u>	<u>0.342 ± 0.098</u>	<u>2.52 ± 1.76</u>	<u>-2.50*</u>	<u>-0.42</u>
<u>Sites in presence of invasive</u>	<u>11</u>	<u>9</u>	<u>0.247 ± 0.0535</u>	<u>0.57 ± 0.65</u>	<u>-2.10*</u>	<u>-9.42*</u>
<u>Sites in absence of invasive</u>	<u>31</u>	<u>22</u>	<u>0.399 ± 0.3976</u>	<u>10.04 ± 5.52</u>	<u>0.22</u>	<u>-0.06</u>
<u>Sites in absence of invasive (without Thau)</u>	<u>16</u>	<u>16</u>	<u>0.179 ± 0.0368</u>	<u>0.38 ± 0.51</u>	<u>-2.38*</u>	<u>< -10*</u>
<u>All populations</u>	<u>37</u>	<u>29</u>	<u>0.356 ± 0.0325</u>	<u>7.63 ± 4.20</u>	<u>-0.81</u>	<u>-4.97</u>

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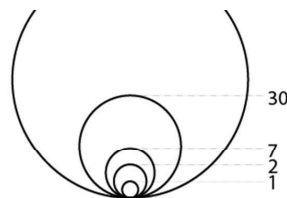
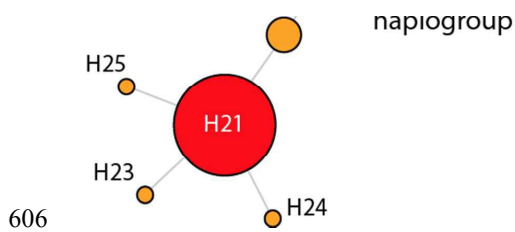
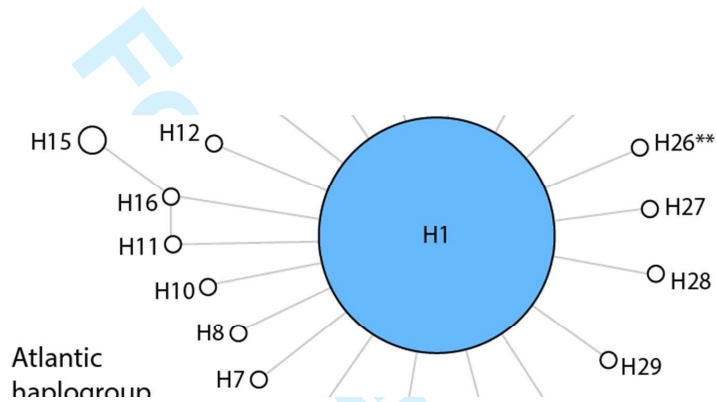
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596 Table 3. Pairwise Φ_{ST} values calculated using the Kimura 2 Parameter model of nucleotide substitution. Only pairwise comparisons involving the
597 population of Thau are statistically significant after sequential Bonferroni correction ($p=0$).
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	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Blainville	0														
2. Chausey	0.0048	0													
3. Saint Malo	0.00242	0.00244	0												
4. Saint Quay	0.01001	0.00356	0.00207	0											
5. Trebeurden	0.02194	0.01045	0.00921	0.01207	0										
6. Crozon	0.05037	0.02772	0.03452	0.04529	0.02682	0									
7. Le Croisic	0.03001	0.01442	0.01577	0.02118	0.01654	0.01135	0								
8. Morbihan	0.03708	0.03371	0.03067	0	0.02156	0.00476	0.01543	0							
9. Bourgneuf	0.00458	0.00021	0.00102	0.00181	0.01083	0.0316	0.01598	0.03274	0						
10. Loix	0.00242	-0.0008	0.00559	0.00747	0.00258	0.02217	0.012	-0.0358	0.01337	0					
11. Aytré	0.03811	0.01838	0.02156	0.0293	0.00956	0.03281	0.02045	0.01049	0.00555	0.01096	0				
12. Oleron	0.28743	0.18725	0.23275	0.28709	0.1342	0.16138	0.13706	0.18898	0.15611	0.12163	0.04004	0			
13. Fouras	0.0004	0.00162	0.00096	0.00611	0.01656	0.04351	0.02394	0.03502	0.00196	0.00096	0.03104	0.26425	0		
14. Arcachon	0.0032	0.00323	0.00014	0.00443	0.00763	0.03266	0.01404	0.02941	0.00153	0.00658	0.01955	0.22606	0.00145	0	
15. Thau	0.96208	0.95559	0.95617	0.95856	0.94988	0.95236	0.95001	0.94928	0.95614	0.9547	0.9484	0.94167	0.95959	0.95541	0

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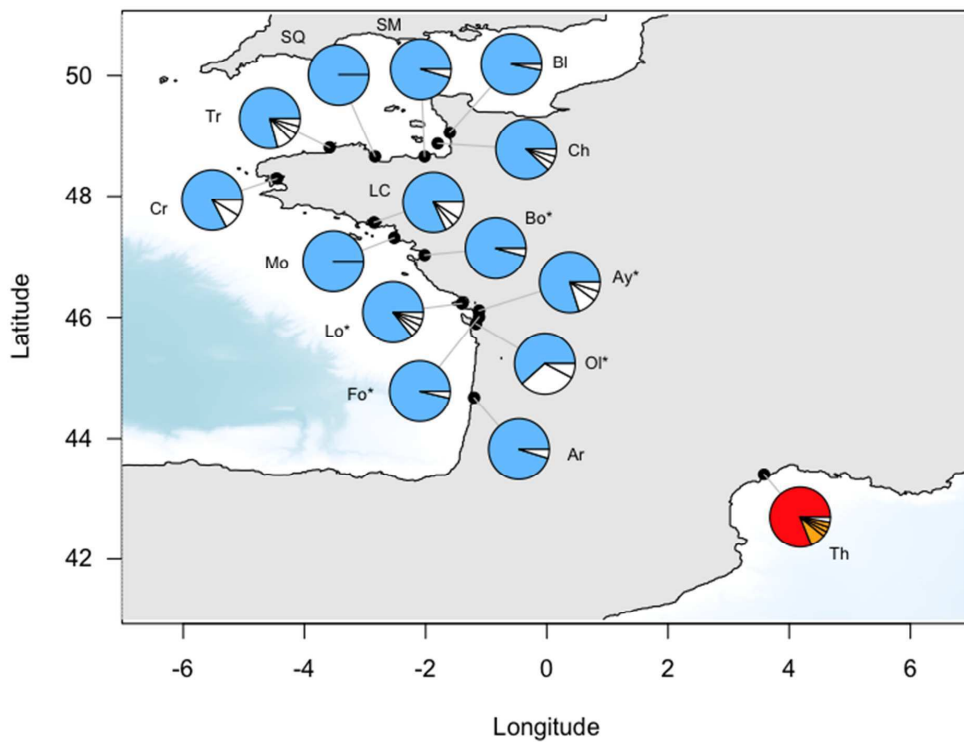
601 Fig. 1. Median-joining haplotype network. Each circle represents a haplotype, which
 602 frequency is proportional to circle diameter (legend: bottom right).
 603 Distances between haplotypes are proportional to the number of mutation events (see text).
 604 The Atlantic haplogroup contains one Mediterranean population, represented by haplotype
 605 H26 (marked with **).



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7 607 Fig. 2. Distribution of haplotype frequencies along the French coast. Abbreviations for site
8 608 names are detailed in Table 1. Haplotype colors correspond to the colors used in Figure 1. The
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10 609 stations where both *Ocenebra- erinacea* and *O. inornatus* were observed
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12 610 are marked with an asterisk. Map constructed with R package marmap (Pante & Simon-
13
14 611 Bouhet 2013).



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