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Invasion of the eastern Bay of Biscay by the nassariid gastropod *Cyclope neritea*: origin and effects on resident fauna

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ABSTRACT: The distribution area of the nassariid gastropod *Cyclope neritea* (L.) includes the Mediterranean and the Black Sea, as well as the Atlantic coasts of the southern Iberian Peninsula. The species has spread north to the eastern Bay of Biscay (Arcachon Bay in 1976, Marennes-Oléron area and Morbihan Gulf in 1983–84). This spread might be explained either by (1) a natural spread favoured by environmental changes (e.g. an increase of temperature) or (2) a sudden range expansion due to the introduction of individuals from distant native populations. Molecular genetic analyses based on mitochondrial markers suggest that the present *C. neritea* population in Arcachon Bay has been introduced, probably unintentionally with oyster transfers, from several source populations, genetically similar to the populations analysed in this study, i.e. those in the western Mediterranean and in south Portugal. Within its new distribution area, *C. neritea* could potentially compete with the autochthonous nassariid *Nassarius reticulatus*, both species being scavengers. Although *C. neritea* tends to occur mainly in relatively clean sands in the intertidal and *N. reticulatus* in subtidal, organic rich sediments, the habitats of both species partially overlap. Laboratory experiments showed that in still water conditions *C. neritea* was more active and reached carrion faster than *N. reticulatus*, thereby having a competitive advantage over the latter; flow conditions (~1 cm s^{-1}) appeared to stimulate the activity of *N. reticulatus*. Analysis of parasite load in both species in Arcachon Bay indicated that *N. reticulatus* was more heavily parasitized by digenean trematodes than *C. neritea*. This suggests that the spread and population dynamics of *C. neritea* along the French Atlantic coast has been favoured by the lack of parasites. Altogether, recurrent introduction, competitive ability and lack of heavy parasitic load might explain the successful settlement of *C. neritea* along the French Atlantic coast.

KEY WORDS: Introduced species · Scavenging gastropods · Population genetics · Interspecific competition · Behaviour · Parasites

INTRODUCTION

The introduction of alien species has been recognized as a major threat to marine ecosystems, with a set of ecological and evolutionary consequences from species to ecosystem levels (Grosholz 2002). In this respect, introduced species are considered one of the most serious threats to the conservation of natural biodiversity (Coblentz 1990, Cohen & Carlton 1998, Mooney & Cleland 2001, Palumbi 2001, Shea & Chesson 2002)

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and are identified as the second cause (after habitat destruction by human activities) of the loss of biological diversity (Vitousek et al. 1997, Wilcove et al. 1998). As recently emphasized by Ruiz et al. (2000), multiple approaches, although rare, are required to understand the patterns and processes of marine biological invasions. For example, to identify the proximate factors for the success or the failure of invasion, studies of life history traits, species interactions (including the consequences on resident species) and the genetic architecture of invasive populations are complementary (Parker et al. 1999, Ruiz et al. 2000, Sakai et al. 2001, Grosholz 2002, Lee 2002).

Dukes & Mooney (1999), Carlton (2000) and Stachowicz et al. (2002) have pointed out the likely relationships between global and climate change and the success of biological invasions. In this context, the nassariid gastropod *Cyclope neritea* (L., 1758) is an interesting model species. This gastropod is 1 of the 87 marine and brackish water species that are known or suspected to have been accidentally introduced to the Atlantic and Channel coasts of France (Goulletquer et al. 2002). *C. neritea* is native to the Mediterranean and Black Seas and to the Atlantic coasts of southern Spain and Portugal as far north as Setubal (Hidalgo 1917, Nobre 1931). Up to the early 1970s, discontinuous populations were also recorded in the north of Spain and on the French Basque coast (Kisch 1950, Morton 1960; see also Sauriau 1991 for a thorough analysis of occurrence records of *C. neritea* on the Atlantic coasts). New *C. neritea* populations appeared on the French Atlantic coasts during the 1970s and 1980s: (1) in 1976 in Arcachon Bay (Bachelet et al. 1980), (2) in 1983–84 further north, in the Marennes-Oléron Bay (Pigeot 1988, Sauriau 1989), at the Isle of Ré (Tardy et al. 1985), and in the Gulf of Morbihan (Le Roux et al. 1988). Arcachon Bay and the Gulf of Morbihan are located approximately 150 km and >500 km, respectively, north of the early 1970s range of the species. It should be noted that, in all these newly colonized areas, *C. neritea* populations are now self-reproducing and abundant, with densities >100 ind m^{-2} (Tardy et al. 1985, Bachelet et al. 1990, Le Roux 1994, Afli & Chenier 2002). A large *C. neritea* population was also observed in 2003 in the Bay of Morlaix, English Channel (B. Simon-Bouhet & F. Viard, pers. obs.).

Two non-exclusive hypotheses can be put forward to explain the present distribution of *Cyclope neritea* along the French Atlantic coasts: (1) a natural range expansion towards the north due to environmental (e.g. climatic) changes, and/or (2) accidental introductions related to shellfish culture (e.g. oyster transfers between the Mediterranean and the Bay of Biscay). As suggested by Sauriau (1991), both processes may have been responsible, since the aforementioned new

populations of *C. neritea* have potentially benefited from a slight warming of coastal waters during the period 1970 to 1990. However, when a species is introduced close to its natural distribution area, it is difficult to discriminate both processes using field survey only. We addressed this issue by sequencing a mitochondrial DNA gene fragment (the Cytochrome C Oxidase I, COI), which is known to be a powerful tool in determining the origin and history of new populations (Sakai et al. 2001) and can trace back historical processes at within-species level (Avise et al. 1987, Avise 1991).

Competitive interactions between native and introduced species, leading to fitness reduction in native populations, have been experimentally demonstrated in marine species and found to be a major determinant of the success of an invasion (see e.g. reviews by Carlton 1992, Ruiz et al. 1999, Grosholz 2002). In European coastal waters an autochthonous species with which *Cyclope neritea* might compete is the netted dogwhelk *Nassarius reticulatus* (L.), as both species belong to the same family and feed as scavengers. We made field observations to determine if both species when living in sympatry occupy a similar habitat, and performed laboratory experiments to determine if there was competition for food between the 2 nassariids.

It has also been suggested that parasite release is important in allowing an introduced species to become invasive (Calvo-Ugarteburu & McQuaid 1998b). Parasitic infection in introduced species is mainly limited by 2 fundamental principles (Torchin et al. 2002): (1) the intensity and prevalence of infection tend to increase with host population density, which suggests that in a recently introduced host-parasite system, host density will often be too low for the parasite to establish and (2) parasites, in particular digenean trematodes that exhibit high prevalence in molluscs (Lauckner 1980), often have a complex life-cycle, including 2 or more host species. Consequently, many introduced species lack most or all of their native natural parasites (Calvo-Ugarteburu & McQuaid 1998a, Torchin et al. 2001), which is advantageous when in potential competition with native species. In the present work we compared the parasite prevalence and richness between *Cyclope neritea* and *Nassarius reticulatus*. The disadvantage in being parasitized was assessed through a survival experiment.

Based on an integrative and pluridisciplinary approach, the aims of this study are therefore to give insights about the history of the introduction, together with the identification of biological factors that may have promoted the settlement and then the maintenance of an exotic marine species, recently introduced along the European North Atlantic coast.

MATERIALS AND METHODS

Study species and area. *Cyclope neritea* is a small mollusc (~15 mm maximum shell width) which lives in shallow and intertidal habitats, predominantly in calm waters, where it usually lies buried a few mm below, or crawls at, the sediment surface. Like most members of the family Nassariidae, it is predominantly a scavenger, feeding on carrion, especially dead bivalves (Morton 1960, Britton & Morton 1994), though it may also behave as a deposit feeder (Southward et al. 1997). *C. neritea* has no dispersive larval stage: the embryos undergo direct development during 2 to 5 wk within egg capsules (each capsule usually containing a single embryo) deposited on hard substrata such as cockle shells, then hatch as benthic juveniles (Kisch 1950, Gomoiu 1964, Massé et al. 1978, Boulhic & Tardy 1986, D'Asaro 1993). The first *C. neritea* population observed outside its common native range (Mediterranean, Black Sea and Atlantic coasts of the Iberian Peninsula) was in Arcachon Bay, where it has been monitored since 1976. We focused on this particular population to develop several parallel studies (population genetics, habitat, competition and parasitic load).

Sampling, DNA extraction, amplification and sequencing. Adult specimens of *Cyclope neritea* were collected from 3 intertidal locations (Table 1): (1) Faro (S Portugal) and (2) Thau Lagoon (French Mediterranean), which are both located within the natural range of the species; these populations may have contributed to the settlement of *C. neritea* in France; and (3) Arcachon Bay (site 'Arguin'), where the first new population of *C. neritea* on the French Atlantic to the north of the Basque coast was recorded. The specimens were attracted with a dead fish, collected, removed from their shell and stored individually in 95% ethanol before DNA extraction. Total DNA was extracted from <15 mg of foot muscle using DNeasy™ Tissue Kit according to the manufacturer's protocol (Quiagen). From preliminary sequences carried out with the universal primers originally defined by Folmer et al. (1994) (i.e. LCO1491 and HCO2198) to amplify the COI gene, we designed a

new set of internal primers more specific to *C. neritea* : Cy2 5′-GTTAAAATTTCGATCTGTTA-3′ (forward) and CyR 5′-GGATTAGTTGGTACAGC-3′ (reverse). The amplifications were performed using these primers in 50 µl reactions containing 5 to 50 ng DNA, 0.5 µM of each primer, 0.2 mM dNTPs, 1.5 mM MgCl$_2$, 1× Reaction Buffer (containing 50 mM Tris HCl pH 8.8 at 25°C, 200 mM (NH$_4$)$_2$SO$_4$, ABgene) and 1.25 U Red Hot® DNA Polymerase (ABgene). A MJResearch PTC 100 Thermal Cycler was used with a cycling profile as follows: initial denaturation step at 93°C (3 min) followed by 40 cycles at 93°C (30 s), 53°C (30 s), 72°C (60 s) and a final extension step at 72°C (10 min). Double stranded PCR products were cleaned using MultiScreen-PCR MANU03010 plates (Millipore) and cycle-sequenced using ABI PRISM® BigDye™ Terminators v3.0 Cycle Sequencing Kit (Heiner et al. 1998) following the manufacturer's protocol (Applied Biosystems). Unincorporated BigDye terminators were removed using MultiScreen MAHVN4510 plates (Millipore). In order to minimize sequencing errors, both strands were sequenced for each individual using an ABI PRISM® 3100 Automated DNA Sequencer (Perkin-Elmer Applied Biosystems). Sequence data were aligned using ClustalW (Thompson et al. 1994) and ambiguities were checked comparing each sequence with its complementary fragment using BioEdit (Hall 1999).

Sequence analysis. A haplotype is defined by a unique stretch of nucleotides. Haplotype diversity H_e (Nei 1987) and average pairwise difference K (i.e. average number of nucleotide differences between pairs of sequences) (Tajima 1983) were calculated for each population using the software DnaSP (Rozas & Rozas 1999). Haplotype frequencies and pairwise F_{ST} values (calculated from frequency values; Weir & Cockerham 1984) were estimated using Arlequin v.2.0 (Schneider et al. 2001). Using a permutation procedure as implemented in Arlequin v.2.0, we assessed the statistical significance of pairwise F_{ST} values under the null hypothesis of no difference between populations.

For polymorphic populations, the mismatch distribution (i.e. the distribution of the observed number of pairwise nucleotide site differences) was computed in DnaSP, in order to examine the occurrence of mixing of evolutionary divergent haplotype groups at the population level. This analysis was used to put forward hypotheses about the number of genetically differentiated populations at the origin of the establishment of a given population.

Finally, phylogenetic analyses were performed with the software Network

Table 1. *Cyclope neritea*. Sampling sites (geographic co-ordinates) and molecular diversity of populations. The number of individuals sequenced (N), the number of haplotypes (*H*), the haplotype diversity (*H*$_e$) and the average pairwise differences (*K*) are given for each studied population and for the entire data set

	Latitude	Longitude	N	*H*	*H*$_e$	*K*
Thau Lagoon	43° 27′ N	3° 38′ E	29	1	0.000	0.000
Faro (Rio Formosa)	37° 00′ N	7° 58′ W	31	1	0.000	0.000
Arcachon Bay (Arguin)	44° 35′ N	1° 14′ W	33	5	0.725	6.576
Total	-	-	93	5	0.602	6.344

which builds haplotypic networks from free-recombinant data, based on the median-joining algorithm (Bandelt et al. 1999). This process combines both minimum spanning trees and maximum parsimony approaches. Such networks show the evolutionary relationships (based on mutational events) between the different haplotypes observed over the whole data set.

Habitat preferences. The habitat, in terms of bathymetric distribution and sediment properties, of *Cyclope neritea* and *Nassarius reticulatus* was assessed in Arcachon Bay in March 2002. In this macrotidal lagoon, 5 bathymetric transects were performed, with 4 to 10 sampling stations on each transect. Stations were distributed over a tidal range from –8.8 to +3.1 m. At each station, a trap (diameter 26 cm), made of a plastic bag with mesh openings of 4 mm on the bottom (to prevent loss of nassariids when raised to the surface) and 15 mm on the sides (to allow nassariids to enter the trap), was immersed at high tide and deposited on the sea bottom. A sampling unit was defined as a trap containing 20 g fresh weight of bait (dead *Crepidula fornicata*) immersed for 30 min. Seven replicates were taken at each station. Nassariids of both species were counted in each sampling unit. At each station, surface sediment was collected with an Ekman grab and analysed for grain size distribution (wet sieving) and organic matter content (loss of weight of dry sediment at 550°C during 2 h).

Experiments on interspecific competition for food. Laboratory experiments were designed to investigate the behaviour and the chemoreception ability of *Cyclope neritea* and *Nassarius reticulatus*. These experiments were performed in a long straight PVC channel ($160 \times 7 \times 3.5$ cm) without sediment and filled to a depth of 3 cm with seawater. No attempt was made to control environmental parameters; however, the experiments were run during the day under natural lighting, and within a short period (16 to 20 May) during which conditions remained largely constant. During the experiments, water temperature and salinity ranges were 17.7 to 18.9°C and 24 to 28‰, respectively. Nassariids were collected in Arcachon Bay and maintained without food in aquaria with running oxygenated seawater for 2 months prior to the experiments. Similar-sized adult *C. neritea* (shell width = 11 to 15 mm) and *N. reticulatus* (shell height = 18 to 27 mm) were used. All experiments were carried under 2 different hydrodynamic conditions: (1) in still water, and (2) in flow conditions, where sea-water flowed unidirectionally at a surface velocity of 0.81 to 0.94 cm s^{-1}.

The first set of experiments was conducted without any prey in order to observe the spontaneous behaviour of both nassariid species. Individuals of the 2 species (4 *Cyclope neritea* and 4 *Nassarius reticulatus*; 8 replicates) were arranged in a line at an equal distance from both ends of the channel. The number of individuals moving spontaneously (in still water and flow conditions) over a distance of at least 10 cm in 30 min was recorded.

In a second set of experiments we measured the ability of both nassariid species to locate and reach a prey (a dead cockle *Cerastoderma edule*). Individuals of the 2 species (4 *Cyclope neritea* and 4 *Nassarius reticulatus*; 8 replicates in still water, 4 replicates in running seawater) were placed at one end of the channel and allowed to feed upon the carrion which was placed at distances of 20, 40 or 60 cm. In flow conditions, seawater flowed unidirectionally towards both the carrion and the nassariids. The times at which each individual reached the prey were recorded and their speed was calculated. Individuals that did not reach the carrion within 30 min were assigned zero.

Survey of parasite abundance. From October 2000 to July 2001, sympatric populations of *Nassarius reticulatus* and *Cyclope neritea* were collected monthly by hand on a sandflat ('Arguin') in Arcachon Bay. In the laboratory, 100 snails of each species were gently crushed and dissected under a stereomicroscope. Mean prevalence (i.e. percentage of infected individuals; Bush et al. 1997) was calculated for each parasite species or family.

Survival experiment in relation to parasites. Due to the low parasite infection in *Cyclope neritea* (see 'Results'), the survival experiment was performed with *Nassarius reticulatus* only. Survival of infected and non-infected *N. reticulatus* individuals was compared to assess the disadvantage of being parasitized for the native species. A large quantity of snails was collected at 'Arguin', and subsequently isolated in dishes with seawater at ~24°C. Each dish was observed under a stereomicroscope to detect digenean larvae (cercariae). Nassariids that did not shed parasites were not always free of infection (Curtis & Hubbard 1990). Therefore, a higher number of control snails (×3) were kept in order to check non-infection when individuals died. Parasitized and control snails had the same size structure. A total of 158 snails (36 parasitized and 122 control) were gathered in a large aquarium, in running seawater at ambient temperature. Each individual was marked to calculate its lifespan during the experiment (total duration: 280 d). Snails were not fed in order to weaken them and exacerbate parasite detrimental effects. Although unrealistic, being starved equalized all gastropods at the same 'diet' and prevented them from trophic infestation (de Montaudouin et al. 2003).

RESULTS

Mitochondrial DNA (mtDNA) polymorphism within and among native and introduced *Cyclope neritea* populations

PCR amplification yielded a 553 base pair (bp) long fragment of the COI gene. Nineteen polymorphic sites were found in the 3 populations, and out of the entire sample (N = 93), 5 different mtDNA haplotypes were identified (Table 2). Over the whole data set, 2 haplotypes were found at very high frequency (*f*), namely A (*f* = 0.462) and B (*f* = 0.430). Haplotypes C, D and E were found more rarely, with frequencies of 0.065, 0.032 and 0.011, respectively. At the population level, Thau and Faro populations revealed only 1 haplotype (A and B, respectively), which was in contrast to the Arcachon population where the 5 haplotypes (A to E) were found, with A and B representing about 70% of the total number of sequences for this population (see haplotype distribution in Fig. 1). Consequently, at Arcachon, the haplotype diversity was very high (H_e = 0.725, Table 1) as well as the average pairwise difference (K = 6.576, Table 1). This high value of K was in agreement with the mismatch distribution from which 2 main peaks were observed, 1 of them centred on values of 13 to 14 nucleotide differences (Fig. 2). Such values are indicative of mixing of groups of individuals characterized by evolutionary divergent haplotypes within the Arcachon population. This pattern can be related to the evolutionary relationships between the observed haplotypes, as shown in the haplotypic network (Fig. 3). From this network, 3 major clusters were identified: (1) Haplotypes A and C, (2) Haplotypes B and D, and (3) Haplotype E. Haplotypes were highly divergent among these groups (13 mutations between A
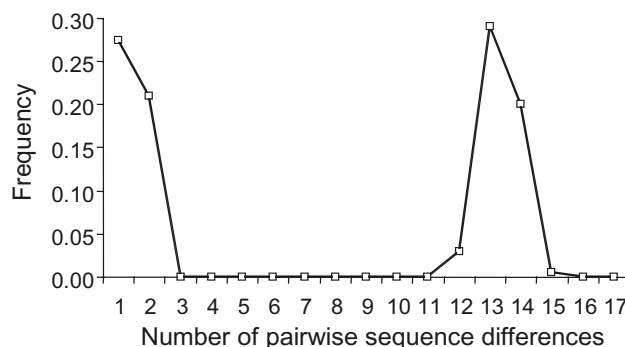

Fig. 2. *Cyclope neritea*. Mismatch distribution for the population of Arcachon Bay. The frequency of each observed value of pairwise difference is plotted

and B, 11 between A and E, and 13 between B and E; Fig. 3) but haplotypes were very close within each group (2 mutations steps between A and C, 1 between B and D; Fig. 3).
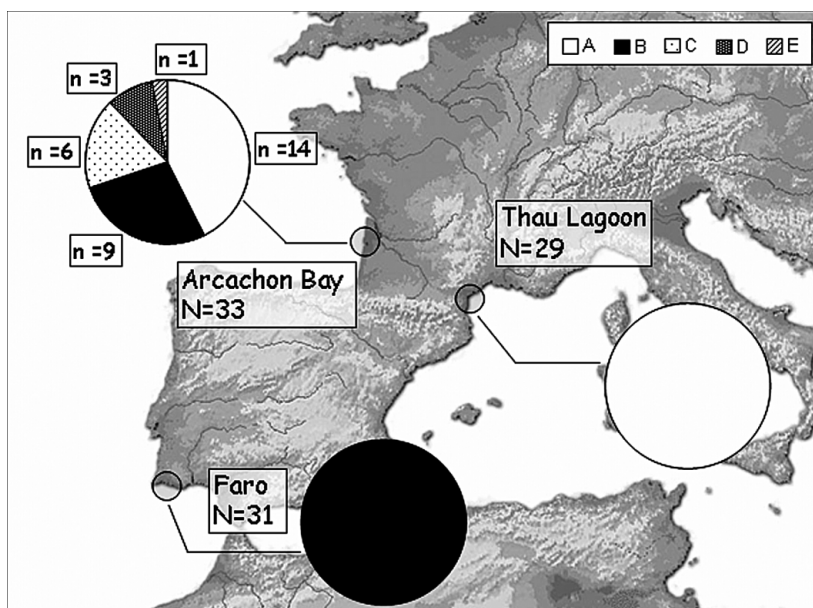

Fig. 1. *Cyclope neritea*. Haplotype distribution within the 3 study populations. Total number of individuals sequenced in each population (N) and number of each haplotype in the population of Arcachon Bay (n) are indicated. Haplotypes A to E are distinguished by different patterns

Table 2. *Cyclope neritea*. Definition of Haplotypes A to E found within the 3 populations of Thau, Faro and Arcachon. Positions of the polymorphic sites are given. '-' indicates that the same character is present in Haplotype A

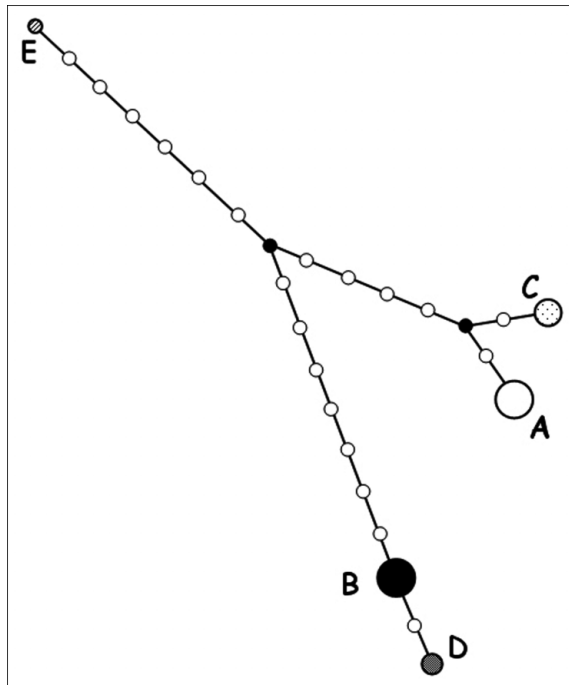| | Positions (bp) | | | | | | | | | | | | | | | | | |
	1	44	50	53	104	218	224	254	266	292	317	347	377	382	398	422	425	479	503
A	A	T	G	G	T	A	A	T	T	G	T	G	G	C	C	C	G	G	C
B	-	C	A	A	-	-	-	C	C	-	C	A	A	-	G	T	-	A	T
C	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-
D	-	C	A	A	-	-	-	C	C	-	C	A	A	A	G	T	-	A	T
E	G	-	-	A	C	G	G	C	C	A	C	-	-	-	-	-	A	A	-

Fig. 3. *Cyclope neritea*. Haplotypic network showing the phylogenetic relationships between the haplotypes. (o) Single mutational events. (●) Missing haplotypes between observed haplotypes in the data set

Pairwise F_{ST} values were all significant (p < 10^{-5}) indicating a large and significant genetic structure among the 3 populations. Because of the lack of common haplotypes, the highest (and maximum) genetic differentiation was found when comparing the populations of Thau and Faro (F_{ST} = 1). High genetic differentiation levels were also found when comparing the populations of Arcachon and Faro (F_{ST} = 0.58) and the populations of Arcachon and Thau (F_{ST} = 0.32).

Habitat of *Cyclope neritea* and *Nassarius reticulatus*

In Arcachon Bay *Cyclope neritea* occurred at tidal levels between –0.8 and +3.1 m, while *Nassarius reticulatus* was found between –5.2 m and +1.9 m (observations at the 5 study transects). As an example, Fig. 4 shows the zonation pattern along the bathymetric transect at 'Arguin', where the abundance of *C. neritea* was the highest at intertidal levels and that of *N. reticulatus* increased with depth down to –4.0 m, with an overlap of both species distribution between –1.0 and +1.3 m. *C. neritea* did not occur in fine, organic-rich sediments, whereas *N. reticulatus* were found in all sediment types sampled at Arcachon (Fig. 5). *C. neritea* did not tolerate sediment organic content > 1%, whereas *N. reticulatus* were still abundant in sediment with organic content as high as 14%.

Competition for food between *Cyclope neritea* and *Nassarius reticulatus*

When placed without any dead prey in the seawater channel, the individuals of both nassariid species either moved erratically or did not move at all, regardless of hydrodynamic conditions. Nevertheless, a 2-way ANOVA indicated that *Cyclope neritea* moved spontaneously in a higher proportion than *Nassarius reticulatus* in both hydrodynamic conditions (p < 0.05) and that this spontaneous moving was greater in flow conditions than in still water for both species (p < 0.05) (Table 3; Fig. 6). Moreover, while the proportion of *C. neritea* moving without the presence of carrion was almost the same in still water (53.1 ± 9.9%) and in flow (59.4 ± 11.5%), a higher proportion of *N. reticulatus* moved spontaneously in flow than in still water conditions (53.1 ± 11.0% and 18.7 ± 12.5%, respectively)
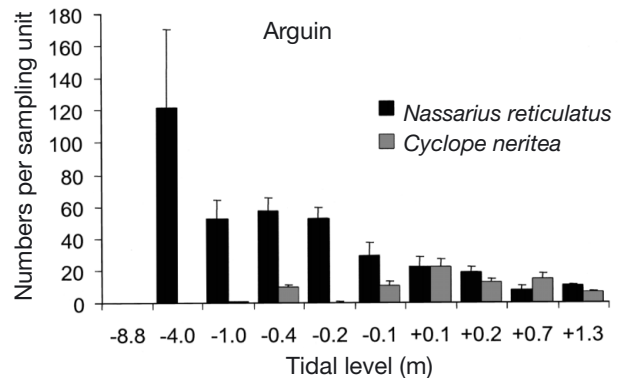

Fig. 4. *Cyclope neritea* and *Nassarius reticulatus*. Mean (+SE) number of individuals of both species per sampling unit (see text for explanations), at various tidal levels along a transect at 'Arguin' (Arcachon Bay)

(Fig. 6) In the presence of carrion, the speed at which individuals of both species moved towards, and reached, food was higher in flow that in still water conditions (Fig. 7), due to chemical stimulus conveyed by water. Due to heteroscedasticity that prevented us from performing a 3-way ANOVA between hydrody-

Table 3. *Cyclope neritea* and *Nassarius reticulatus*. Results of a 2-way ANOVA, testing for the effect of species and hydrodynamic conditions on the spontaneous moving of individuals of both species when no carrion was available for 30 min. Data were previously arcsin-transformed and homogeneity of variances was checked by Cochran test. ns: p > 0.05; *: p < 0.05

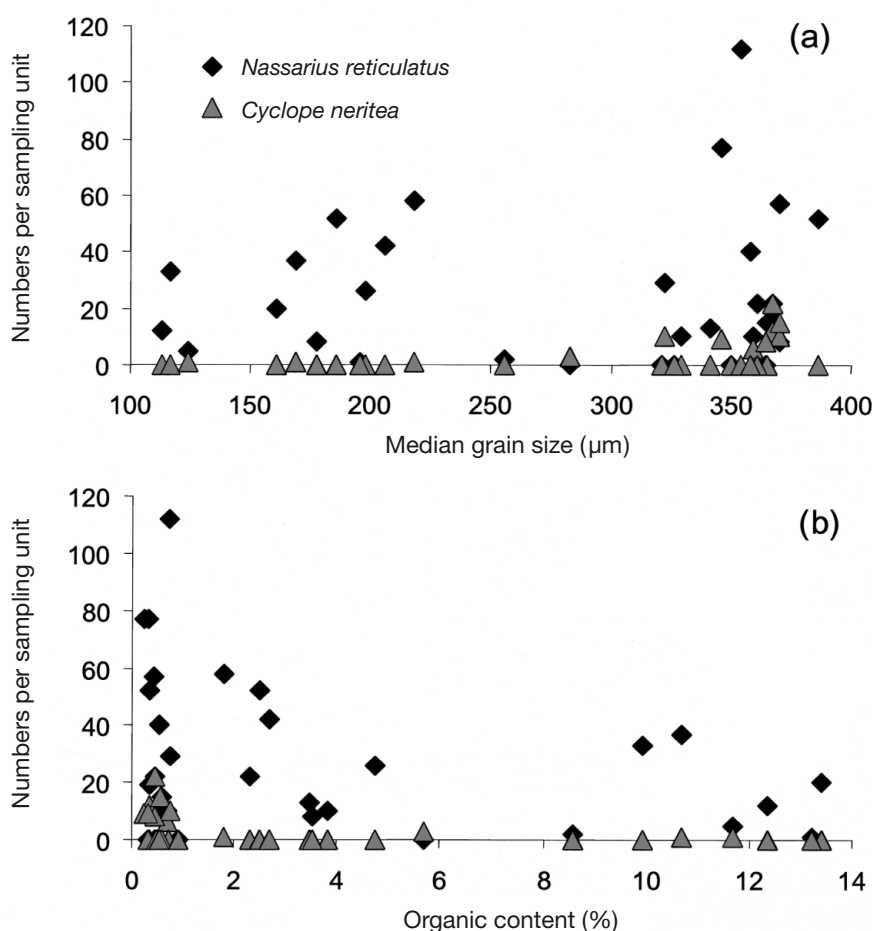| Source of variation	df	MS	F
Species	1	0.848	4.330*
Hydrodynamism	1	1.027	5.244*
Species × Hydrodynamism	1	0.303	1.548 ns
Error	28	0.196	

Fig. 5. *Cyclope neritea* and *Nassarius reticulatus*. Numbers of individuals of both species per sampling unit (see text for explanations) in relation to (a) median grain size and (b) organic content of the sediment at 35 stations located along 5 transects in Arcachon Bay

namic conditions, species and distance to the prey, the results were analysed separately for each hydro-dynamic condition by means of a 2-way ANOVA

(Table 4). In still water conditions, *N. reticulatus* took longer to arrive at carrion (i.e. speed was slower) than *C. neritea* (p < 0.001) and the speeds of both species
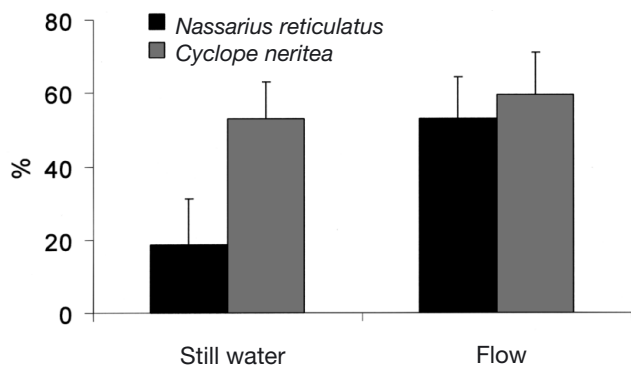

Fig. 6. *Cyclope neritea* and *Nassarius reticulatus.* Mean (+SE) percentage of individuals of both species moving sponta-neously (i.e. without bait) over a minimum distance of 10 cm during 30 min, in laboratory experiments with still water or flow conditions

Table 4. *Cyclope neritea* and *Nassarius reticulatus.* Results of 2-way ANOVAs, testing for the effect of species and distance to the prey on the speed of individuals of both species to reach their prey (a) in still water and (b) in flow conditions. Data were previously log(x+1) transformed and homogeneity of variances was checked by Cochran test. ns: p > 0.05; **: p < 0.01; ***: p < 0.001

Source of variation	df	MS	F
(a) in still water conditions:			
Species	1	1.656	18.202***
Distance	2	3.022	33.214***
Species × Distance	2	0.043	0.471 ns
Error	281	0.091	
(b) in flow conditions:			
Species	1	36.741	9.949**
Distance	2	74.702	20.228***
Species × Distance	2	30.783	8.335***
Error	90	3.693	

decreased the further away the carrion was placed from the nassariids (p < 0.001) (Table 4a; Fig. 7). In flow conditions, the speed of both species was similar when nassariids were at distances of 20 and 40 cm from the carrion, but at 60 cm *N. reticulatus* arrived at prey more quickly than *C. neritea* (Fig. 7); as a consequence, the species × distance interaction was highly significant (p < 0.001) (Table 4b).

Parasite load in *Cyclope neritea* and *Nassarius reticulatus*

Five digenean families were found in *Nassarius reticulatus* from Arcachon Bay (site 'Arguin'): Schistosomatidae, Zoogonidae, Lepocreadiidae, Echinostomatidae (mainly *Himasthla quissetensis*), Microphallidae, and unidentified sporocysts. Total prevalence fluctuated between 6 and 18% (Fig. 8). In contrast, *Cyclope neritea* was hardly parasitized, with only 1% infection by Microphallidae in November, February and April (Fig. 8). At experiment completion (280 d), the percentage of parasitized *N. reticulatus* among surviving individuals was identical to that of the dead ones, i.e. 50%. However, the peak of mortality was reached earlier in parasitized snails, i.e. 100–120 d as opposed to 140–160 d in uninfected snails (Fig. 9). From the 60th day to the mortality peak, parasitized snails had a higher mortality rate.

DISCUSSION

Vacant habitat and/or high competitive ability, low parasitic load, high genetic variance and climate change are among the factors shown to be most critical in the establishment of an exotic species (Williamson 1996, Carlton 2000, Sakai et al. 2001, Grosholz 2002, Stachowicz et al. 2002). From field, empirical and experimental studies, we analyzed patterns and processes associated with the recent settlement (less than 30 yr) of *Cyclope neritea* populations along the Atlantic French coast. We particularly studied the first established population (1976) in Arcachon Bay.

We first tackled the genetic architecture of the Arcachon population compared with populations from the original range of the species. Following accidental introductions of alien species, significant decreases of population genetic diversity compared to the native range have been regularly reported, e.g. in plants (Amsellem et al. 2000), in insects (Villablanca et al. 1998, Tsutsui et al. 2000), and in marine invertebrates (Grosholz 2002). In contrast with these studies, the recently settled French Atlantic population of *Cyclope neritea* (Arcachon) exhibited a far greater genetic
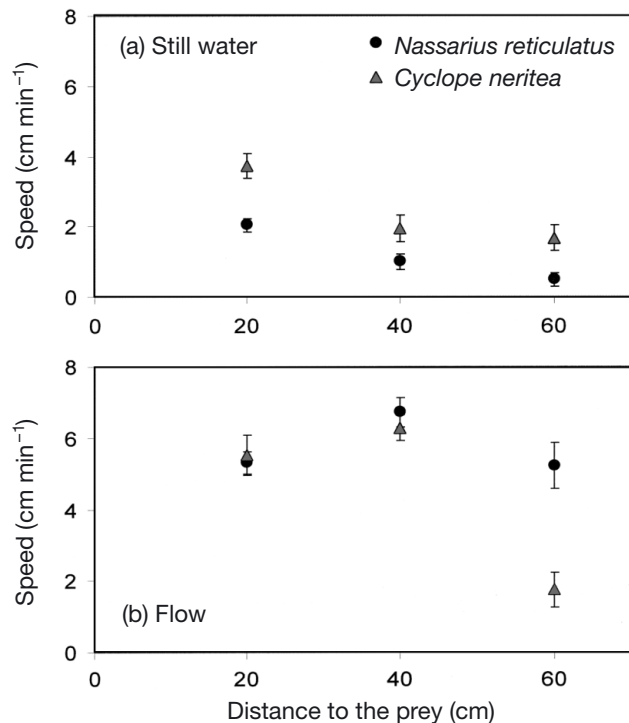

Fig. 7. *Cyclope neritea* and *Nassarius reticulatus*. Mean (± SE) speed by which individuals of both species reached carrion placed at 20, 40 or 60 cm in laboratory experiments with (a) still water or (b) flow conditions

diversity than the populations located within the natural range of the species (Thau and Faro). In contrast to the populations of Thau and Faro, for which a total lack of polymorphism was found (only 1 haplotype per population, H_e = 0), the recently settled population of Arcachon showed a very high level of polymorphism (5 haplotypes, H_e = 0.725).

The high genetic diversity observed at Arcachon could either be due to an introduction from a genetically diverse source population or to recurrent introductions from genetically differentiated sources (Bohonak et al. 2001). Our data support the latter hypothesis. Indeed, the 5 haplotypes found in this population were highly divergent with a mean number of pairwise differences (*K*) equal to 6.576. Together with the mismatch analysis, this is indicative of a genetic admixture within the Arcachon population and suggests the establishment of individuals originating from genetically distant populations. Moreover, haplotypes found most frequently in this population were fixed in the population of Thau (Haplotype A) and in the population of Faro (Haplotype B). This suggests that independent introductions from either these 2 sites or from genetically related sites occurred. In addition, Haplotype E, genetically divergent from the other haplotypes, was not found in the 2 populations sampled within the natural range of the species. This could indi-
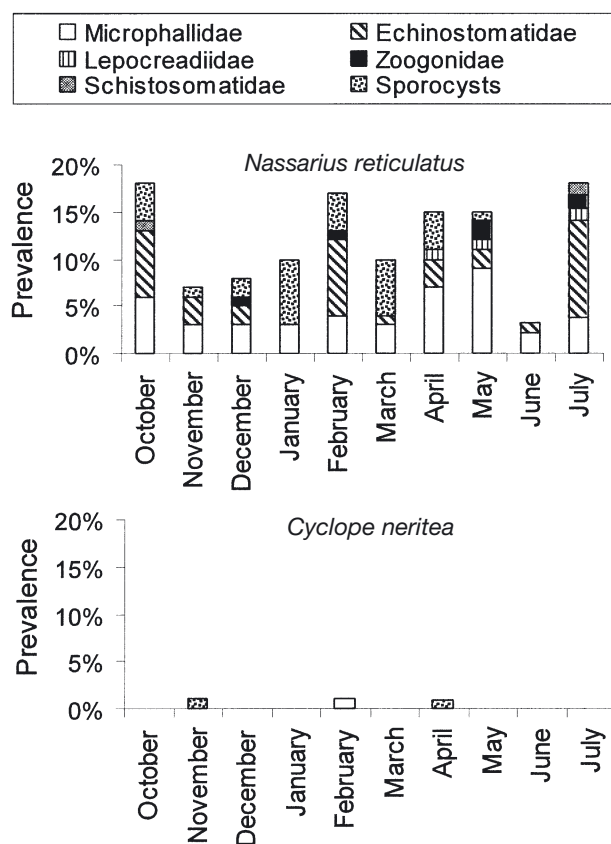
Fig. 8. *Cyclope neritea* and *Nassarius reticulatus*. Digenean prevalence (% of infected snails) in *N. reticulatus* (top) and *C. neritea* (bottom), from October 2000 to July 2001, in Arcachon Bay ('Arguin')
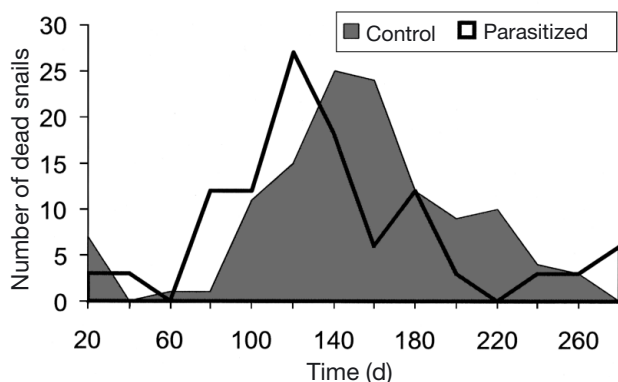

Fig. 9. *Nassarius reticulatus*. Mortality of parasitized and healthy (control) individuals during a 280 d experiment in running seawater

cate that other sources (e.g. eastern Mediterranean or Adriatic Sea) may have contributed to the settlement of the Arcachon population. The Adriatic Sea, where there are high densities of *Cyclope neritea*, is suspected as being at the origin of several gastropod introductions on the French Atlantic coasts because of

the Manila clam *Tapes philippinarum* shellfish trade (Goulletquer et al. 2002). Examples of possible introductions are *Gibbula albida*, noticed in the Morbihan Gulf in 1973 (Delemarre & Le Neuthiec 1995) and in Arcachon Bay in 1986 (Bachelet et al. 1990), and *Rapana venosa*, first introduced in the Black Sea during the 1940s and the Adriatic Sea during the 1970s (Zibrowius 1991), then in southern Brittany in the late 1990s (Goulletquer et al. 2002).

The recently settled *Cyclope neritea* population at Arcachon probably has multiple origins: a Mediterranean source with the transfer of Haplotype A (and probably C) and an Atlantic source with the transfer of Haplotype B (and probably D). However, because we lack other populations from the native range of the species, it is difficult to specify the true number of sources for the French populations as well as the number of independent introduction events (the origin of Haplotype E is yet unknown). It would be interesting to examine more closely the reasons for the observed divergence between haplotypes A and B which could be indicative of a phylogeographic break across Gibraltar and signal the occurrence of morphologically identical cryptic species. The presence at Arcachon of the haplotype found in Thau Lagoon implies human mediated dispersal mechanisms related to shellfish culture (e.g. transfers of *Crassostrea gigas* since the 1960s between French oyster farms; Sauriau 1991). However, the presence at Arcachon of the haplotype found in Faro could be due either to a natural range expansion of the species towards the north or to introduction events related to human activities (e.g. imports of *Crassostrea angulata* from Portugal since the 1860s; Gruet & Baudet 1997). These 2 processes (natural expansion and introduction) are characterized by different evolutionary time scales (Ribera & Boudouresque 1995). Because of the low dispersal ability of *C. neritea* (i.e. direct development), a natural range expansion towards the north would probably be associated with a step by step process contributing to a cline of the frequency of the haplotype found in Faro. Conversely, recurrent introductions are likely to leave jump dispersal genetic signatures with random fluctuations of frequencies between populations. It is therefore critical to analyze other populations located along the Atlantic coasts of Portugal, Spain and France to discriminate these 2 hypotheses (i.e. natural expansion or human-mediated dispersal from Portugal). The analysis of other recently settled French populations of *C. neritea* would also be useful to check for the consistency of the pattern observed in the Arcachon population.

Recent studies documented the importance of the genetic architecture (genetic variance, response to natural selection, etc.) of invading populations as being a crucial mechanism of biological invasions

(Reznick & Ghalambor 2001, Lee 2002). In several cases, bottleneck effects have been correlated with rapid rates of evolution (Tsutsui et al. 2000, Lee 2002). The large genetic diversity in neutral markers of *Cyclope neritea* from Arcachon cannot be directly correlated with adaptative evolution. However, the studied population is likely to originate from individuals coming from different geographic origins, and may have evolved under various environmental conditions; this would favour its adaptation to a wide range of environmental conditions and promote its long-term establishment and spread.

Mechanisms responsible for biological invasions are not only linked to intrinsic characteristics of the invaders, but also to interspecific interactions at the community/ecosystem level. Both competitive interaction and parasitism are considered as critical factors for the success of an invasion (Mooney & Cleland 2001, Keane & Crawley 2002). Non-indigenous species introduced in a new environment are expected to interfere with native species that exploit common food resources. We therefore hypothesized that *Cyclope neritea*, as a scavenger, would interact with the autochthonous dogwhelk *Nassarius reticulatus* which is probably the commonest scavenger gastropod in shallow marine waters of western Europe (Britton & Morton 1994). The *N. reticulatus* complex actually includes 2 closely related species, *N. reticulatus* (L.) and *N. nitidus* (Jeffreys) (Sanjuan et al. 1997); however, we found only *N. reticulatus* in field sampling in Arcachon Bay and used this species in competition experiments. A prerequisite to any study on interspecific competition is that species encounter each other so they must occur in the same habitat. Both *C. neritea* and *N. reticulatus* are extremely eurythermic and euryhaline but, according to Mars (1950), *N. reticulatus* is slightly less euryhaline than *C. neritea* (15 to 39‰ vs. 6 to 42‰, respectively). Actually, *C. neritea* occurs everywhere in Arcachon Bay, whereas *N. reticulatus* does not inhabit the most eastern parts of the bay where salinity may fall well below 15‰ in winter, due to large inputs of freshwater from rivers and drainage trenches (Bachelet & Dauvin 1993). The zonation pattern found in Arcachon Bay, i.e. −0.8 to +3.1 m for *C. neritea* and −5.2 to +1.9 m for *N. reticulatus*, is in agreement with literature data which indicate that *C. neritea* occurs in the Atlantic mainly in the intertidal (Morton 1960, Sauriau 1989) while *N. reticulatus* extends from low tide level to 36 m depth (Fretter & Graham 1962). Although both species may occur on a wide range of sediments, *C. neritea* in Arcachon Bay seems to be restricted to sands with low organic content, whereas *N. reticulatus* generally thrives best in substrates with high organic content (Tallmark 1980). Therefore, it appears that the habitats of both gastropods partially overlap in Arcachon Bay, with populations of both species being in sympatry in relatively clean medium and coarse sands located at tidal levels between ca. −1 and +1 m, where some interspecific competition may be expected.

Competition for food between benthic scavengers has seldom been demonstrated experimentally. To our knowledge, this has been attempted only by Morton & Yuen (2000) who used a combination of methods such as the assessment of food preferences, speed to bait, consumption rates, and the observation of individual behaviour when species were feeding together. We tested neither different bait species nor consumption rates by the 2 nassariid species. Behavioural observations (not reported here) in aquaria showed that up to 20 individuals of each species could be found aggregated on a single dead cockle without interfering with each other, probably because both species possess an extendible proboscis that allows feeding at a distance of 2 to 3 cm; similar observations were made by Morton (1960) on *Cyclope neritea* and by Morton & Yuen (2000) on *Nassarius festivus*. In the present study, we chose the speed of individuals to reach carrion and begin feeding as a method to assess competition. Our laboratory experiments clearly showed that, in static water, *C. neritea* moved towards food faster than *N. reticulatus*, whatever the distances (20, 40, or 60 cm) between the prey and the nassariids were. This trend changed in flow conditions, at least when the nassariids were at distances of 20 and 40 cm from the prey, when both species moved with a similar speed. These results were consistent with the spontaneous moving observed without prey, *C. neritea* being active in both static and moving water, and *N. reticulatus* moving spontaneously mainly in flow conditions. It appears that: (1) in still water experimental conditions, similar to those prevailing on intertidal sand flats with slowly running water at low tide (i.e. the habitat preferred by *C. neritea*), *C. neritea* was more active and reached its prey faster than *N. reticulatus*, thus having a competitive advantage over the latter; (2) in flow conditions, similar to those prevailing in subtidal levels (i.e. the habitat preferred by *N. reticulatus*), the activity of *N. reticulatus* increased compared to static conditions and its efficiency to reach a prey became similar to that of *C. neritea*. Nevertheless, even in low hydrodynamic conditions, competitive exclusion of *N. reticulatus* by *C. neritea* is unlikely, due to the abundance of bivalve carrion in the field, especially in shellfish areas such as Arcachon Bay. The fact that the 2 species basically have different substrate preferences would be an additional enhancing factor for the establishment of *C. neritea* because it can occupy an open niche in its new distribution area.

The present study demonstrated that the native *Nassarius reticulatus* was much more heavily parasitized than the introduced *Cyclope neritea*. A similar result was found in South Africa with the native mussel *Perna perna* and the introduced mussel *Mytilus galloprovincialis* (Calvo-Ugarteburu & McQuaid 1998a). The lack of infection in *C. neritea* could be due to a natural resistance to digenean parasites, as observed in the gastropod *Crepidula fornicata* in its native area (Pechenik et al. 2001). However, this is not the case because *C. neritea* in the Mediterranean can be heavily parasitized with the same digenean species as those observed at Arcachon in *N. reticulatus*, e.g. *Himasthla quissetensis* (Prévot 1974). Therefore, it is difficult to explain why *C. neritea* was not parasitized in Arcachon Bay where both parasite and host species (final host: lariid birds; second intermediate host: cockles) were all present. Two hypotheses can be proposed. The first one is a misidentification of the parasites. For example, is *Himasthla quissetensis* really the same species that parasitizes *C. neritea* (Prévot 1974), *N. reticulatus* (Russell-Pinto 1993, Desclaux et al. 2002) and *Ilyanassa obsoleta* (Stunkard 1938)? If not, the lack of infection of *C. neritea* in the introduction site could be due to the absence of the different hosts of the parasite cycle (the lariid birds and the cockles in the Mediterranean belong to the same genus but not to the same species). An alternative hypothesis in explaining the lack of parasites in *C. neritea* is that populations of the introduced nassariid have acquired a higher resistance to digenean infections.

When an introduced species does not bring its natural suite of enemies/parasites with it, it should experience a type of release or 'freedom' in its new environment. This release will be of a magnitude proportional to the ecological importance of the natural enemies left behind (Torchin et al. 2002). In the case of a competition between introduced and indigenous species, parasite release can be an important advantage (Calvo-Ugarteburu & McQuaid 1998b). Indeed, parasitic infection is recognized as a stressful factor which may lower the resistance of the host and its ability to challenge an unparasitized species. The experiment comparing mortality rates of infected and non-infected *Nassarius reticulatus* emphasized the disadvantage of being parasitized, as already suggested in a similar experiment with the mudsnail *Hydrobia ulvae* (de Montaudouin et al. 2003).

By using a multidisciplinary approach, this paper investigated several features expected to promote the long-term establishment of a recent invader that colonized Arcachon Bay 30 yr ago. Our results suggest that the combination of various factors, i.e. recurrent introduction of individuals originating from different environments, a significant competitive ability and the lack of heavy parasitic load, explains the settlement of *Cyclope neritea* in Arcachon Bay. Whether these factors can promote a greater invasion success in the near future and the long-term establishment of this species along the Atlantic French coast, although likely, is still an opened issue. In particular, the role played by climate change is still to be investigated since, as already reported for marine benthic communities (Glémarec 1979) and invasive species such as *C. neritea* (Sauriau 1991) and *Crassostrea gigas* (Drinkwaard 1999), climate warming is expected to favour population dynamics and further northwards expansion of warm-temperate species. Experimental studies and genetic analysis of other French populations of *C. neritea* would be useful to check for this effect as well as to verify the consistency of the patterns observed in the Arcachon population.

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